Toxin-Antitoxin Systems as Phage Defense Elements

Toxin-Antitoxin Systems as Phage Defense Elements

Michele LeRoux and Michael T. Laub[1,2,#]

[1] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

[2] Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139

[#] correspondence: laub@mit.edu; 617-324-0418

Abstract

Toxin-antitoxin (TA) systems are ubiquitous genetic elements in bacteria that consist of a growth-inhibiting toxin and its cognate antitoxin. These systems are prevalent in bacterial chromosomes, plasmids, and phage genomes, but individual systems are not highly conserved, even among closely related strains. The biological functions of TA systems have been controversial and enigmatic, although a handful of these systems have well-documented roles in defending bacteria against their viral predators, bacteriophage. Additionally, their patterns of conservation – ubiquitous, but rapidly acquired and lost from genomes – as well as the co-occurrence of some TA systems with known phage defense elements are suggestive of a broader role in mediating phage defense. Here, we review the existing evidence for phage defense mediated by TA systems, highlighting how toxins are activated by phage infection and how toxins disrupt phage replication. We also discuss phage-encoded systems that counteract TA systems, underscoring the ongoing coevolutionary battle between bacteria and phage. We anticipate that TA systems will continue to emerge as central players in the innate immunity of bacteria against phage.

Introduction

Toxin-antitoxin (TA) systems are genetic modules found in most bacterial (and archaeal) genomes, with individual species often encoding dozens of distinct systems (37). Despite their abundance and prevalence, the functions of TA systems have remained poorly defined and even controversial (16, 37, 41, 52, 65, 78, 89, 108, 109, 112). However, there is accumulating evidence that they play critical roles in protecting bacteria against their ubiquitous and relentless predators, bacteriophage (35, 37, 103). Here, we review the evidence for TA systems as a major form of defense against phage. Like other immune mechanisms, TA systems are likely subject to frequent and rapid diversification as bacteria seek to stay one step ahead of their predators. We argue that this coevolutionary dynamic helps to explain the abundance, patterns of conservation, diversity, and mechanistic hallmarks of TA systems. We begin by briefly introducing the key features and aspects of TA systems most relevant to their consideration as phage defense elements, and refer the reader to other reviews for details on the many variations and differences between TA systems (26, 30, 37, 84, 116).

TA systems typically feature a protein toxin that inhibits host cell growth – often reversibly – if not restrained by its cognate antitoxin (116). There are four major types of TA systems, classified based on the nature of their antitoxins (Fig. 1) (37). For type I systems, the antitoxins are antisense RNAs that block toxin synthesis (31), and the type I toxins are typically small peptides that create membrane pores to dissipate the proton motive force necessary for ATP synthesis (29) (Fig. 2). Type II systems, which are the most extensively characterized TA systems, consist of an antitoxin protein that directly binds and neutralizes its cognate toxin. These antitoxins also typically contain a DNA-binding domain through which they negatively autoregulate their own transcription (104). Type II toxins include endoribonucleases that target mRNA, rRNA, and tRNA (16, 18, 114, 118); ribosome poisoning proteins (64); acetyltransferases that target tRNAs (15); topoisomerase inhibitors (5, 39, 47); ppGpp/ppApp synthetases (48); tRNA pyrophosphorylating proteins (55); mono-ADP-ribosyltransferases (87), and cell wall inhibitors (76) (Fig. 2). Type III systems feature an RNA antitoxin that directly binds and neutralizes its cognate toxin (25); all known type III toxins are endoribonucleases (25, 94, 100). Finally, type IV systems consist of an antitoxin protein that indirectly antagonizes the enzymatic activity of its cognate toxin rather than directly binding the toxin. These toxins exhibit a wide range of functions including DNA ADP-ribosyltransferases (46), predicted nucleotidyltransferases (22), FtsZ inhibitors (43, 105), and ppGpp/ppApp synthetases (48) (Fig. 2). Although these classifications are a useful framework, there are some TA systems that do not fit neatly into any of these designations (1, 48, 57, 111).

TA systems have been found in virtually every sequenced bacterium, with many species encoding dozens. For instance, *Escherichia coli* K12 encodes more than 30 TA systems and the pathogen *Mycobacterium tuberculosis* encodes at least 80 systems (37, 88, 91). However, TA systems are poorly conserved. *E. coli* O157:H7 is predicted to encode at least 31 TA systems, but only about half appear to be orthologs of those in *E. coli* K12 (Fig. 3). For

many organisms, the number of TA systems currently annotated is likely an underestimate of the actual number, as most are identified by homology searches using known systems, which will miss distantly related homologs or novel systems unrelated to those used as queries in annotation efforts (58, 69, 85, 98, 115). TA systems are also sometimes found in phage genomes, particularly prophages, as will be discussed later, and on many plasmids (69, 98).

Functions of TA systems

The first TA system identified, the type II system CcdAB, can function as a plasmid-stabilization element (79). The protein antitoxin CcdA is relatively unstable and must be continuously synthesized to prevent liberation of the toxin, CcdB, which is a gyrase poison (5, 71, 107). If a plasmid carrying *ccdAB* is lost, the antitoxin cannot be replenished and the existing pool of CcdA is cleared from the cell, leading to liberation of the CcdB toxin that kills the cell (107) (Fig. 4). Thus, only cells that inherit the plasmid, and can replenish CcdA, survive. This simple yet elegant mechanism does not, however, apply to the vast majority of TA systems, which are found on bacterial chromosomes and do not play an analogous role in chromosome inheritance.

TA systems are often purported to function in bacterial stress responses, largely because many systems are transcriptionally upregulated following exposure to diverse abiotic stresses (17, 75, 89) (Fig. 4). Notably however, there are few reported cases of TA systems having significant null phenotypes in the stress conditions that induce their transcription. A recent systematic study of the relationship between stress and type II TA systems in *E. coli* found that diverse stresses can drive substantial increases in TA transcription, often 10-fold or greater, but without liberating active toxin (59). Some of these stresses accelerate the degradation rates of antitoxins, which relieves the auto-repression of their operons. However, increased transcription leads to concomitant synthesis of new toxin and its neutralizing antitoxin, helping to ensure that no free, active toxin is produced. Additionally, the increased proteolysis of antitoxin following stress preferentially impacts pools of free antitoxin (typically in excess relative to toxin) while the toxin-antitoxin complexes remain relatively stable. Thus, abiotic stress can dramatically increase TA transcription, but without producing detectable levels of active toxin.

TA systems have frequently been implicated in the formation of so-called persisters, which are growth-arrested cells that transiently, but non-heritably, tolerate antibiotics (89). This proposed function for TA systems came initially from the discovery of high-incidence of persister (*hip*) mutants of *E. coli* that mapped to a TA system, *hipAB* (6, 50, 74). However, although these mutations, *e.g. hipA7*, likely lead to elevated levels of HipA toxin that can promote a non-growing persister state, deleting the *hipAB* locus does not measurably impact persister cell formation (36, 53). Similarly, overexpressing many toxins can block growth and render cells more tolerant to antibiotics, particularly those that target actively growing cells, but these observations do not mean these toxins normally promote persistence. A role for TA systems in persister cell formation in *E. coli* was suggested by two

studies showing that the deletion of 10 TA systems substantially decreased persister levels (67, 68). However, this work could not be replicated and was retracted after subsequent studies revealed that the originally-reported phenotypes arose from prophage contamination of the Δ10TA strain, not the deleted TA systems (33, 38). There are, however, other studies supporting a role for some TA systems in persister cell formation by wild-type strains. For instance, *E. coli* lacking the type I *tisAB* system was reported to produce fewer persister cells in response to ciprofloxacin challenge (21), and deleting individual TA systems from *Salmonella* produces fewer persisters within macrophages (42).

A general role for TA systems in persister cell formation is uncertain, and the genomic distribution of TA systems suggests an alternative, and possibly broader, function. Although TA systems are nearly ubiquitous within bacterial and phage genomes, individual TA systems show limited conservation and are typically part of the non-conserved bacterial "accessory genome" (37). As noted above, many TA systems encoded by *E. coli* strain MG1655 are not found in other *E. coli* strains (Fig. 3). This and similar observations in other species suggest that TA systems are rapidly acquired and lost over the course of evolution. Such a distribution pattern – ubiquitous, but highly variable – is often seen with phage immunity systems (70), and some TA systems are associated with clusters of genes related to phage defense, referred to as defense islands (60, 66, 70). Additionally, TA systems are frequently encoded in prophages, possibly to help them compete with or fend off other phages or to ensure the survival of their host (58). Together, these genomic distribution patterns suggest that many TA systems could function in phage defense (Fig. 4). There are also a handful of TA systems directly shown to provide immunity against phage. This includes several systems originally identified as so-called abortive infection systems (Abi), in which a host-encoded protein triggers cell death in response to phage infection, that were subsequently classified as TA systems.

TA systems as phage defense elements

Here, we review experimental studies of TA systems that provide phage defense (summarized in Table 1), focusing on the cases that have been best characterized. In each case, we highlight, when possible, four key aspects of TA systems as phage defense elements. First, we discuss the mechanisms responsible for activating TA systems and liberating toxin following phage infection. In contrast to phage defense elements like CRISPR and restriction-modification (RM) systems, which are effectively always on but able to distinguish self from non-self, TA systems must remain off until phage infection occurs and then somehow be rapidly activated. Second, we discuss the diverse mechanisms that toxins use to thwart a phage infection. Again, in contrast to CRISPR and RM systems that predominantly target phage DNA and occasionally phage RNA, the toxins of TA systems exhibit a wide range of biochemical activities that can target many different phage processes to block an infection cycle. In some cases, the toxins may also target host factors to kill the cell and abort the infection, thereby saving neighboring cells

from infection. Third, we highlight cases where phages are known to have evolved anti-TA mechanisms. Such mechanisms underscore the coevolutionary battle being waged between phages and their hosts and the role that TA systems play in this battle. These mechanisms may also have limited the identification of phage-defensive TA systems. Fourth, we include discussion of the phage specificity of individual defensive TA systems. Whereas CRISPR and RM systems are frequently effective against many different phages, TA systems appear to be more specific, and we discuss the possible mechanistic bases of this specificity.

E. coli RnlAB

One particularly well studied case of phage defense by a TA system involves the type II system RnlAB in *E. coli* K12. This system provides robust defense against T4 phage lacking the gene *dmd*, with the efficiency of plaquing for *dmd*- phage reduced ~1,000-10,000-fold on strains harboring *rnlAB* (51, 82). Yonesaki and colleagues had initially found that the T4 gene *dmd* was required to stabilize mRNA expressed late during T4 development (49). The RNase activity responsible was subsequently found to depend on the host-encoded toxin, RnlA (81), which is normally restrained by its cognate antitoxin, RnlB (51). Following infection with a T4 *dmd* mutant, the antitoxin RnlB is rapidly degraded, presumably leading to release of the RnlA endoribonuclease toxin (51). RnlB is also rapidly degraded in uninfected cells in a Lon- and ClpX-dependent manner. Because RnlB is intrinsically unstable, the shutoff of host transcription induced by T4 infection, including *rnlAB*, may enable release of active RnlA as RnlB cannot be replenished. T4 has a variety of mechanisms for shutting down host transcription as it commandeers cellular resources for itself, so this model is plausible and reminiscent of the post-segregational killing mechanism discussed above that activates the type II toxin CcdB (107). However, whether a shutdown of host transcription is necessary or sufficient to activate RnlA has not yet been established.

When activated after infection by a T4 *dmd* mutant, RnlA toxin cleaves phage RNAs (80, 81), though the sequence specificity of RnlA remains uncertain. Another twist in the RnlAB system is that RnlA activity somehow depends on host-encoded RNase HI, which degrades RNA-DNA hybrids (77). In fact, RNase HI was found to promote both the activity of RnlA following T4 infection and the inhibition of RnlA by RnlB, likely through a direct interaction, but precisely how RNase HI impacts the RnlAB system remains to be elucidated.

