Endoribonuclease type II toxin–antitoxin systems: functional or selfish?

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Abstract
Most bacterial genomes have multiple type II toxin–antitoxin systems (TAs) that encode two proteins which are referred to as a toxin and an antitoxin. Toxins inhibit a cellular process, while the interaction of the antitoxin with the toxin attenuates the toxin’s activity. Endoribonuclease-encoding TAs cleave RNA in a sequence-dependent fashion, resulting in translational inhibition. To account for their prevalence and retention by bacterial genomes, TAs are credited with clinically significant phenomena, such as bacterial programmed cell death, persistence, biofilms and anti-addiction to plasmids. However, the programmed cell death and persistence hypotheses have been challenged because of conceptual, methodological and/or strain issues. In an alternative view, chromosomal TAs seem to be retained by virtue of addiction at two levels: via a poison–antidote combination (TA proteins) and via transcriptional reprogramming of the downstream core gene (due to integration). Any perturbation in the chromosomal TA operons could cause fitness loss due to polar effects on the downstream genes and hence be detrimental under natural conditions. The endoribonucleases encoding chromosomal TAs are most likely selfish DNA as they are retained by bacterial genomes, even though TAs do not confer a direct advantage via the TA proteins. TAs are likely used by various replications as ‘genetic arms’ that allow the maintenance of themselves and associated genetic elements. TAs seem to be the ‘selfish arms’ that make the best use of the ‘arms race’ between bacterial genomes and plasmids.

INTRODUCTION
Toxin–antitoxin systems (TAs) are operons that are widely distributed on plasmids and prokaryotic genomes [1, 2] and consist of two/three adjacent genes encoding a toxin and an antitoxin. Toxins, when free of antitoxins, inactivate their corresponding cellular targets, resulting in metabolic regression. Diverse molecular targets, like mRNA, tRNA, rRNA, DNA gyrase, etc., are inactivated by various toxin proteins [3]. Antitoxins prevent the expression of the toxin or attenuate its toxic effects [4]. TAs are classified into six types, based on the type of gene products and the mode of toxin neutralization [3, 5]. Type II TAs, the most prevalent type on prokaryotic genomes and plasmids, are bicistronic operons which code for a toxin protein and an antitoxin protein. Most of the type II TAs are typically less than 1 kb, encode endoribonuclease toxins [2] and propagate through horizontal gene transfer [6–8]. The antitoxin physically forms a toxin–antitoxin complex (TA complex), resulting in toxin sequestration from the cellular target. The antitoxins have a high turnover rate due to rapid degradation by proteases like Lon, while toxins are relatively stable. Furthermore, the TA complex also acts as an auto-repressor which can bind to the operator present upstream of the TA operon. In most cases, the antitoxin acts as the repressor by virtue of its DNA binding motif and the toxin acts as the co-repressor. The TA complex binds to the promoter/operator region of the TA operon, thus inducing transcriptional repression. This auto-regulatory mechanism prevails in most of the type II TAs characterized to date [9, 10]. When the cellular antitoxin/toxin ratio falls beyond a threshold, the toxin protein is rendered free of antitoxin and hence inactivates its cognate molecular targets. Although toxin activity is the sole limiting factor for the manifestation of a TA-dependent phenotype, the relative concentration of the antitoxin compared to the toxin is the decisive factor in the regulation of a typical type II TA. Complex regulatory mechanisms for TA activation and numerous physiological roles for chromosomal TAs have been proposed by different groups. At least with regard to plasmid-borne TAs, most researchers agree that TAs are ‘addiction systems’ which reduce the proportion of cells cured of TA-encoding-plasmids by a phenomenon referred to as post-segregational killing (PSK) [11, 12]. Proposed chromosomal TA-dependent phenotypes
include persistence [4, 13], bacterial programmed cell death (PCD) [14–16], biofilm formation [17–19] and anti-addiction modules [6, 20]. Because of the implications of the above clinical phenomena, TAs have been proposed as molecular targets for drug development [21–23]. In this paper, we discuss several discrepancies with regard to the proposed functions of endoribonuclease-encoding type II TAs and address some unanswered questions.