Notably, the RnlAB system only protects *E. coli* against a T4 *dmd* mutant (82). Biochemical and structural studies showed that Dmd, a 61 amino acid protein, directly binds and inactivates RnlA like an antitoxin, although it binds a slightly different region of RnlA compared to RnlB (113). Dmd also binds and inhibits the toxin of another, closely related, type II TA system called LsoAB found on a plasmid in *E. coli* O157:H7 (82, 110). LsoAB can, if introduced into *E. coli* K12, also provide robust protection against a T4 *dmd* mutant (82). Although Dmd promiscuously inhibits both toxins, RnlB and LsoB, the antitoxins of each system only neutralize their cognate toxin. Dmd homologs are found in the T-even phages T2, T6, and RB69, suggesting that each of these phages

have encountered RnlAB- or LsoAB-like defense systems in the wild and have consequently been under pressure to maintain mechanisms that overcome them.

ToxIN systems

Type III TA systems, featuring an RNA antitoxin, were first discovered in the context of phage infection (25). These systems have now been extensively characterized, and thus provide a detailed case study of TA-mediated phage defense (Fig. 5). The Salmond lab first demonstrated that a *toxIN* system found on a cryptic plasmid could provide the phytopathogen *Erwinia carotovora* (now *Pectobacterium atrosepticum*) potent resistance against the phages ΦA2 and ΦM1 – with EOP values of ~10^{-6} in both cases – likely through an Abi mechanism (25). In canonical Abi systems, phage infection triggers release of a toxin that poisons the infected cell. Although the infected cell does not survive the infection, as would happen with true 'immunity' mechanisms like CRISPR-Cas systems, this cell "suicide" effectively spares neighboring, clonal cells from infection and prevents viral spread in the population. However, ToxIN (and likely other TA systems) are not necessarily canonical Abi systems. Like many of the type II toxins, ToxN is only bacteriostatic when overproduced, indicating that it blocks cell growth rather than killing cells (25). The death of infected cells likely results from the phages triggering lethal processes such as chromosome degradation well before the ToxIN system is activated.

Further characterization of the *P. atrosepticum toxIN* locus, *toxIN*$_{Pa}$, demonstrated that *toxI*$_{Pa}$ is a non-coding RNA comprised of 5.5 repeats of a 36-nucleotide sequence (9, 25). ToxN$_{Pa}$ is an endoribonuclease that processes the *toxI*$_{Pa}$ precursor into monomers that can assume a pseudoknot structure (100). Structural studies indicated that three ToxN$_{Pa}$ monomers bind three *toxI*$_{Pa}$ monomers in a complex that possibly holds the ToxN in an inert state. However, recent work suggests that this complex is dynamic, and that ToxN$_{Pa}$ is, in fact, not stably bound to *toxI*$_{Pa}$ (99). Rather, *toxI*$_{Pa}$ is synthesized, processed by ToxN$_{Pa}$, and degraded at relatively fast rates, but that toxin is effectively always occupied processing *toxI*$_{Pa}$.

After their initial discovery, type III TA systems were subsequently found to be relatively widespread (10, 32). In fact, *P. atrosepticum* ToxN is homologous to AbiQ, a protein produced from a plasmid in some strains of *Lactococcus lactis* that provides phage defense (92). Although AbiQ was reported before the discovery of *toxIN*, the upstream non-coding antitoxin RNA was not identified and thus it was not initially classified as a TA system (24). Further bioinformatic studies found that type III TA systems, now categorized into three separate sub-families (ToxIN, TenpIN, and CptIN), are found in a wide variety of bacteria though the phage defense role of most remains untested (10).

More recently, a *toxIN* locus from an environmental isolate of *E. coli*, strain GCA_001012275, was shown to provide *E. coli* K12 with very strong (EOP ~10^{-7}) protection against the model coliphage T4 (34). RNA-seq analysis of *E. coli* cells harboring this *toxIN* system demonstrated that infection initially proceeds normally with

no major detectable differences in the expression of T4 genes until ~10 min post-infection. At that point, several T4 transcripts show regions with lower read counts relative to a control strain lacking the *toxIN*$_{Ec}$ system indicating that they are likely directly cleaved by liberated ToxN$_{Ec}$. By 20 min post-infection, more than 50% of all T4 transcripts show significant cleavage, particularly those transcripts harboring the 5-mer GAAAU, which was also identified as the sequence motif for ToxN in overexpression studies. The widespread cleavage of T4 transcripts leads to a major decrease in T4 protein production and a lack of mature virion development. Notably, most host transcripts are undetectable by RNA-seq following T4 infection either in the presence or absence of *toxIN,* most likely due to shutoff of host transcription by the phage. Thus, ToxN$_{Ec}$ is not triggering cell death, as in canonical Abi systems, but is deployed to disrupt the late stages of phage development in a cell already destined to die in order to save clonal neighbors from infection.

The study of this *E. coli* ToxIN system during T4 infection provided insights into the physiologically relevant activity of a TA system following its native induction. This stands in contrast to most prior studies of toxin activity, which have predominantly been through artificial overexpression in uninfected cells. For TA systems that function as phage defense elements, the study of their effects on uninfected cells could be missing key targets, even though most are toxic to their bacterial hosts. For instance, when *E. coli* ToxN is overproduced in *E. coli,* it inhibits growth by targeting a range of bacterial transcripts, but, as noted, few of those transcripts are present when ToxN is naturally activated by phage. This may also be the case for other types of toxins. For example, MazF homologs from *Mycobacterium tuberculosis* cleave host tRNAs when overproduced (96). As many phages encode tRNAs, it is possible that these are important, consequential targets if and when these TA systems are natively activated by phages. Similarly, overexpression studies indicate that many toxins target gyrase (5, 39, 47) (Fig. 2), but phages often encode their own topoisomerases that could be alternative or even the preferred targets of these toxins.

The protection of *E. coli* against T4 infection by ToxIN has also provided insight into the mechanism of toxin activation (34) (Fig. 5). This ToxIN$_{Ec}$ system, like that of *P. astrosepticum*, features a relatively unstable antitoxin. Treatment of uninfected cells with rifampicin leads to the almost complete clearance of full-length *toxI* precursor in ~5 minutes. The decay rate of full-length *toxI* is nearly identical following T4 infection, consistent with the notion that *toxI* (and *toxN*) transcription is shutoff by T4, leading to its rapid decay and subsequent release of the more stable ToxN protein. The transcriptional shutoff of *toxIN* was demonstrated to be both necessary and sufficient for ToxN activation. If an ectopic copy of *toxI* is placed under the control of a T4 middle promoter, and therefore actively transcribed during infection around the time ToxN is normally active, the *toxIN* system no longer provides robust protection against T4. Placing *toxI* under the control of an early or late promoter has no such effect, indicating that the extra *toxI* came too early or too late to block ToxN. Thus, the inhibition of *toxI* transcription by T4 is necessary for ToxN activation. Conversely, forcing a shutoff of *toxIN* is sufficient to activate

ToxN, even in the absence of phage infection. Either adding rifampicin to globally block transcription or specifically inhibiting *toxIN* transcription via CRISPR interference releases active ToxN.

The identification of a mechanism for activation of the ToxIN system is noteworthy as TA systems differ relative to phage defense systems like CRISPR and RM systems. These latter defense systems are always on and in surveillance mode, using sequence-specific spacers and PAM sequences or DNA modifications, respectively, to discriminate between host and virus. In contrast, the toxins of TA systems must remain off until phage infection occurs to prevent inhibiting growth of the bacteria. Most toxins are bacteriostatic, particularly on short time-scales, possibly to ensure that their inadvertent release would not kill a host cell. There are some exceptions like CcdB, which is a bacteriocidal gyrase poison (5), although as noted above, this system functions in plasmid inheritance potentially making bacteriocidal activity an advantage. The need to keep phage-defensive TA systems off until infection occurs may also explain other common features, including the excess levels of antitoxin relative to toxin and negative autoregulation, both of which help prevent toxin release in uninfected cells. Indeed, bacteria devote considerable cellular resources to maintaining these systems in an off state under non-infection conditions (59): transcribing and constantly producing excess antitoxin to prevent toxin release.

Whether other type III TA systems use a mechanism similar to *E. coli* ToxIN – transcription-shutoff coupled to intrinsically fast antitoxin turnover – is not yet known, but seems plausible given that most antitoxin RNAs are probably labile and not stably associated with their cognate toxins (99). This mechanism would potentially allow a type III TA system to function in a wide range of hosts, thereby facilitating its horizontal spread, and to respond to a wide variety of phages, many of which shutdown host transcription. However, not all phages inhibit transcription as rapidly and completely as T4. For instance, T7 produces an inhibitor of the host RNA polymerase and encodes its own RNA polymerase, but the overall shutdown of host transcription is not as rapid and complete as during T4 infection (13, 102). Consequently, the ToxIN$_{Ec}$ system only modestly reduces T7 burst size, without impacting the efficiency of plaquing (34). However, forcing the shutoff of *toxIN* transcription just prior to infection substantially improves its defensive capabilities against T7. Thus, phage may be faced with a tradeoff. Shutting down host transcription helps them to commandeer host resources, such as nucleotides, to promote their own replication, but comes at the cost of potentially triggering defense systems such as *toxIN*. Similarly, host defense systems may need to balance or tune their sensitivity to phage-induced shutdown of transcription. A TA system that is highly sensitive to transcription inhibition may potentiate its phage defensive capability, but could suffer spurious toxin activation in response to non-phage related perturbations and transcriptional changes.

As with RnlAB/LsoAB, phage have acquired or can evolve mechanisms to counteract type III TA systems. The phage ΦTE cannot infect *P. atrosepticum* 1043, which harbors *toxIN*$_{Pa}$. A selection for 'escape' phages that can infect this strain produced phages with expansions of a repeat region bearing some similarity to *toxI* (8). This pseudo-*toxI* locus was amplified in the phage genome and then acquired additional mutations, enabling the phage

to infect the previously resistant ToxIN-encoding host. A second lineage of escape mutants acquired *toxI* repeats from the host's plasmid-borne *toxIN* locus. Another selection for escape mutants of T4-like phages that infect a *Serratia* species engineered to harbor $toxIN_{Pa}$ revealed point mutations in the genes *asiA* and *orf84*, and a large (6.5-10 kb) deletion (14). How these mutations enable the phage to escape ToxIN-mediated defense is not clear. However, if the *P. atrosepticum* ToxIN system is activated like the *E. coli* ToxIN system, *i.e.* by transcription shutoff, the *asiA* mutation may work by preventing T4 from fully shutting off host transcription. AsiA, along with MotA, normally helps T4-like phages appropriate the host's primary sigma factor, σ^{70}, to promote phage transcription and block host transcription (45).

Retron-based TA systems

Retron systems, first discovered in 1984, have recently been identified as phage defense elements that are effectively tripartite toxin-antitoxin modules (11, 12, 27, 72). Retrons contain three elements: a noncoding RNA, a reverse transcriptase (RT) that generates a covalently linked DNA-RNA hybrid, and an effector protein of varying function (101). Maturation of the RNA-DNA hybrid requires the cognate RT, as well as RNase H, and sometimes Exo VII (11, 56). The mature RNA-DNA hybrid, likely along with the RT enzyme itself, comprise an antitoxin for the cognate effector protein of the system (11). These retron systems vary substantially in the length and identity of the RNA-DNA hybrid, as well as in the functions of the toxin, which include endonucleases, ribosyltransferases, and transmembrane proteins (27, 72). A recent characterization of the Ec48 retron in *E. coli* found that its effector protein has two predicted transmembrane helices and likely compromises membrane integrity shortly after phage infection, leading to an abortive infection (72).

To become activated following phage infection, the Ec48 retron somehow "monitors" the state of the bacterial RecBCD complex, which is normally involved in homologous recombination and can degrade linear, double-stranded DNA, including phage genomes (4, 73). In the absence of phage infection, the retron is thought to bind RecBCD, though a direct interaction has not been shown. Many phage, such as λ and T7 produce RecB inhibitors (Gam and gp5.9, respectively) that are thought to siphon RecBCD away from the retron (63, 83, 90), somehow triggering release of the effector protein. Thus, phage may face a major dilemma: inhibiting RecBCD will promote their replication within a host lacking a retron system, but will activate a defensive retron system, if present. A separate study that screened for activators and inhibitors of retrons identified a number of prophage-encoded (as well as host) proteins that impact retrons, suggesting these systems may recognize or respond to other phage proteins and raising the possibility of phage counter-defense mechanisms (12).

Other phage-defensive TA systems

In addition to RnlAB, *E. coli* K12 encodes at least 12 other type II TA systems (37) (Fig. 2) that could, in principle, protect against phage infection. One of the first type II TA systems identified on the *E. coli* K12 chromosome,

mazEF, was suggested to participate in phage defense (40). In *E. coli* strains MC4100 and JM109, cells harboring *mazEF* were reported to produce ~10-15-fold fewer phage upon induction of, or infection with, the temperate phage P1. However, no efficiency of plaquing data were reported, nor was a mechanism of MazF activation determined, and a recent study could not reproduce these initial findings in *E. coli* K12 (34). Additionally, the Δ*mazEF* strains examined in the original study were in an MC4100 background later shown to harbor mutations in the adjacent gene *relA* (106), which is critical to growth control in *E. coli*. Thus, the role of MazEF, as well as the other type II TA systems in *E. coli* K12 remains uncertain. However, as coliphage have historically been isolated on K12 *E. coli*, there has been an intrinsic selection bias against finding phage that these TA systems may defend against. As noted earlier, a phage defense function for RnlAB was only revealed by working with a T4 *dmd* mutant; it could be that many of the coliphages isolated on K12 strains have mechanisms such as Dmd that counteract the type II TA systems in this strain.

If the other *E. coli* type II TA systems protect against phages, their mechanisms of activation may differ substantially from RnlAB, which likely involves transcriptional shutoff of *rnlAB* coupled to fast turnover of RnlB by Lon and ClpXP (51). Although it is often assumed that all type II antitoxins have fast turnover rates, a recent study measuring degradation rates *in vivo* under native expression conditions found that some antitoxins, such as MqsA and YefM, have half-lives longer than 20 minutes (59). Given that the time from genome injection to new particle release for many phages is ~20-40 minutes, these antitoxins are unlikely to decay fast enough to release toxin unless their degradation is somehow stimulated.

Type I TA systems may also participate in phage defense. An early study showed that the *hok/sok* system from the R1 plasmid provides *E. coli* modest (< 10-fold protection by EOP) levels of protection against T4 phage if carried on a high-copy plasmid (86). The entire, lower-copy R1 plasmid also provided some, but reduced, protection against T4. The mechanism by which T4 infection activates Hok, a membrane pore forming peptide, has not been elucidated. It was suggested that the inhibition of transcription by T4 may be responsible, similar to the mechanism proposed for RnlAB and demonstrated for ToxIN, but a direct test of this mechanism was not reported to our knowledge. *E. coli* K12 encodes at least 4 *hok/sok*-like systems and ~15 other type I TA systems (Fig. 2A); whether any of these chromosomal loci protect against phage is not yet known.

A recent bioinformatic analysis revealed that members of the DarTG family of TA systems are frequently encoded in defense islands, leading to the prediction that these systems may be phage defense elements (60). Although *E. coli* K12 does not encode any DarTG homologs, two distinct members of this family from environmental *E. coli* isolates, termed DarTG1 and DarTG2, provided the *E. coli* MG1655 strain strong defense against RB69 and T5 phage, respectively, with EOP values of $< 10^{-6}$ when expressed from their native promoters. Both systems also defend against other types of phage, albeit with lesser protection. Prior to this finding, the DarTG systems had only been characterized *in vitro* and through artificial induction of the toxin in bacterial cells (46, 57, 97, 117).

These studies demonstrated that the DarT toxins ADP-ribosylate single-stranded DNA, which inhibits DNA synthesis and induces a DNA damage response in bacterial cells (46, 57). Additionally, DarG antitoxins were shown to possess ADP-glycohydrolase activity, which allows them to enzymatically reverse the modification made by their cognate DarT toxin. For at least one DarTG system, the antitoxin also interacts directly with its cognate toxin and this activity contributes equally to its ability to neutralize its cognate DarT (57), making these hybrid type II and type IV TA systems.

During phage infection, the DarT toxin of both the DarTG1 and DarTG2 systems is liberated and subsequently ADP-ribosylates phage DNA (60). This modification leads to the inhibition of DNA synthesis, and to a lesser degree, RNA synthesis, preventing the phage from producing viable progeny. Even though DarT indiscriminately modifies DNA, host DNA is unlikely to be the biologically relevant target of DarT as phage like RB69 and T5 likely trigger host chromosome degradation even in the presence of DarTG. This chromosome degradation means that a host cell carrying DarTG does not survive infection, but because the DarT toxin inhibits phage replication, it effectively prevents spread of an infection through a population, similar to other TA-mediated defense mechanisms. The same study also found that evolving the phage SECϕ18 to escape the DarTG2 system resulted in mutations in the phage DNA polymerase, indicating that the evolved SECϕ18 phages overcome DarT2 not by preventing toxin activation, but by adapting to replicate ADP-ribosylated DNA.

Beyond *E. coli*, there are several examples of TA systems providing phage defense (62, 95). In *Lactococcus lactis*, a two gene locus (AbiEi and AbiEii) on the plasmid pNP40 was found to provide defense against the phages ϕc2 and ϕ712 (28). However, it was not recognized until later that this locus represents a type IV TA system. A recent study demonstrated that for the *L. lactis* AbiE system, and a homologous system in *Streptococcus agalactiae,* the toxin, AbiEii, is a GTP-binding nucleotidyltransferase, and the antitoxin, AbiEi, somehow offsets the activity of AbiEii, but without binding it directly (23). The AbiEii toxin interferes with the packaging – but not replication – of phage DNA, although the precise target is unknown (28). AbiEii is toxic when overexpressed in *E. coli* in the absence of phage infection, indicating that it targets host factors as well in these conditions.

Another TA system implicated in phage defense was identified in a study of temperate phage in mycobacteria (19). Ten lysogens generated from ten unique temperate phage were tested for their ability to withstand infection by a collection of 80 phage. This study revealed extensive prophage-mediated defense, with subsequent investigation revealing the prophage genes responsible in a handful of cases. For the lysogen containing the prophage Phrann, which protects against infection by the phages Tweety and Gaia, the genes providing defense were identified as gp29 and gp30. The sequence of gp29 revealed similarity to (p)ppGpp synthases and it could not be expressed in *M. smegmatis* unless co-expressed with gp30, indicating that gp29/30 likely constitute a TA system. A subsequent study identified this family as a new class of TA systems featuring toxins with similarity to

(p)ppGpp synthases (48). These systems are broadly distributed, including outside the mycobacteria, with some capable of synthesizing (p)ppGpp, (p)ppApp, or a combination of both. Some of the antitoxins directly bind and neutralize their cognate toxin (as in type II systems), but others act indirectly (as in type IV systems), likely as hydrolases that degrade the pyrophosphorylated nucleotides synthesized by their cognate toxins. However, the enzymatic activity of Phrann gp29, the only predicted (p)ppGpp-related toxin shown thus far to function in phage defense, has not been studied, either *in vitro* or in the context of phage infection. Whether producing (p)ppGpp can interfere with phage development is not clear. The nucleotide (p)ppApp could, in principle, function as an Abi system as a dedicated (p)ppApp synthase was recently found to be a potent type VI secretion system effector in *Pseudomonas aeruginosa*, capable of rapidly killing target cells (2). Even more recently, a (p)ppGpp-like synthase was shown to pyrophosphorylate the 3' adenosine of tRNAs, which could also potentially disrupt some phage infections (55). Whatever the case, the discovery of phage defensive TA systems with similarity to (p)ppGpp synthases underscores the remarkable diversity of toxin activities and the clever mechanisms that bacteria have evolved to thwart their viral predators.

Phage counter-defense systems and their coevolution with TA systems

Not to be outdone by bacteria, phage have evolved sophisticated mechanisms to counteract TA systems (Fig. 6). In fact, the identification of anti-TA system mechanisms in phage represents some of the strongest evidence that phage defense is a *bona fide*, and possibly primary, function of TA systems. TA-mediated defense can be overcome through three mechanisms: 1) preventing activation of the system, 2) modifying the toxin or its target, and 3) acquiring antitoxins or antitoxin mimics (Fig. 6). The latter category is possibly the simplest route to overcoming TA-mediated defense. As already noted, the protein Dmd in T4 normally inhibits the toxin RnlA or its homolog LsoA (82). How Dmd arose is not clear. In one sense it mimics the antitoxin RnlB and directly binds RnlA to neutralize its activity. However, Dmd shares no similarity to RnlB and structural studies indicate that it binds a different, but partially overlapping site on RnlA (110). As also discussed above, *P. atrosepticum* strains harboring *toxIN* normally protect against phage ϕTE, but escape phage can emerge by amplifying a locus harboring repeats that resemble *toxI* (8). These *pseudo-toxI* repeats were likely acquired originally to counteract a different but related *toxIN* system in another host of phage ϕTE and could then be effectively repurposed through amplification to counteract the *toxIN* of *P. atrosepticum*.

Some of the T-even phages, including T4, T6, and RB69 (but not T2), also encode a putative anti-DarT1 factor at gene *61.2* (60). The *61.2* gene is encoded near *dmd*, the gene encoding the pseudo-antitoxin that inhibits RnlA activity, suggesting that this may be an anti-TA defense region of T-even phage genomes. The function of *61.2* was revealed when RB69, which is susceptible to DarTG1-mediated defense, was evolved to overcome its defense and all resistant clones isolated had a single point mutation in the *61.2* gene. This mutation resulted in a

substitution of arginine with a histidine at position 164 in gp61.2, which rendered the phage entirely resistant to DarTG1. The gp61.2 homologs in T4 and T6 (phage which are unaffected by DarTG) both naturally contain histidine at this position, strongly suggesting that this residue plays a critical role in resistance of these phages to DarTG1.

More generally, the presence of antitoxins and antitoxin mimics in phage genomes probably reflects an ongoing arms race between, and consequent coevolution of, phage and bacteria. Defensive TA systems constitute a strong selective pressure for susceptible phage to acquire or express an antitoxin to neutralize the toxin. In cases where a related, but non-cognate antitoxin has been acquired, it may need to be amplified or modified, as seen with the *pseudo-toxI* amplifications in ϕTE and for the gp61.2 substitution in RB69, to boost efficacy or specificity against the toxin (7, 60). The rise of resistant escape phage would then drive selection for mutations in the host that circumvent phage inhibition, such as mutations in the toxin that prevent interaction with the phage antitoxin mimic. However, any mutations in the toxin must retain an interaction with the cognate, host-encoded antitoxin or be accompanied by changes in the antitoxin that will tolerate the variant toxin. In this way, the phage and its host may coevolve, with each driving changes in the other.

In addition to directly binding and inhibiting toxins, phage may have evolved other means of indirectly blocking their activation following phage infection. The *sanaTA* system was suggested to only protect against T7 lacking the gene gp4.5, which was proposed to inhibit the protease Lon (95). The model posited that the protein gp4.5 allows T7 to block Lon from degrading antitoxins, including *sanaA*, thereby preventing liberation of *sanaT*. However, beyond a possible interaction between gp4.5 and Lon in uninfected cells, this speculative model remains untested. Nevertheless, blocking the proteases important for type II antitoxin degradation is a plausible mechanism for disrupting toxin-mediated phage defense. A Lon inhibitor, PinA, encoded by T4 was discovered twenty years ago (44) and it could, in principle, help T4 block TA defense systems that require Lon, but an *in vivo* function for *pinA* has not been reported. There are also hints of other possible mechanisms by which phage antagonize TA systems. For instance, the ADP-ribosyltransferase Alt from T4, which modifies host RNA polymerase to help promote viral transcription, was suggested to also modify the toxin MazF (3).