DISCOVERY

The instances of TAs discoveries influenced the path and progression of TAs research and its implications. Plasmid TAs were shown to confer plasmid addiction, thereby increasing the propagative potential of the plasmid. This phenomenon is referred to as PSK [11, 12, 24], wherein any bacterium that does not inherit the TAs-encoding plasmid from its parent becomes metabolically static or is killed. When the plasmid is not inherited, the antitoxin is degraded, allowing the toxin to inactivate vital targets, resulting in killing/stasis of the cured daughter bacterium. Hence, plasmidic TAs were thought to increase the plasmid-harbouring cells in a population [11, 12, 24, 25]. The discovery of chromosomal TAs during studies on stringent response and persistence has influenced how we view the involvement of TAs in bacterial physiology. Under stress conditions such as amino acid starvation, RelA-mediated accumulation of (p)ppGpp (guanosine tetra- and pentaphosphate) alarmone occurs [26]. (p)ppGpp is known to modulate RNA polymerase to inhibit rRNA synthesis [27] and enhance the transcription of the biosynthetic operons. During these studies some mutants, later mapped to the relB locus, were isolated which were deficient/alted in stringent response [28, 29]. Later, relB was characterized as encoding RelB protein, the antitoxin of RelBE TAs. RelB binds and incapacitates ribosome-dependent endoribonuclease toxin, RelE [30]. Chromosomal TAs were also discovered in persistence studies [31, 32], a phenomenon characterized by transient and non-inheritable tolerance to antibiotic stress by a small fraction (referred to as persisters) of an isogenic population. Some mutants, referred to as high-persister mutants (hip), had a higher persister frequency than the wild-type. This locus of this mutant was named hipA [33], and was later characterized as a typical type II TA, HipBA, encoding HipA toxin and HipB antitoxin [34–36]. The genetic architecture and mechanism of the regulation of RelE and HipA are similar (although different in their molecular targets) and hence belong to type II TAs. These observations were the first indicators that chromosomal type II TAs are involved in stringent response and persistence [9, 36–39].

ENDORIBONUCLEASE-ENCODING TAS ARE HORIZONTALLY TRANSFERRING OPERONS

The endoribonuclease encoding TAs like HicAB [40] and MazEF [7] propagates through horizontal gene transfer (HGT) mechanisms [8]. It was shown that the distribution of several chromosomal TAs is uneven and random in various strains of E. coli [6, 41]. It was observed that the closest homologues of these TAs are found on plasmids, indicating that these TAs were acquired from plasmids. Using sequence analysis, it was shown that TAs integrate on the bacterial chromosomes by de novo non-homologous integration from plasmids or by homologous mechanisms from other bacterial chromosomes. The minimal propagating TAs consist of a promoter/operator, an antitoxin gene and a toxin gene, but not the terminator [6]. TAs are well suited for HGT because they are small and encode a poison–antidote combination which could confer ‘addiction’. As the minimal TAs organization does not encode a terminator, TAs are suited to integrating within close proximity of core genes and they are less likely to compromise the expression of the downstream gene.

FUNCTIONS OF CHROMOSOMAL TYPE II TAS

Understandably, molecular microbiologists are driven to assign a highly significant physiological function to TAs due to their diversity and prevalence on prokaryotic genomes/plasmids. A multitude of physiological roles, which supposedly increased bacterial ecological fitness, were ascribed to TAs by various groups [42, 43]. Unfortunately, reports showing that the deletion of chromosomal TAs does not cause a loss of a detectable phenotype [13, 44] were ignored.