In sum, there are at least a few examples of phage proteins that interfere with TA systems. There are also now many well-documented cases of phage proteins that antagonize CRISPR and RM proteins (35, 54, 61, 93). Thus, if TA systems play a broader, but currently underappreciated, role in phage defense, more examples are likely to emerge. Notably, many anti-CRISPR genes cluster in phage genomes and this property has been leveraged to find more. If anti-TA genes also cluster, as seems to be the case in T-even phage, either with each other or with other anti-defense genes, a similar approach could help in finding more examples. Many phage genomes encode dozens of genes of unknown function, and many are not required for the production of mature virions. Instead, they may equip phage with critical anti-TA, or other anti-defense, capabilities.

Do most TA systems function in phage defense?

Although some TA systems can and do protect against phage predation, it not yet clear whether most TA systems play such a role. Consider, for example, *E. coli* K12, which encodes at least 40 TA systems of different types (Fig. 3); only RnlAB has a confirmed role in phage defense. As noted, RnlAB was only found to provide protection against T4 phage lacking *dmd*, so it may well be that other coliphage counterdefense systems overcome the existing TA systems in *E. coli* K12. Alternatively, or in addition, TA systems may provide protection against only a very narrow spectrum of phage, and the phage that some individual TA systems in *E. coli* K12 defend against may not have been identified or tested yet given the tremendous diversity of phage that exist (20). Moreover, phage are often isolated by selecting for those that infect *E. coli* K12, which will, by definition, exclude those that TA systems can defend against. In short, issues of sampling and selection bias may have obscured a role for many TA systems in phage defense.

TA systems involved in phage defense may rapidly become obsolete and non-functional, either because phage have evolved potent counter-defense mechanisms or because a host has not encountered the relevant phage over long periods of time. Consistent with such a possibility, the toxins of some annotated TA systems have lost their toxicity, and TA systems are notorious for their poor conservation, implying relatively high rates of gene loss (69, 88). The notion that TA systems may generally function in phage defense would help explain both their widespread distribution and the poor conservation of individual systems.

Notably, many TA systems, including the gp29/30 system in mycophages and the DarTG systems discussed above, are found on prophages or in genomic regions featuring phage-related genes. These TA systems may be used by prophages to defend their host against predation by other phages. Some TA systems are found on plasmids and other mobile genetic elements. Although the TA systems on plasmids are often assumed to promote plasmid maintenance through a post-segregational killing mechanism, many could function instead, or in addition, in phage defense.

The notion that TA systems, and other phage defense factors, allow prophage to protect themselves and their host cell could underlie the clustering of phage defense systems into islands. Because phage genomes are typically highly organized, with genes related to a given function – like replication or capsid formation – clustered and co-expressed, accessory genes like defense systems can only be carried in a limited number of genomic locations. This may lead to a natural clustering of defense systems within prophages. If those prophages lose the ability the excise and form a functional phage particle, the core phage genes may be lost from the genome, but the clustered defense systems, which would provide a benefit to the host, could be retained.

Outlook

Future studies are needed to further explore and characterize the role of TA systems in phage defense. Certainly not all TA systems will function in phage defense, but there is growing evidence that many do provide potent phage defense. However, there is much we do not yet understand about them as defense systems. In particular, there are likely to be diverse mechanisms that drive the liberation of toxins from their cognate antitoxins specifically upon phage infection. Phage-induced shutdown of host transcription is one mechanism, but there are likely other ways in which TA systems recognize specific components of infecting phage or other phage-triggered cellular events. How TA systems thwart phage replication and development must also be further examined. As noted earlier, the toxins of most TA systems have been characterized by overexpressing them in uninfected hosts, which will miss any critical phage targets. Future studies to elucidate the mechanisms used by phage to overcome TA systems may be critical in revealing the role of many TA systems in phage defense, and also promises to reveal the sophisticated molecular innovations that inevitably arise in the context of a host-pathogen interface.

Toxin-antitoxin systems have long fascinated, but also stymied and confused, microbiologists. We anticipate that further investigating their role in phage defense will bring new clarity and insight into these ubiquitous genetic elements in bacteria. We anticipate that TA systems will emerge as a major category of phage defense elements alongside RM and CRISPR systems. Like those systems, the study of TA systems may unveil or inspire precision molecular tools for modifying cells and genomes. In addition to exploring this rich area of biology, understanding the myriad ways in which bacteria defend themselves against phage, and the ways in which phage fight back, promises to inform future efforts to deploy phage as therapeutic agents. With the alarming rise of bacterial resistance to conventional small molecule antibiotics, there is an urgent need for alternative approaches and phage therapy has emerged as one such alternative. The prospect of using phages to manipulate microbiomes and thereby impact health and disease has also risen to prominence in recent years. The long-term efficacy and success of these phage-based clinical approaches will demand a fuller understanding of the many mechanisms and systems used by bacteria to defend themselves, including TA systems.

Figure Legends

Figure 1 - The major types of TA systems.

The schematics show the four major types of TA systems, which are classified based on how an antitoxin (blue) neutralizes its cognate toxin (red).

Figure 2 - The diversity of toxin targets.

The toxins of TA systems target a diverse set of cellular proteins and processes, including DNA replication, topoisomerases, RNA, translation, the cell wall and envelope, and growth. In each category, specific toxin targets are shown with examples of toxins in each case listed in parentheses.

Figure 3 - Summary of TA systems in *E. coli* strains MG1655 (a K12 derivative) and O157:H7.

The approximate genomic location of known or predicted TA systems of any type are shown outside the circle representing the genome with the location of prophage (orange), including cryptic prophage, shown inside. *E. coli* O157:H7 also harbors two plasmids. Orthologous TA systems shared between the two strains are colored green and those unique in this pairwise comparison are in dark blue.

Figure 4 - Possible functions for TA systems.

Three major categories of proposed functions for TA systems: plasmid maintenance, stress response, and phage defense. For plasmid maintenance (left panel), loss of a TA-encoding plasmid can lead to degradation of the antitoxin, thereby liberating the toxin, which then prevents growth of a cell lacking the plasmid. In response to abiotic stress (middle panel), antitoxins can be degraded, leading to transcriptional induction; however, antitoxin can be replenished and free toxin is not liberated. For some TA systems, phage infection (right panel) can lead to release of active toxin, which can inhibit phage replication.

Figure 5 - Model of ToxIN-mediated phage defense.

For *E. coli* cells lacking *toxIN* (left) infected with T4 phage, host transcription is inhibited as the phage commandeers host resources to produce new phage particles, before lysing the cell. For cells harboring *toxIN*, the antitoxin *toxI* is normally produced and degraded at relatively high rates. Following their infection by T4, the shutoff of host transcription leads to the loss of *toxI* and consequent liberation of ToxN, an endoribonuclease. Active ToxN directly cleaves most phage transcripts, preventing the production of mature virions. The infected cell still dies as T4 triggers host chromosome degradation, but it does not spread the infection to neighbor cells.

Figure 6 - Phage mechanisms for counteracting TA systems.

Three major categories of phage-encoded mechanisms for counteracting TA systems are shown, with specific examples of each indicated. Phage can produce antitoxins or antitoxin mimics, express factors that directly overcome/bypass the effects of a toxin, or block toxin activation, *e.g.* by inhibiting antitoxin degradation.

Table 1. TA systems involved in phage defense

System	Experimentally characterized systems		Toxin activity/mechanism of defense	Mechanism of activation	References
	Host	Phage			
Hok/sok	R1 plasmid, *E. coli*	T4	pore forming	unknown; hypothesized transcriptional shutoff	86
RnlAB	*E. coli* MG1655	T4 *dmd* mutant	endonuclease cleavage of mRNA; dependent on host RNase H	unknown; hypothesized transcriptional shutoff	51, 77, 80, 81, 82
DarTG	from environmental *E. coli* isolates, characterized in *E. coli* MG1655	T5, SECϕ18, RB69	ADP-ribosylation of phage DNA inhibits DNA synthesis	unknown	60
PfiAT	*Pseudomonas aeruginosa*	Pf4	unknown	unknown	62
ToxIN	*Pectobacterium atrosepticum*; from environmental *E. coli* isolates characterized in *E. coli* MG1655	ϕA2 ϕM1 T4, T5	endonuclease cleavage of mRNA	phage-induced host transcriptional shutoff	9, 10, 25, 32, 34, 99 100,
TenpIN	*Photorhabdus luminescens*	ϕF6, ϕTB27, and ϕTB28	endonuclease cleavage of mRNA	not directly studied, but likely host transcriptional shutoff	7, 10
SanaTA	*Shewanella sp. ANA-3,* characterized in *E. coli*	T7 Δ*gp4.5*	predicted nucleotidyltransferase	unknown	95
AbiEi AbiEii	*Lactococcus lactis*	ϕc2 and ϕ712	nucleotidyltransferase prevents phage packaging, target unknown	unknown	23, 28
Gp29/30	*Mycobacterium smegmatis*	Tweety, Gaia	Precise mechanism unknown, related proteins are (p)ppGpp/ppApp synthases or tRNA pyrophosphorylases	unknown	19, 48
Retron systems	many, including EC48 from environmental *E. coli* characterized in *E. coli* MG1655	T7, T4, T6, SECϕ4, SECϕ6, and SECϕ18	toxin activity varies (*e.g.* endonucleases, ribosyltransferases, transmembrane proteins); Ec48 effector targets membrane	RecB inhibition by phage proteins activates Ec48	11, 12, 27, 72

Acknowledgements

We thank Thúy-Lan Lite for the design of Figure 1; and L. van Melderen and N. Fraikin for input on Figure 2 and the text. We thank C. Guegler, C. Vassallo, and T. Zhang for comments on the manuscript and all members of the Laub lab for helpful discussions. This work was supported by a Charles A. King Trust fellowship to M.L. and the Howard Hughes Medical Institute (M.T.L.).

References

1. Aakre CD, Phung TN, Huang D, Laub MT. 2013. A bacterial toxin inhibits DNA replication elongation through a direct interaction with the β sliding clamp. *Molecular cell*. 52(5):617–28
2. Ahmad S, Wang B, Walker MD, Tran H-KR, Stogios PJ, et al. 2019. An interbacterial toxin inhibits target cell growth by synthesizing (p)ppApp. *Nature*. 575(7784):674–78
3. Alawneh AM, Qi D, Yonesaki T, Otsuka Y. 2015. An ADP-ribosyltransferase Alt of bacteriophage T4 negatively regulates the E scherichia coli MazF toxin of a toxin-antitoxin module. *Molecular Microbiology*. 99(1):188–98
4. Behme MT, Lilley GD, Ebisuzaki K. 1976. Postinfection control by bacteriophage T4 of Escherichia coli recBC nuclease activity. *J Virol*. 18(1):20–25
5. Bernard P, Couturier M. 1992. Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. *J Mol Biol*. 226(3):735–45
6. Black DS, Kelly AJ, Mardis MJ, Moyed HS. 1991. Structure and organization of hip, an operon that affects lethality due to inhibition of peptidoglycan or DNA synthesis. *J Bacteriol*. 173(18):5732–39
7. Blower TR, Chai R, Przybilski R, Chindhy S, Fang X, et al. 2017. Evolution of Pectobacterium Bacteriophage ΦM1 To Escape Two Bifunctional Type III Toxin-Antitoxin and Abortive Infection Systems through Mutations in a Single Viral Gene. *Appl Environ Microbiol*. 83(8):
8. Blower TR, Evans TJ, Przybilski R, Fineran PC, Salmond GPC. 2012. Viral evasion of a bacterial suicide system by RNA-based molecular mimicry enables infectious altruism. *PLoS Genet*. 8(10):e1003023
9. Blower TR, Pei XY, Short FL, Fineran PC, Humphreys DP, et al. 2011. A processed noncoding RNA regulates an altruistic bacterial antiviral system. *Nat Struct Mol Biol*. 18(2):185–90
10. Blower TR, Short FL, Rao F, Mizuguchi K, Pei XY, et al. 2012. Identification and classification of bacterial Type III toxin-antitoxin systems encoded in chromosomal and plasmid genomes. *Nucleic Acids Res*. 40(13):6158–73
11. Bobonis J, Mateus A, Pfalz B, Garcia-Santamarina S, Galardini M, et al. 2020. Bacterial retrons encode tripartite toxin/antitoxin systems. Microbiology
12. Bobonis J, Mitosch K, Mateus A, Kritikos G, Elfenbein JR, et al. 2020. Phage proteins block and trigger retron toxin/antitoxin systems. *bioRxiv*. 2020.06.22.160242