mazEF systems do not confer PCD in E. coli

One of the earliest proposals, which was popular for more than 18 years and is highly controversial, is that mazEF mediates PCD in E. coli [16, 45, 46]. The presence of mazEF caused dramatic loss of viability upon induction of stresses, while deletion of mazEF did not induce loss of viability. A plethora of stress conditions, such as nutrient starvation, heat shock, antibiotics, etc., were shown to induce mazEF-mediated PCD [15, 47, 48]. At this stage, it seems that it is an artefact or an erroneous interpretation. Firstly, the strains are not isogenic and seem to have numerous problems, including several deletions of the adjacent genes [44, 49, 50]. Secondly, it is highly improbable that we can obtain relA derivatives of MC4100 and MC4100ΔmazEF strains as described by the PCD proponents [14, 51]. Thirdly, the concept of PCD, wherein the majority of the cells of the population die, is probably counterproductive for the goal of spatiotemporal propagation [14]. Paradoxically, according to the proposed PCD, it is better for bacteria not to harbour mazEF TAs. It must also be noted that the observations reported by the proponents of the PCD hypothesis were not reproducible [44, 50]. Apart from a few recent reviews that invoke mazEF-mediated PCD to exemplify bacterial PCD [52, 53], there have been no research publications on TA-mediated PCD since 2013, which allows the assumption of ‘no contention’ to be made in support of the mazEF-mediated PCD hypothesis.

Role of endoribonuclease TAs in persistence

Bacterial persistence, a transient drug-tolerant phenotype, has been linked with TAs based on observations of higher
persister frequency in HipA7 (a kinase of GltX) mutants [33, 54]. Later it was reported that persistence is caused by several factors, in which endoribonuclease TAs are predominantly implicated [13, 37, 39, 55]. The implication of endoribonuclease TAs in persistence is entrenched through two methodologies: (i) ectopic overexpression of toxins and (ii) construction of TAs deletion strains. TAs were deduced to be mediators of persister after observations of increased persister frequency upon ectopic expression of toxin and/or loss of persister frequency upon deletion of TAs [13]. It was shown by several researchers that persister frequency increases upon overexpression of toxin genes [13, 56]. However, the implication of TAs in persistence using ectopic expression methodology was challenged by a study in which the overexpression of genes that were not related to toxins also induced persistence [57]. Any factor or process that strains the metabolism is likely to cause a reduction in the metabolic rate and hence is likely to increase persister frequency [58]. Hence, this methodology for determining persistence factors could be misleading. In the other methodology, a decrease in persister frequency upon deletion of chromosomal TAs (multiple or single) indicated that TAs are the molecular determinants of persistence. It was shown that as more chromosomal TAs are deleted, persister frequency also decreases, which suggests that persistence is the reason for the accumulation of TAs on bacterial genomes [13]. Moreover, RelBE has been identified during studies on stringent response and HipAB has been identified during persistence studies. Hence, it was predicted by some researchers that type II TAs are involved in persistence through stringent response. In the currently accepted model, the stochastic activation of RelA and the consequent accumulation of ppGpp are thought to result in the accumulation of inorganic polyphosphate (polyP). PolyP is thought to modulate Lon protease to specifically degrade anti-toxins and thus cause the activation of toxins, resulting in dormancy and the persister phenotype [38, 39]. However, an increasing number of reports are contradicting this model. Persistence is observed even in the absence of (p)ppGpp [59]. Moreover, it was shown that (p)ppGpp is not required for the regulation of relBE [30], mazEP [60] and yefM/yoeb operons [61]. It was also shown that polyP is not required for transcriptional regulation or toxin activity (of yefM/yoeb TAs), which suggests that polyP is not required for degradation of antitoxin YefM [61]. In stark contradiction to the proposed model, polyP was shown to inhibit the proteolytic activity of Lon proteases [62]. Recently, it was shown that persister frequency is dependent on cellular ATP levels and is independent of TAs [63]. Hence, the link between stringent response, TAs regulation and persistence is refuted citing issues pertaining to fitness, possible polar effects and inadvertent mutations of the constructed strains (such as Δ10 strain, a MG1655 mutant with 10 endoribonuclease TAs deletions [13]). It was reported that there are multiple mutations (160 single nucleotide polymorphisms) in the Δ10 strain [63]. It is conceivable that multiple deletions of genes could result in abnormal gene expression of the adjacent core genes [61, 64]. The possibility that endoribonuclease TAs can induce persistence cannot be ruled out at this stage. With the existing evidence, however, the role of chromosomal TAs in bacterial persistence is inconclusive and needs to be approached with scepticism.