13. Brunovskis I, Summers WC. 1971. The process of infection with coliphage T7: V. Shutoff of host RNA synthesis by an early phage function. *Virology*. 45(1):224–31
14. Chen B, Akusobi C, Fang X, Salmond GPC. 2017. Environmental T4-Family Bacteriophages Evolve to Escape Abortive Infection via Multiple Routes in a Bacterial Host Employing "Altruistic Suicide" through Type III Toxin-Antitoxin Systems. *Front Microbiol*. 8:1006
15. Cheverton AM, Gollan B, Przydacz M, Wong CT, Mylona A, et al. 2016. A Salmonella Toxin Promotes Persister Formation through Acetylation of tRNA. *Molecular cell*. 63(1):86–96
16. Christensen SK, Pedersen K, Hansen FG, Gerdes K. 2003. Toxin–antitoxin Loci as Stress-response-elements: ChpAK/MazF and ChpBK Cleave Translated RNAs and are Counteracted by tmRNA. *Journal of molecular biology*. 332(4):809–19
17. Christensen-Dalsgaard M, Jørgensen MG, Gerdes K. 2010. Three new RelE-homologous mRNA interferases of Escherichia coli differentially induced by environmental stresses. *Molecular Microbiology*. 75(2):333–48
18. Culviner PH, Laub MT. 2018. Global Analysis of the E. coli Toxin MazF Reveals Widespread Cleavage of mRNA and the Inhibition of rRNA Maturation and Ribosome Biogenesis. *Molecular cell*. 70(5):868-880.e10
19. Dedrick RM, Jacobs-Sera D, Bustamante CAG, Garlena RA, Mavrich TN, et al. 2017. Prophage-mediated defence against viral attack and viral counter-defence. *Nature Microbiology*. 2(3):1–13
20. Dion MB, Oechslin F, Moineau S. 2020. Phage diversity, genomics and phylogeny. *Nature Reviews Microbiology*. 18(3):125–38
21. Dörr T, Vulić M, Lewis K. 2010. Ciprofloxacin Causes Persister Formation by Inducing the TisB toxin in Escherichia coli. *PLoS Biol*. 8(2):e1000317
22. Dy RL, Przybilski R, Semeijn K, Salmond GPC, Fineran PC. 2014. A widespread bacteriophage abortive infection system functions through a Type IV toxin–antitoxin mechanism. *Nucleic Acids Research*. 42(7):4590–4605
23. Dy RL, Przybilski R, Semeijn K, Salmond GPC, Fineran PC. 2014. A widespread bacteriophage abortive infection system functions through a Type IV toxin–antitoxin mechanism. *Nucleic Acids Research*. 42(7):4590–4605
24. Emond E, Dion E, Walker SA, Vedamuthu ER, Kondo JK, Moineau S. 1998. AbiQ, an Abortive Infection Mechanism fromLactococcus lactis. *Appl. Environ. Microbiol.* 64(12):4748–56

25. Fineran PC, Blower TR, Foulds IJ, Humphreys DP, Lilley KS, Salmond GPC. 2009. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. *Proc Natl Acad Sci U S A*. 106(3):894–99
26. Fraikin N, Goormaghtigh F, Van Melderen L. 2020. Type II Toxin-Antitoxin Systems: Evolution and Revolutions. *J Bacteriol*. 202(7):e00763-19
27. Gao L, Altae-Tran H, Böhning F, Makarova KS, Segel M, et al. 2020. Diverse enzymatic activities mediate antiviral immunity in prokaryotes. *Science (New York, N.Y.)*. 369(6507):1077–84
28. Garvey P, Fitzgerald GF, Hill C. 1995. Cloning and DNA sequence analysis of two abortive infection phage resistance determinants from the lactococcal plasmid pNP40. *Applied and environmental microbiology*. 61(12):4321–28
29. Gerdes K, Bech FW, Jørgensen ST, Løbner-Olesen A, Rasmussen PB, et al. 1986. Mechanism of postsegregational killing by the hok gene product of the parB system of plasmid R1 and its homology with the relF gene product of the E. coli relB operon. *EMBO J*. 5(8):2023–29
30. Gerdes K, Maisonneuve E. 2012. Bacterial persistence and toxin-antitoxin loci. *Annual review of microbiology*. 66(1):103–23
31. Gerdes K, Thisted T, Martinussen J. 1990. Mechanism of post-segregational killing by the hoklsok system of plasmid R1: sok antisense RNA regulates formation of a hok mRNA species correlated with killing of plasmid-free cells. *Molecular Microbiology*. 4(11):1807–18
32. Goeders N, Chai R, Chen B, Day A, Salmond GPC. 2016. Structure, Evolution, and Functions of Bacterial Type III Toxin-Antitoxin Systems. *Toxins (Basel)*. 8(10):282
33. Goormaghtigh F, Fraikin N, Putrinš M, Hallaert T, Hauryliuk V, et al. 2018. Reassessing the Role of Type II Toxin-Antitoxin Systems in Formation of Escherichia coli Type II Persister Cells. *mBio*. 9(3):1479
34. Guegler CK, Laub MT. 2021. Shutoff of host transcription triggers a toxin-antitoxin system to cleave phage RNA and abort infection. *Mol Cell*. 81(11):2361-2373.e9
35. Hampton HG, Watson BNJ, Fineran PC. 2020. The arms race between bacteria and their phage foes. *Nature*. 577(7790):327-336.
36. Hansen S, Lewis K, Vulic M. 2008. Role of Global Regulators and Nucleotide Metabolism in Antibiotic Tolerance in Escherichia coli□. *Antimicrob Agents Chemother*. 52(8):2718–26

37. Harms A, Brodersen DE, Mitarai N, Gerdes K. 2018. Toxins, Targets, and Triggers: An Overview of Toxin-Antitoxin Biology. *Molecular cell*. 70(5):768–84
38. Harms A, Fino C, Sørensen MA, Semsey S, Gerdes K. 2017. Prophages and Growth Dynamics Confound Experimental Results with Antibiotic-Tolerant Persister Cells. *mBio*. 8(6):e01964-17–18
39. Harms A, Stanger FV, Scheu PD, de Jong IG, Goepfert A, et al. 2015. Adenylylation of Gyrase and Topo IV by FicT Toxins Disrupts Bacterial DNA Topology. *Cell Rep*. 12(9):1497–1507
40. Hazan R, Engelberg-Kulka H. 2004. Escherichia coli mazEF-mediated cell death as a defense mechanism that inhibits the spread of phage P1. *Molecular Genetics and Genomics*. 272(2):227–34
41. Hazan R, Sat B, Engelberg-Kulka H. 2004. Escherichia coli mazEF-Mediated Cell Death Is Triggered by Various Stressful Conditions. *Journal of Bacteriology*. 186(11):3663–69
42. Helaine S, Cheverton AM, Watson KG, Faure LM, Matthews SA, Holden DW. 2014. Internalization of Salmonella by macrophages induces formation of nonreplicating persisters. *Science*. 343(6167):204–8
43. Heller DM, Tavag M, Hochschild A. 2017. CbtA toxin of Escherichia coli inhibits cell division and cell elongation via direct and independent interactions with FtsZ and MreB. *PLoS Genet*. 13(9):e1007007
44. Hilliard JJ, Maurizi MR, Simon LD. 1998. Isolation and characterization of the phage T4 PinA protein, an inhibitor of the ATP-dependent lon protease of Escherichia coli. *Journal of Biological Chemistry*. 273(1):518–23
45. Hinton DM. 2010. Transcriptional control in the prereplicative phase of T4 development. *Virol J*. 7:289
46. Jankevicius G, Ariza A, Ahel M, Ahel I. 2016. The Toxin-Antitoxin System DarTG Catalyzes Reversible ADP-Ribosylation of DNA. *Molecular cell*. 64(6):1109–16
47. Jiang Y, Pogliano J, Helinski DR, Konieczny I. 2002. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of Escherichia coli gyrase. *Mol Microbiol*. 44(4):971–79
48. Jimmy S, Saha CK, Kurata T, Stavropoulos C, Oliveira SRA, et al. 2020. A widespread toxin-antitoxin system exploiting growth control via alarmone signaling. *PNAS*. 117(19):10500–510
49. Kai T, Selick HE, Yonesaki T. 1996. Destabilization of bacteriophage T4 mRNAs by a mutation of gene 61.5. *Genetics*. 144(1):7–14

50. Kaspy I, Rotem E, Weiss N, Ronin I, Balaban NQ, Glaser G. 2013. HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA-synthetase. *Nature communications*. 4:3001
51. Koga M, Otsuka Y, Lemire S, Yonesaki T. 2011. Escherichia coli rnlA and rnlB Compose a Novel Toxin-Antitoxin System. *Genetics*. 187(1):123–30
52. Kolodkin-Gal I, Engelberg-Kulka H. 2006. Induction of Escherichia coli Chromosomal mazEF by Stressful Conditions Causes an Irreversible Loss of Viability. *Journal of Bacteriology*. 188(9):3420–23
53. Korch SB, Henderson TA, Hill TM. 2003. Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis. *Molecular Microbiology*. 50(4):1199–1213
54. Krüger DH, Bickle TA. 1983. Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of their hosts. *Microbiol Rev*. 47(3):345–60
55. Kurata T, Brodiazhenko T, Alves Oliveira SR, Roghanian M, Sakaguchi Y, et al. 2021. RelA-SpoT Homolog toxins pyrophosphorylate the CCA end of tRNA to inhibit protein synthesis. *Mol Cell*. 81(15):3160-3170.e9
56. Lampson BC, Inouye M, Inouye S. 1989. Reverse transcriptase with concomitant ribonuclease H activity in the cell-free synthesis of branched RNA-linked msDNA of Myxococcus xanthus. *Cell*. 56(4):701–7
57. Lawarée E, Jankevicius G, Cooper C, Ahel I, Uphoff S, Tang CM. 2020. DNA ADP-Ribosylation Stalls Replication and Is Reversed by RecF-Mediated Homologous Recombination and Nucleotide Excision Repair. *Cell reports*. 30(5):1373-1384.e4
58. Leplae R, Geeraerts D, Hallez R, Guglielmini J, Drèze P, Van Melderen L. 2011. Diversity of bacterial type II toxin-antitoxin systems: a comprehensive search and functional analysis of novel families. *Nucleic Acids Res*. 39(13):5513–25
59. LeRoux M, Culviner PH, Liu YJ, Littlehale ML, Laub MT. 2020. Stress Can Induce Transcription of Toxin-Antitoxin Systems without Activating Toxin. *Molecular cell*. 79(2):280-292.e8
60. LeRoux M, Srikant S, Littlehale ML, Teodoro G, Doron S, et al. 2021. The DarTG toxin-antitoxin system provides phage defense by ADP-ribosylating viral DNA. *bioRxiv*