**Chromosomal TAs confer anti-addiction to TA-encoding plasmids**

Unfortunately, less appealing hypotheses, such as ‘chromosomal TAs confer anti-addiction on TA-encoding plasmids’ and ‘TAs are selfish DNA’, are largely overshadowed by exuberant hypotheses such as ‘mediators of PCD’, ‘stress-response elements’ and ‘inducers of persistence’. Plasmid TAs were shown to induce addiction to enforce cell stasis/death of cells cured of TA-encoding plasmids during non-selective conditions, resulting in the increased prevalence of plasmids within a population [11, 24, 65, 66]. Logically, if a bacterium were to be cured of a TA-encoding plasmid and yet survive peer pressure in the limiting conditions, it must have acquired an antidote for the toxin. Hence, it is highly rational to assume that the function of chromosomal TAs is to provide anti-addiction to plasmids. During non-selective conditions for plasmid-encoded phenotypes, the acquisition of TAs on the chromosome will allow the loss of the plasmid, thereby lessening the metabolic burden and increasing fitness. There are very limited experimental means of proving this ecological-cum-evolutionary phenomenon. It is indeed difficult to experimentally test the acquisition of TAs by bacterial genomes from plasmids. This ingenious phenomenon was proven using chromosomally encoded control of cell death (ccd) TAs, from *Erwinia chrysanthemi*, in which it protected the bacterium against PSK mediated by its F-plasmid ccd homologue [20]. Alternatively, the enumeration of identical TA occurrence on the genomes and plasmids of natural isolates allows us to test this hypothesis. If the anti-addiction hypothesis were true, the occurrence of identical TAs on the plasmid as well as the host genome, in non-selective conditions for plasmid-encoded phenotypes, should be mutually exclusive. It was shown that natural isolates of *E. coli*, the species with the largest available set of sequenced plasmids and natural isolates, do not harbour plasmids which encode TAs that are identical to those of the chromosomal TAs [6]. This strengthens the notion that chromosomal TAs play a role in anti-addiction to plasmids [6, 20]. Another hypothesis, referred to as the ‘competition hypothesis’, proposes that PSK conferring TAs favours the selection of TA-encoding plasmids over TA-deficient plasmids when there is plasmid–plasmid competition within the host [67]. It would be interesting to test such a hypothesis under suboptimal growth conditions, as the burden of extra DNA could be overwhelming in demanding conditions. Using a transposon-based approach, it was shown that under the conditions tested, plasmids are more likely to accumulate TAs than chromosomes [68]. However, in the genomes and plasmids sequenced to date, the multiplicity of TAs is greater in bacterial genomes compared to their plasmid counterparts, which could be due to size of the DNA. Interestingly, high TA density (the number of TAs per unit length of DNA) is usually associated with superintegrons [69].
In a recent study it was found that the toxin genes are lost, but the antitoxin ORFs are retained, in *Xanthomonas* species, which is indicative of the selective retention of antitoxins to confer anti-addiction [70]. It is possible that the greater the number of TA-encoding plasmids to which a bacterium is exposed, the more chromosomal TAs there are. This partly explains the diversity and multitude of TAs on genomes of free-living bacteria. The same may not be true for *Mycobacterium tuberculosis*, which has coevolved with humans (and other corresponding hosts) and hence its pan-genome is not as diverse as that of free-living bacteria. It is known that various strains of *M. tuberculosis* share most of the TAs of the *vapBC* family. Hence, more experimental evidence is required to support the anti-addiction hypothesis.

**TAs are selfish DNA**

The null hypothesis [42] in horizontal gene transfer is that the acquired DNA is selfish DNA (or junk), i.e. it does not contribute to the propagative potential but propagates along with the host replicon. There is a major inconsistency in the functional roles of type II TAs as ascribed by several researchers. Critical evaluation of the PCD and persistence hypotheses highlighted the 'strain problems' [44, 50, 71]. The anti-addiction hypothesis is short of experimental evidence. Moreover, once the bacterium is cured of the plasmid, there is no need for the retention of the chromosomal TAs, unless for an unlikely anticipated repetitive invasion by a plasmid with identical TAs. Therefore, with current knowledge, it is reasonable to be inclined to the null hypothesis that chromosomal TAs are selfish DNA. Due to the general tendency for optimal minimization of the host replicon over generations, those elements that do not contribute to the propagative potential are lost. However, surprisingly, multiple TAs are retained by bacterial genomes, even though a clear benefit is not observed [44]. TAs could induce 'selfishness' or 'addiction' in at least two ways: (i) by virtue of poison–antitoxin combination [20] and/or (ii) by irreversible reprogramming of the adjacent host genes due to TA integration [6] (Fig. 1). The host genome will have to retain the TAs or face extinction due to a similar phenotype upon loss of the TA operon. TAs are horizontally propagating entities and are integrated in the intergenic regions. Any perturbations in the TA operon could also negatively influence the downstream core gene, which is usually co-transcribed with the TA ORFs [71] (Fig. 1). Hence, the bacterial genomes are seemingly 'resigned' to retaining TAs. A few examples of chromosomal TA disintegration have also been reported, such as partially degraded HicB [40], toxin-deleted CcdB [72], etc. If a TA should be deleted, the only possibility is to start with a mutation/deletion in the toxin gene, as observed in *Xanthomonas* species [70].

Fig. 1. Manifestation of toxin–antitoxin system-mediated selfishness through addiction and integration. A typical TA could be acquired from exogenous DNA. Step 1. Those TAs that integrate within intergenic regions and do not result in deleterious effects on the host genes at the locus of the integration are usually tolerated within a bacterial population. The regulation of the downstream core gene of the host replicon is now under the control of the TA promoter, which is referred to as irreversible transcriptional reprogramming. Step 2. Toxin and antitoxin proteins are produced and these will result in addiction. Deletion of all or part of the TAs will usually result in fitness issues for the host replicon. If the antitoxin gene alone is deleted, the antitoxin protein is degraded and the free toxins are activated and will result in dormancy/death. Moreover, since the limiting factor for repression (the antitoxin) is not present, the toxin gene could be overexpressed and may result in death. Step 3. If both the toxin and antitoxin genes are deleted, the promoter (if retained) could result in overexpression of the downstream core gene. If the promoter is deleted along with the TA genes, the downstream core gene will not be expressed appropriately and consequently there will be a loss of fitness. Step 4. If the toxin gene alone is deleted/mutated, there is no longer addiction. However, since the toxin protein also acts as a corepressor (as in many cases), the regulation of the downstream core gene could be altered.

**UNANSWERED QUESTIONS**

What are the triggers and consequences of toxin activity?

Owing to the potential of the TAs to regulate the bacterial metabolism, various groups have proposed different applications in basic and applied research [21]. For these purposes the regulation of TAs must be understood with much greater clarity. Unfortunately, there have been very few holistic studies in which assays are reported for the transcriptional activity of TAs, toxin activity and a consequent phenotype upon induction of a stress. The closest study in this regard concerned the observation of RelE- and MazF-dependent RNA cleavage upon the induction of amino acid starvation [60]. It must be noted that transcriptional activation is not necessarily followed by detectable mRNA cleavage. For example, overproduction of Lon protease, although it cleaves antitoxins such as YefM, RelB and MazE, only causes YoeB-dependent RNA cleavage [71, 73]. Intriguingly, the total toxin concentration in the cell during various rates of translation and the required toxin concentration to manifest a phenotype are not known. It is more important to know whether the toxin activity causes stasis or death when attempting to understand and assign a physiological function to TAs.