61. Li Y, Bondy-Denomy J. 2020. Anti-CRISPRs go viral: the infection biology of CRISPR-Cas inhibitors. *Cell Host Microbe*. 29(5):704–14
62. Li Y, Liu X, Tang K, Wang W, Guo Y, Wang X. 2020. Prophage encoding toxin/antitoxin system PfiT/PfiA inhibits Pf4 production in Pseudomonas aeruginosa. *Microbial biotechnology*. 41(11):73–13
63. Lin L. *Study of bacteriophage T7 gene 5.9 and gene 5.5*. Ph.D. thesis. State University of New York at Stony Brook. 172 pp.
64. Liu M, Zhang Y, Inouye M, Woychik NA. 2008. Bacterial addiction module toxin Doc inhibits translation elongation through its association with the 30S ribosomal subunit. *Proceedings of the National Academy of Sciences of the United States of America*. 105(15):5885–90
65. Magnuson RD. 2007. Hypothetical functions of toxin-antitoxin systems. *Journal of Bacteriology*. 189(17):6089–92
66. Maikova A, Peltier J, Boudry P, Hajnsdorf E, Kint N, et al. 2018. Discovery of new type I toxin-antitoxin systems adjacent to CRISPR arrays in Clostridium difficile. *Nucleic Acids Res*. 46(9):4733–51
67. Maisonneuve E, Gerdes K. 2014. Molecular mechanisms underlying bacterial persisters. *Cell*. 157(3):539–48
68. Maisonneuve E, Shakespeare LJ, Jørgensen MG, Gerdes K. 2011. Bacterial persistence by RNA endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*. 108(32):13206–11
69. Makarova KS, Wolf YI, Koonin EV. 2009. Comprehensive comparative-genomic analysis of Type 2 toxin-antitoxin systems and related mobile stress response systems in prokaryotes. *Biology Direct*. 4(1):19
70. Makarova KS, Wolf YI, Koonin EV. 2013. Comparative genomics of defense systems in archaea and bacteria. *Nucleic Acids Res*. 41(8):4360–77
71. Miki T, Park JA, Nagao K, Murayama N, Horiuchi T. 1992. Control of segregation of chromosomal DNA by sex factor F in Escherichia coli. Mutants of DNA gyrase subunit A suppress letD (ccdB) product growth inhibition. *J Mol Biol*. 225(1):39–52
72. Millman A, Bernheim A, Stokar-Avihail A, Fedorenko T, Voichek M, et al. 2020. Bacterial Retrons Function In Anti-Phage Defense. *Cell*. 183(6):1551-1561.e12

73. Millman A, Melamed S, Amitai G, Sorek R. 2020. Diversity and classification of cyclic-oligonucleotide-based anti-phage signalling systems. *Nature Microbiology*. 5(12):1608–15
74. Moyed HS, Bertrand KP. 1983. hipA, a newly recognized gene of Escherichia coli K-12 that affects frequency of persistence after inhibition of murein synthesis. *Journal of Bacteriology*. 155(2):768–75
75. Muthuramalingam M, White J, Bourne C. 2016. Toxin-Antitoxin Modules Are Pliable Switches Activated by Multiple Protease Pathways. *Toxins*. 8(7):214–16
76. Mutschler H, Gebhardt M, Shoeman RL, Meinhart A. 2011. A novel mechanism of programmed cell death in bacteria by toxin-antitoxin systems corrupts peptidoglycan synthesis. *PLoS Biol*. 9(3):e1001033
77. Naka K, Koga M, Yonesaki T, Otsuka Y. 2014. RNase HI stimulates the activity of RnlA toxin in Escherichia coli. *Molecular Microbiology*. 91(3):596–605
78. Nigam A, Ziv T, Oron-Gottesman A, Engelberg-Kulka H. 2019. Stress-Induced MazF-Mediated Proteins in Escherichia coli. *mBio*. 10(2):6059–9
79. Ogura T, Hiraga S. 1983. Mini-F plasmid genes that couple host cell division to plasmid proliferation. *PNAS*. 80(15):4784–88
80. Otsuka Y, Ueno H, Yonesaki T. 2003. Escherichia coli Endoribonucleases Involved in Cleavage of Bacteriophage T4 mRNAs. *Journal of Bacteriology*. 185(3):983–90
81. Otsuka Y, Yonesaki T. 2005. A novel endoribonuclease, RNase LS, in Escherichia coli. *Genetics*. 169(1):13–20
82. Otsuka Y, Yonesaki T. 2012. Dmd of bacteriophage T4 functions as an antitoxin against Escherichia coli LsoA and RnlA toxins. *Molecular Microbiology*. 83(4):669–81
83. Pacumbaba R, Center MS. 1975. Partial purification and properties of a bacteriophage T7 inhibitor of the host exonuclease V activity. *J Virol*. 16(5):1200–1207
84. Page R, Peti W. 2016. Toxin-antitoxin systems in bacterial growth arrest and persistence. *Nat Chem Biol*. 12(4):208–14
85. Pandey DP, Gerdes K. 2005. Toxin–antitoxin loci are highly abundant in free-living but lost from host-associated prokaryotes. *Nucl. Acids Res.* 33(3):966–76
86. Pecota DC, Wood TK. 1996. Exclusion of T4 phage by the hok/sok killer locus from plasmid R1. *J Bacteriol*. 178(7):2044–50

87. Piscotta FJ, Jeffrey PD, Link AJ. 2019. ParST is a widespread toxin-antitoxin module that targets nucleotide metabolism. *Proceedings of the National Academy of Sciences of the United States of America*. 116(3):826–34
88. Ramage HR, Connolly LE, Cox JS. 2009. Comprehensive Functional Analysis of Mycobacterium tuberculosis Toxin-Antitoxin Systems: Implications for Pathogenesis, Stress Responses, and Evolution. *PLoS Genet*. 5(12):e1000767
89. Ronneau S, Helaine S. 2019. Clarifying the Link between Toxin-Antitoxin Modules and Bacterial Persistence. *J Mol Biol*. 431(18):3462–71
90. Sakaki Y, Karu AE, Linn S, Echols H. 1973. Purification and properties of the gamma-protein specified by bacteriophage lambda: an inhibitor of the host RecBC recombination enzyme. *Proc Natl Acad Sci U S A*. 70(8):2215–19
91. Sala A, Bordes P, Genevaux P. 2014. Multiple Toxin-Antitoxin Systems in Mycobacterium tuberculosis. *Toxins*. 6(3):1002–20
92. Samson JE, Bélanger M, Moineau S. 2013. Effect of the abortive infection mechanism and type III toxin/antitoxin system AbiQ on the lytic cycle of Lactococcus lactis phages. *J Bacteriol*. 195(17):3947–56
93. Samson JE, Magadán AH, Sabri M, Moineau S. 2013. Revenge of the phages: defeating bacterial defences. *Nature Reviews Microbiology*. 11(10):1–13
94. Samson JE, Spinelli S, Cambillau C, Moineau S. 2013. Structure and activity of AbiQ, a lactococcal endoribonuclease belonging to the type III toxin-antitoxin system. *Mol Microbiol*. 87(4):756–68
95. Sberro H, Leavitt A, Kiro R, Koh E, Peleg Y, et al. 2013. Discovery of Functional Toxin/Antitoxin Systems in Bacteria by Shotgun Cloning. *Molecular cell*. 50(1):136–48
96. Schifano JM, Cruz JW, Vvedenskaya IO, Edifor R, Ouyang M, et al. 2016. tRNA is a new target for cleavage by a MazF toxin. *Nucleic Acids Res*. 44(3):1256–70
97. Schuller M, Butler RE, Ariza A, Tromans-Coia C, Jankevicius G, et al. 2021. Molecular basis for DarT ADP-ribosylation of a DNA base. *Nature*. 596(7873):597–602
98. Shao Y, Harrison EM, Bi D, Tai C, He X, et al. 2011. TADB: a web-based resource for Type 2 toxin-antitoxin loci in bacteria and archaea. *Nucleic Acids Res*. 39(Database issue):D606-611

99. Short FL, Akusobi C, Broadhurst WR, Salmond GPC. 2018. The bacterial Type III toxin-antitoxin system, ToxIN, is a dynamic protein-RNA complex with stability-dependent antiviral abortive infection activity. *Sci Rep*. 8(1):1013
100. Short FL, Pei XY, Blower TR, Ong S-L, Fineran PC, et al. 2013. Selectivity and self-assembly in the control of a bacterial toxin by an antitoxic noncoding RNA pseudoknot. *Proc Natl Acad Sci U S A*. 110(3):E241-249
101. Simon AJ, Ellington AD, Finkelstein IJ. 2019. Retrons and their applications in genome engineering. *Nucleic Acids Research*. 47(21):11007–19
102. Snyder L, Gold L, Kutter E. 1976. A gene of bacteriophage T4 whose product prevents true late transcription on cytosine-containing T4 DNA. *Proceedings of the National Academy of Sciences*. 73(9):3098–3102
103. Song S, Wood TK. 2020. A Primary Physiological Role of Toxin/Antitoxin Systems Is Phage Inhibition. *Front. Microbiol.* 11:
104. Tam JE, Kline BC. 1989. Control of the ccd operon in plasmid F. *J Bacteriol*. 171(5):2353–60
105. Tan Q, Awano N, Inouye M. 2011. YeeV is an Escherichia coli toxin that inhibits cell division by targeting the cytoskeleton proteins, FtsZ and MreB. *Mol Microbiol*. 79(1):109–18
106. Tsilibaris V, Maenhaut-Michel G, Mine N, Van Melderen L. 2007. What is the benefit to Escherichia coli of having multiple toxin-antitoxin systems in its genome? *Journal of Bacteriology*. 189(17):6101–8
107. Van Melderen L, Bernard P, Couturier M. 1994. Lon-dependent proteolysis of CcdA is the key control for activation of CcdB in plasmid-free segregant bacteria. *Molecular Microbiology*. 11(6):1151–57
108. Van Melderen L, Saavedra De Bast M. 2009. Bacterial Toxin–Antitoxin Systems: More Than Selfish Entities? *PLoS Genet*. 5(3):e1000437
109. Van Melderen L, Wood TK. 2017. Commentary: What Is the Link between Stringent Response, Endoribonuclease Encoding Type II Toxin-Antitoxin Systems and Persistence? *Frontiers in Microbiology*. 8:R81-3
110. Wan H, Otsuka Y, Gao Z-Q, Wei Y, Chen Z, et al. 2016. Structural insights into the inhibition mechanism of bacterial toxin LsoA by bacteriophage antitoxin Dmd. *Molecular Microbiology*. 101(5):757–69

111. Wang X, Lord DM, Cheng H-Y, Osbourne DO, Hong SH, et al. 2012. A new type V toxin-antitoxin system where mRNA for toxin GhoT is cleaved by antitoxin GhoS. *Nat Chem Biol*. 8(10):855–61
112. Wang X, Wood TK. 2011. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. *Applied and environmental microbiology*. 77(16):5577–83
113. Wei Y, Gao Z, Zhang H, Dong Y. 2016. Structural characterizations of phage antitoxin Dmd and its interactions with bacterial toxin RnlA. *Biochemical and Biophysical Research Communications*. 472(4):592–97
114. Winther KS, Gerdes K. 2011. Enteric virulence associated protein VapC inhibits translation by cleavage of initiator tRNA. *Proc Natl Acad Sci U S A*. 108(18):7403–7
115. Xie Y, Wei Y, Shen Y, Li X, Zhou H, et al. 2018. TADB 2.0: an updated database of bacterial type II toxin-antitoxin loci. *Nucleic Acids Res*. 46(D1):D749–53
116. Yamaguchi Y, Park J-H, Inouye M. 2011. Toxin-antitoxin systems in bacteria and archaea. *Annual review of genetics*. 45(1):61–79
117. Zaveri A, Wang R, Botella L, Sharma R, Zhu L, et al. 2020. Depletion of the DarG antitoxin in Mycobacterium tuberculosis triggers the DNA-damage response and leads to cell death. *Molecular Microbiology*. 114(4):641–52
118. Zhang Y, Zhang J, Hoeflich KP, Ikura M, Qing G, Inouye M. 2003. MazF Cleaves Cellular mRNAs Specifically at ACA to Block Protein Synthesis in Escherichia coli. *Molecular Cell*. 12(4):913–23