The cumulative phenotype that is dependent on all the type II TAs of a strain seems to be complicated, according to
several observations. Different stress conditions seem to activate transcription from different TAs [74]. Even though there is transcriptional activation of TAs, there is no detectable phenotype [63, 73]. Furthermore, several TAs have been shown to cross-talk, resulting in the regulation of other TAs via toxin neutralization, cleavage of TA mRNA and/or repression of other TA operons. [75, 76]. Since it is difficult to assign a single function to all TAs, it is safe to assume that different TAs may cause different phenotypes, based on the triggers for activity, the type of molecular target and the extent of target inactivation. However, a robust genetic analysis and concepts of higher rationality should be developed to explain the role of TAs in bacterial physiology and evolution.

What are the genetic effects of TA integration?
Over the decade of TA research, most researchers have focused on the direct impact of the gene products of TA operons on bacterial physiology. Apart from bringing in new genetic information, the integration of any exogenous DNA into a replicon will cause variation within the already existing genetic information of the host replicon. The association of TAs with plasmids has increased the stable reach of TAs to various species, thus allowing the prevalence of TAs in bacterial genomes. The indirect effects, like the alterations caused within the host genome, should also be considered, as these may increase fitness. In E. coli, TAs integration also caused variations in the regulation and/or coding regions of the host genes [6]. TAs are a gene pair with high potential for propagation driven by at least two rationalities. They induce ‘addiction’ of the host replicon to retain TAs by virtue of a poison–antidote (toxin and antitoxin proteins) combination. Secondly, the minimal TAs does not encode a terminator. Hence, the downstream gene is usually co-transcribed as a part of the TA operon. A bacterium with a deletion of TAs, that was integrated near core genes, is likely to be outcompeted as the expression of the downstream gene will be compromised (Fig. 1). Hence, this irreversible transcriptional reprogramming of the downstream gene will ensure that the TA genes are retained within a population. Integration of dinJ/yafQ in the E. coli K12 strain has caused the replacement of the N-terminal 24 amino acids of YafL. Similarly, the integration of the priF/yhaV TA operon adjacent to agaR has resulted in replacement of the last six codons (the codons for Asp-His-Ala-Ser-Ser-Leu), with one codon for Glu [6]. YafL seems to be a membrane-associated protein with cysteine-type peptidase activity [77], which may have important functions in bacterial cellular physiology through altering the stability of various other proteins. Similarly, AgaR is a known transcriptional regulator [78, 79] of several operons [80] involved in N-acetyl-galactosamine metabolism. In order to ascertain the significance of the primary structural alterations of YafL and AgaR, we may need to study the properties and consequences of both the ancestral and the modified protein (altered due to TA integrations). It is even more important to evaluate the genomes of all bacteria with a focus on the alterations induced by TAs integration.

Regulation of other genes/proteins by TAs?
All the antitoxins of endoribonuclease-encoding type II TAs have a DNA-binding motif, which primarily functions in the autoregulation of TAs. It was shown that DinJ antitoxin can bind to the promoter of the cspD gene. CspE, a cold-shock protein, enhances the translation of RpoS mRNA encoding the stationary-phase sigma factor [81]. This implies that the DNA-binding ability of antitoxin DinJ could influence bacterial physiology simply because of operator sequence similarities. Similarly, MqsA was shown to enhance biofilm formation and persistence by acting as a transcriptional repressor for cspD [82], rpoS and csgD [83]. This is another level at which TAs could influence bacterial physiology, and many more such examples could surface in the future. Since most antitoxins are loosely folded and/or have extended structures, one cannot rule out the possibility that antitoxins could interact with other proteins in addition to their cognate toxins, albeit with lower affinity.

Why are TAs so prevalent and divergent?
Type II endoribonuclease-encoding TAs are the most prevalent, due to low target diversity. Toxins that act on protein targets may have a restricted host range due to variations among species. However, RNA is present in all the cells and the probability of cleavage site occurrence is high in diverse bacterial species and consequently endoribonuclease toxins are effective even in eukaryotes [84, 85]. Similarly, the prevalence of endoribonuclease-encoding TAs on plasmids can also be explained. Low target diversity is advantageous for heterologous plasmids to establish addiction over a broad host range. Hence, endoribonuclease-encoding TAs are effective in very divergent cells and highly prevalent in bacterial genomes and plasmids. Once a member of a species acquires a TA on its chromosome, TAs could propagate within the species via transformation, transduction and conjugation. Based on the inter-species interactions, different members of a bacterial species might accumulate different TAs.

TAs enhance the potential of the associated replicon (such as plasmids) to establish addiction in bacterial cells thereby propagation of the TAs and the associated replicon is accomplished (Fig. 2). If the autonomous replicon (the genome of the cellular entity, i.e. bacteria) acquires an identical TAs, the cellular genome is no longer addicted to the plasmid, but to the TAs alone, which is economical than bearing the costs of propagating the whole plasmid. However, any plasmid that has significantly diversified TAs, such that the host antitoxin can no longer neutralize the plasmid-encoded toxins, can induce addiction to the plasmid. The only path available to the host genome is to acquire the diversified TAs as well. It could also be the case that different TA-encoding-plasmids have contributed different TAs to a bacterial genome. Thus the accumulation of diverse TAs on the genomes of free-living bacteria is highly likely. Genetic elements like plasmids [12], superintegrons [86, 87] and chromosomal loci [88] that are associated with TAs are usually prevalent, which could imply that TAs confer stable maintenance within a species. Hence, TAs could
be viewed as ‘genetic arms’ of various replicons such as genomes and plasmids. TAs seem to be ‘selfish arms’, similar to restriction-modification systems [89, 90], in the arms race for competitive propagation between bacterial genomes and plasmids, in which TAs have ‘made the best of it’. This biological system exemplifies the notion that the inevitable consequence of any war is the propagation of arms.

**CONCLUSIONS**

Although, the molecular details of TAs functioning are highly accurate, the holistic understanding of TAs significance is riddled with inaccuracies. TAs research to date seems to be a search for complexity and clinical relevance. The proposed physiological and/or ecological functions of endoribonuclease TAs, such as PCD and persistence, seem to be artefactual due to mutant and/or methodological issues. TAs should be reclassified based on evolution and molecular activity rather than mere architecture. Different type II TAs may be similar in genetic architecture and regulation, but the consequences of toxin activity could be different, based on the target that is inactivated, and hence could result in phenotypic variation. However, we must realize that ‘stress’ is a natural part of the bacterial life cycle and TA-inflicted metabolic stasis or killing would be counterproductive for competition and colonization. Hence, it is opined that unless there is a loss or dysregulation of TA operons there might not be an authentic TA-dependent phenotype. Endoribonuclease TAs are horizontally propagating operons and hence must initially be treated as selfish DNA. In the current state of knowledge, it is difficult to explain the retention of multiple endoribonuclease-encoding TAs on bacterial genomes. Hence, we cannot rule out the possibility that these TAs might have been domesticated by the prokaryotic genomes for an elusive physiological purpose. Based on the location of TAs integration and the resultant variation in the host genome, TAs integration may enhance bacterial fitness. It is also possible that the TAs integration could be neutral but never negative in terms of...
bacterial fitness. The multiplicity of TAs on the bacterial genomes could influence ecological fitness and drive the evolution of bacterial genomes. Our understanding of the chromosomal TAs significance could be enhanced by holistic studies of the evolutionary basis and consequences of multiple TAs on each of the bacterial genomes.

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