Figure 1

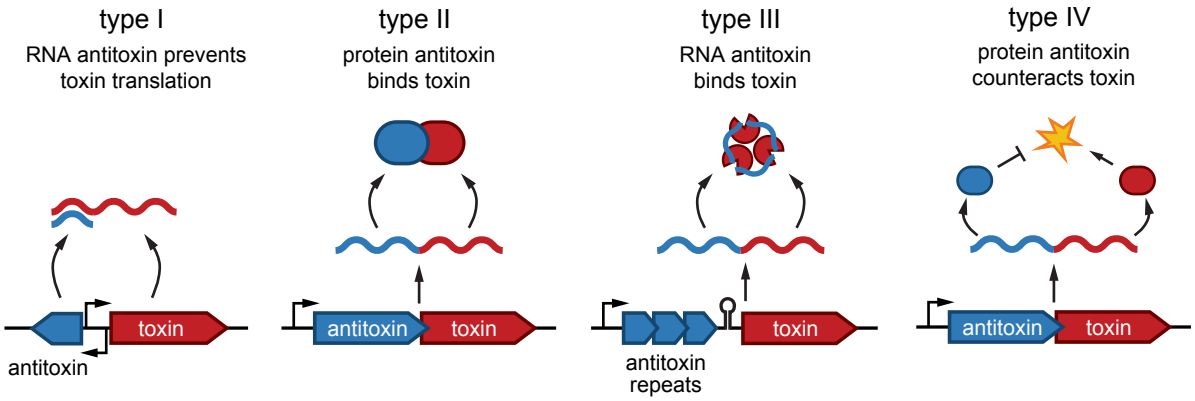

type I
RNA antitoxin prevents toxin translation
toxin
antitoxin
type II
protein antitoxin binds toxin
antitoxin
toxin
type III
RNA antitoxin binds toxin
toxin
antitoxin repeats
type IV
protein antitoxin counteracts toxin
antitoxin
toxin

Figure 2

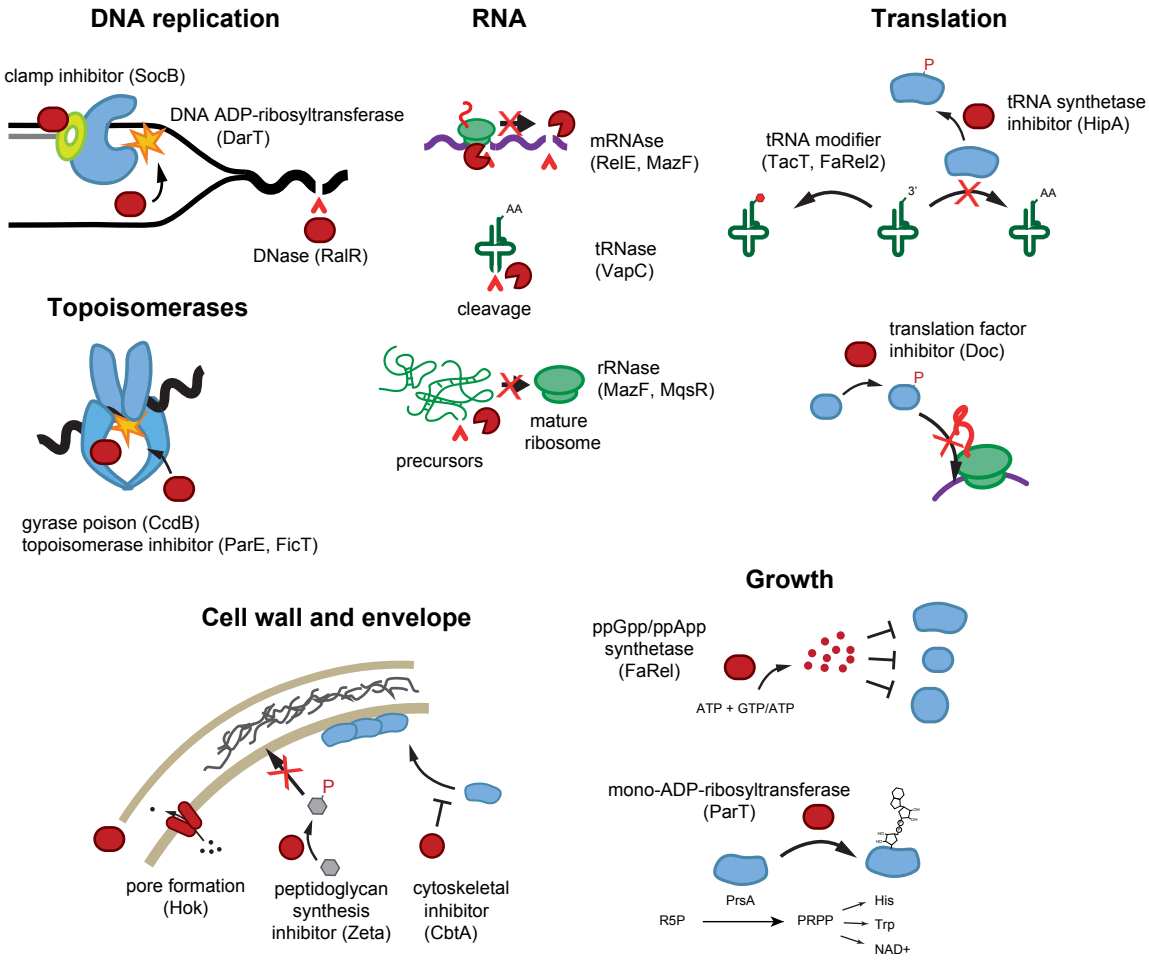

DNA replication
clamp inhibitor (SocB)
DNA ADP-ribosyltransferase (DarT)
DNase (RalR)
RNA
mRNAse (RelE, MazF)
AA
tRNase (VapC)
cleavage
Translation
P
tRNA synthetase inhibitor (HipA)
tRNA modifier (TacT, FaRel2)
3'
AA
Topoisomerases
gyrase poison (CcdB)
topoisomerase inhibitor (ParE, FicT)
rRNase (MazF, MqsR)
mature ribosome
precursors
translation factor inhibitor (Doc)
P
Cell wall and envelope
P
pore formation (Hok)
peptidoglycan synthesis inhibitor (Zeta)
cytoskeletal inhibitor (CbtA)
Growth
ppGpp/ppApp synthetase (FaRel)
ATP + GTP/ATP
mono-ADP-ribosyltransferase (ParT)
PrsA
R5P
PRPP
His
Trp
NAD+

Figure 3

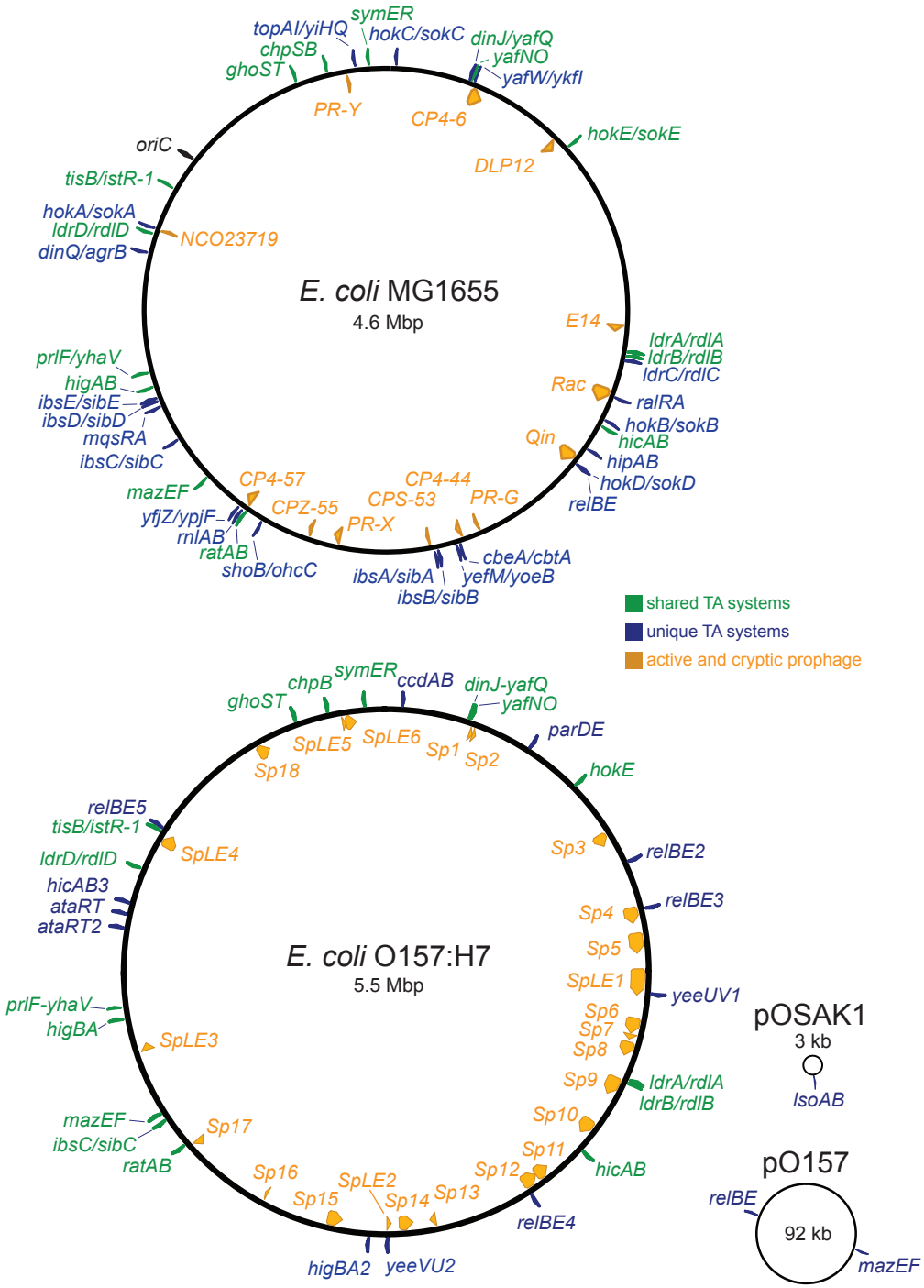

E. coli MG1655
4.6 Mbp
oriC
tisB/istR-1
hokA/sokA
ldrD/rdlD
dinQ/agrB
NCO23719
topAI/yiHQ
symER
hokC/sokC
chpSB
ghoST
PR-Y
dinJ/yafQ
yafNO
yafW/ykfI
CP4-6
DLP12
hokE/sokE
E14
ldrA/rdlA
ldrB/rdlB
ldrC/rdlC
Rac
ralRA
Qin
hokB/sokB
hicAB
hipAB
hokD/sokD
relBE
prlF/yhaV
higAB
ibsE/sibE
ibsD/sibD
mqsRA
ibsC/sibC
mazEF
yfjZ/ypjF
rnlAB
ratAB
shoB/ohcC
CP4-57
CPZ-55
PR-X
CPS-53
CP4-44
PR-G
ibsA/sibA
ibsB/sibB
cbeA/cbtA
yefM/yoeB
shared TA systems
unique TA systems
active and cryptic prophage
E. coli O157:H7
5.5 Mbp
chpB
symER
ccdAB
ghoST
dinJ-yafQ
yafNO
parDE
SpLE5
SpLE6
Sp1
Sp2
Sp18
hokE
relBE5
tisB/istR-1
SpLE4
ldrD/rdlD
hicAB3
ataRT
ataRT2
Sp3
relBE2
Sp4
relBE3
Sp5
SpLE1
yeeUV1
Sp6
Sp7
Sp8
prlF-yhaV
higBA
SpLE3
Sp9
ldrA/rdlA
ldrB/rdlB
Sp10
Sp11
Sp12
hicAB
mazEF
ibsC/sibC
ratAB
Sp17
Sp16
SpLE2
Sp15
Sp14
Sp13
relBE4
higBA2
yeeVU2
pOSAK1
3 kb
lsoAB
pO157
relBE
92 kb
mazEF

Figure 4

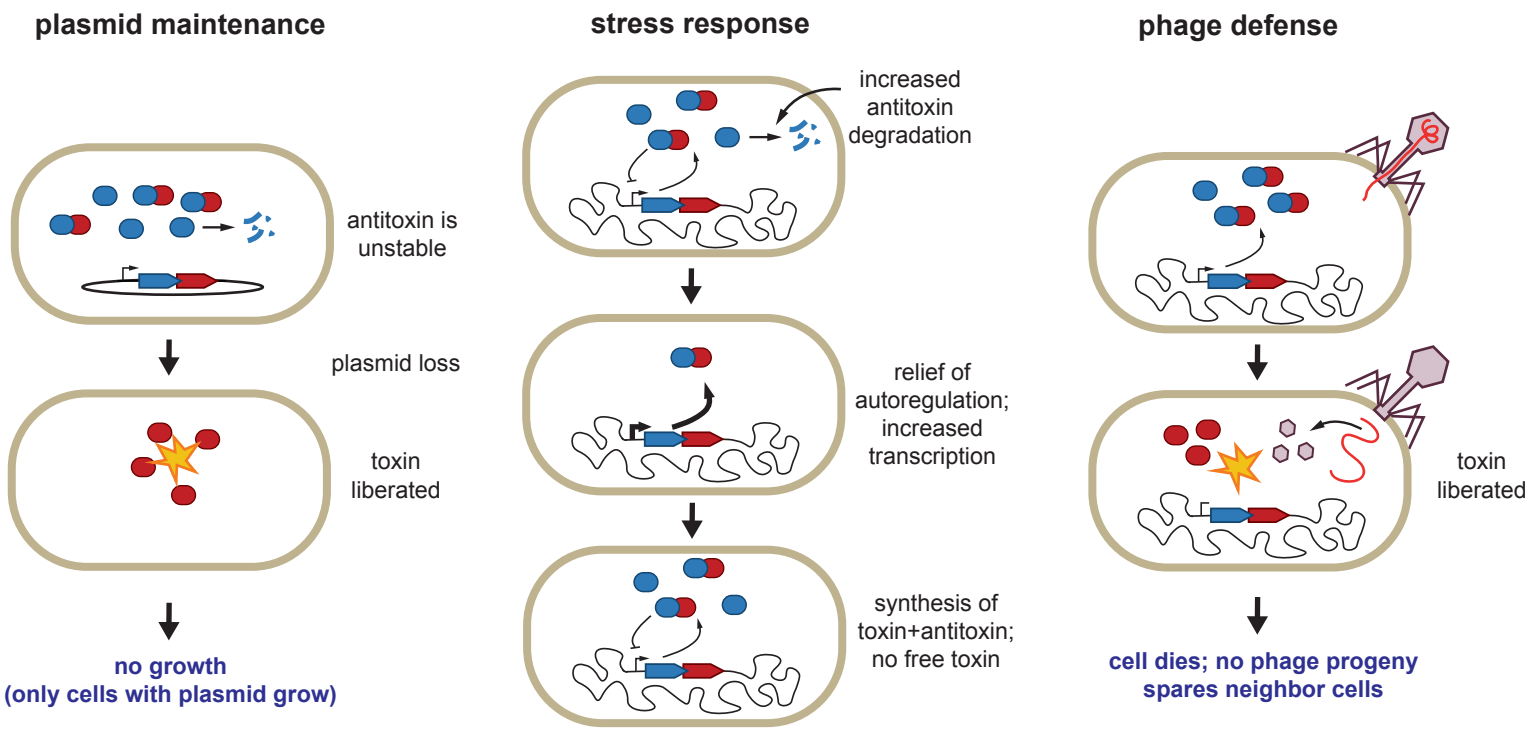

plasmid maintenance
antitoxin is unstable
plasmid loss
toxin liberated
no growth
(only cells with plasmid grow)
stress response
increased antitoxin degradation
relief of autoregulation; increased transcription
synthesis of toxin+antitoxin; no free toxin
phage defense
toxin liberated
cell dies; no phage progeny
spares neighbor cells

Figure 5

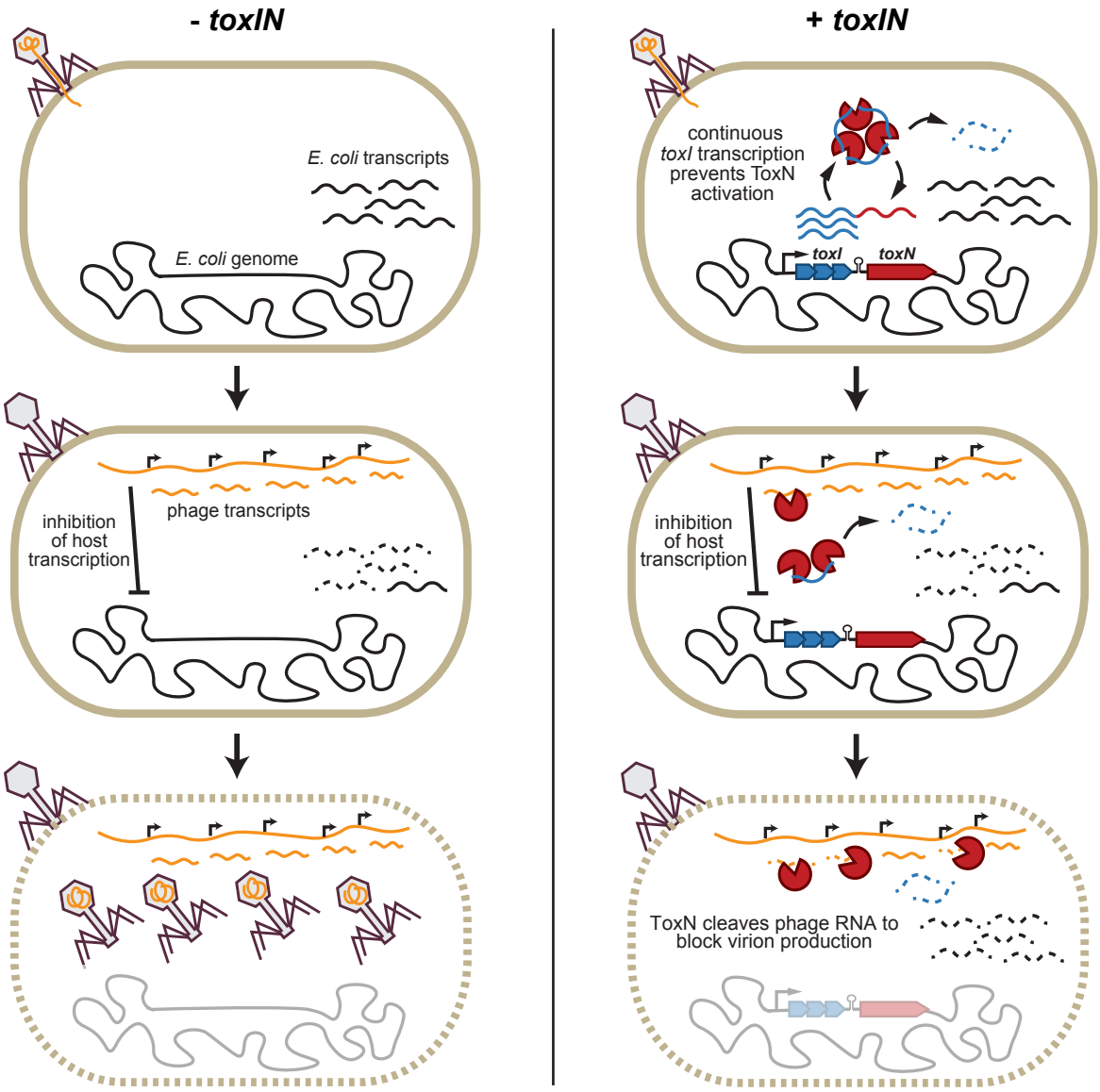
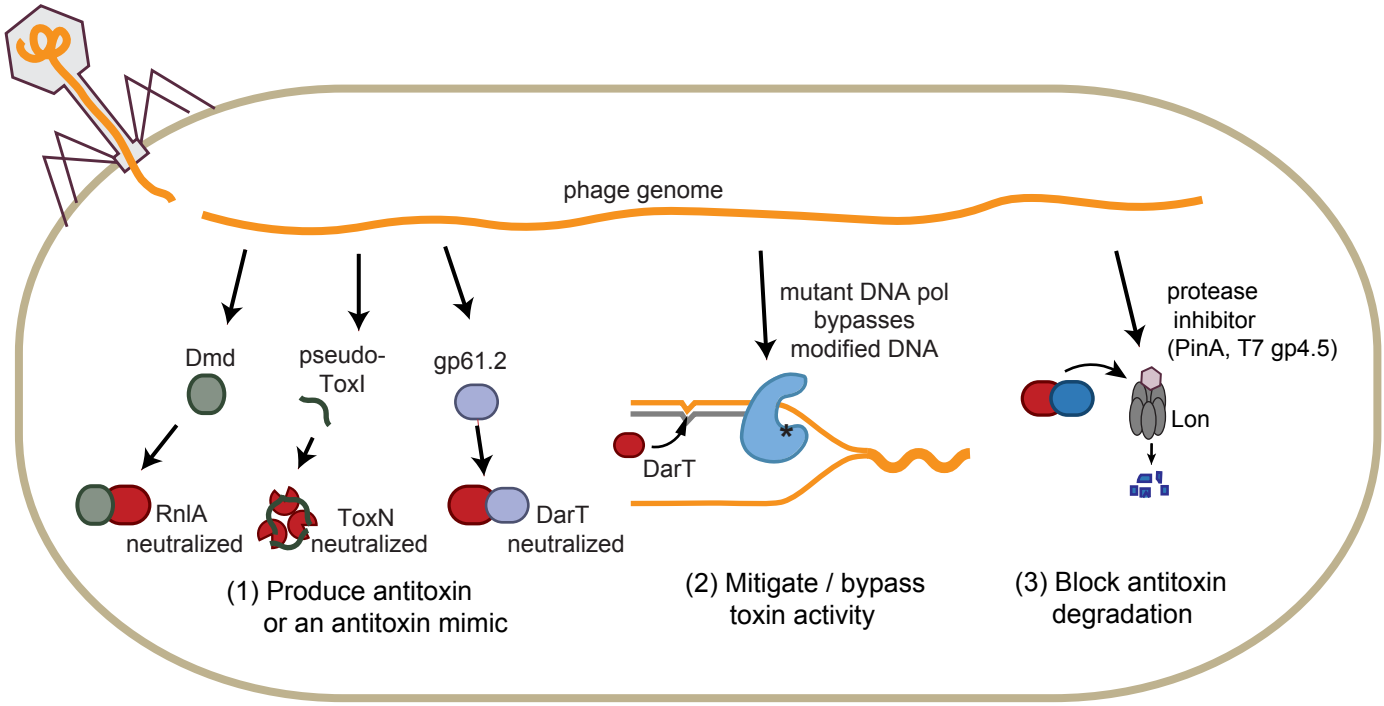

- toxIN
E. coli transcripts
E. coli genome
inhibition of host transcription
phage transcripts
+ toxIN
continuous toxI transcription prevents ToxN activation
toxI
toxN
inhibition of host transcription
ToxN cleaves phage RNA to block virion production

Figure 6


phage genome
Dmd
pseudo-
ToxI
gp61.2
RnlA
neutralized
ToxN
neutralized
DarT
neutralized
(1) Produce antitoxin
or an antitoxin mimic
mutant DNA pol
bypasses
modified DNA
DarT
(2) Mitigate / bypass
toxin activity
protease
inhibitor
(PinA, T7 gp4.5)
Lon
(3) Block antitoxin
degradation