Engineering Microbiology 3 (2023) 100069

Contents lists available at ScienceDirect

Engineering Microbiology

journal homepage: www.elsevier.com/locate/engmic

Review

Applications of toxin-antitoxin systems in synthetic biology

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ARTICLE INFO

Keywords:
Toxin
Antitoxin
Toxin-antitoxin system
Classification
Application

ABSTRACT

Toxin-antitoxin (TA) systems are ubiquitous in bacteria and archaea. Most are composed of two neighboring genetic elements, a stable toxin capable of inhibiting crucial cellular processes, including replication, transcription, translation, cell division and membrane integrity, and an unstable antitoxin to counteract the toxicity of the toxin. Many new discoveries regarding the biochemical properties of the toxin and antitoxin components have been made since the first TA system was reported nearly four decades ago. The physiological functions of TA systems have been hotly debated in recent decades, and it is now increasingly clear that TA systems are important immune systems in prokaryotes. In addition to being involved in biofilm formation and persister cell formation, these modules are antiphage defense systems and provide host defenses against various phage infections via abortive infection. In this review, we explore the potential applications of TA systems based on the recent progress made in elucidating TA functions. We first describe the most recent classification of TA systems and then introduce the biochemical functions of toxins and antitoxins, respectively. Finally, we primarily focus on and devote considerable space to the application of TA complexes in synthetic biology.

1. Introduction

TA systems are broadly distributed in bacterial and archaeal chromosomes and mobile genetic elements and usually consist of two genetic components, including typically stable toxins that function as bactericidal or bacteriostatic agents, while the adjacent labile antitoxins act as antagonists to mask their toxicity [1]. In model strains, such as *Escherichia coli* K12 and *Mycobacterium tuberculosis*, dozens or even hundreds of TA systems have been identified [2–4].

TA systems were originally discovered in conjugative plasmids in the 1980s, and the first three TA systems described were CcdB/CcdA of the F plasmid [5,6], and Hok/Sok and ParD (kis/kid) of the conjugative plasmid R1 [7,8]. The function of plasmid-encoded TA systems in maintaining the vertical inheritance of plasmids is well documented by strong evidence obtained in different labs, but the exact mechanisms remain debatable. TA systems in plasmids have generally recognized “plasmid addition modules” which control the stability of plasmids in bacterial populations by a mechanism known as postsegregational killing (PSK) [5,9,10]. Other mechanisms have been found to explain the advantages of harboring TA systems in plasmids, including increased fitness during plasmid-plasmid competition [11] and the direct control of plasmid replication by the antitoxins [12]. In the past decade, the rapid expansion in the number of sequenced plasmids has revealed that many plasmids harbor multiple TA pairs, including many conjugative plasmids carrying multiple antibiotic resistance genes [13,14].

TA systems have also been discovered in some other mobile elements in chromosomes, such as integrative and conjugative elements, transposons and prophages [15–18]. In most cases, toxin- and antitoxin-encoding genes are located on the same operon, and antitoxin genes are located upstream of toxin genes [19]. Type II TA systems are negatively autoregulated either by antitoxin alone or by toxin-antitoxin complexes [1]. Currently, the mechanisms by which toxin proteins exert toxicity include binding to DNA helicases or ribosomes and RNA degradation, leading to disruption of DNA replication or mRNA translation [20–22]. Structural studies also help to uncover the molecular basis of enzymatic toxins and how antitoxins counteract toxins by direct interactions in type II and type III TA systems or by posttranslational modifications in type VII TA systems [23–25]. The physiological roles of TA systems are also diverse. Studies to date have shown that TA systems are crucial in plasmid stability maintenance [12], phage inhibition [26], biofilm formation and stress response [27] and programmed cell death (PCD) [28].

In this review, we summarize the most recent classification of TA systems, including the discovery of two new types of TA systems since 2016. We then explore the biochemical functions of toxins and antitoxins, and the application of TA systems in synthetic biology, including

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https://doi.org/10.1016/j.engmic.2023.100069
Received 26 November 2022; Received in revised form 1 January 2023; Accepted 3 January 2023
Available online 18 January 2023

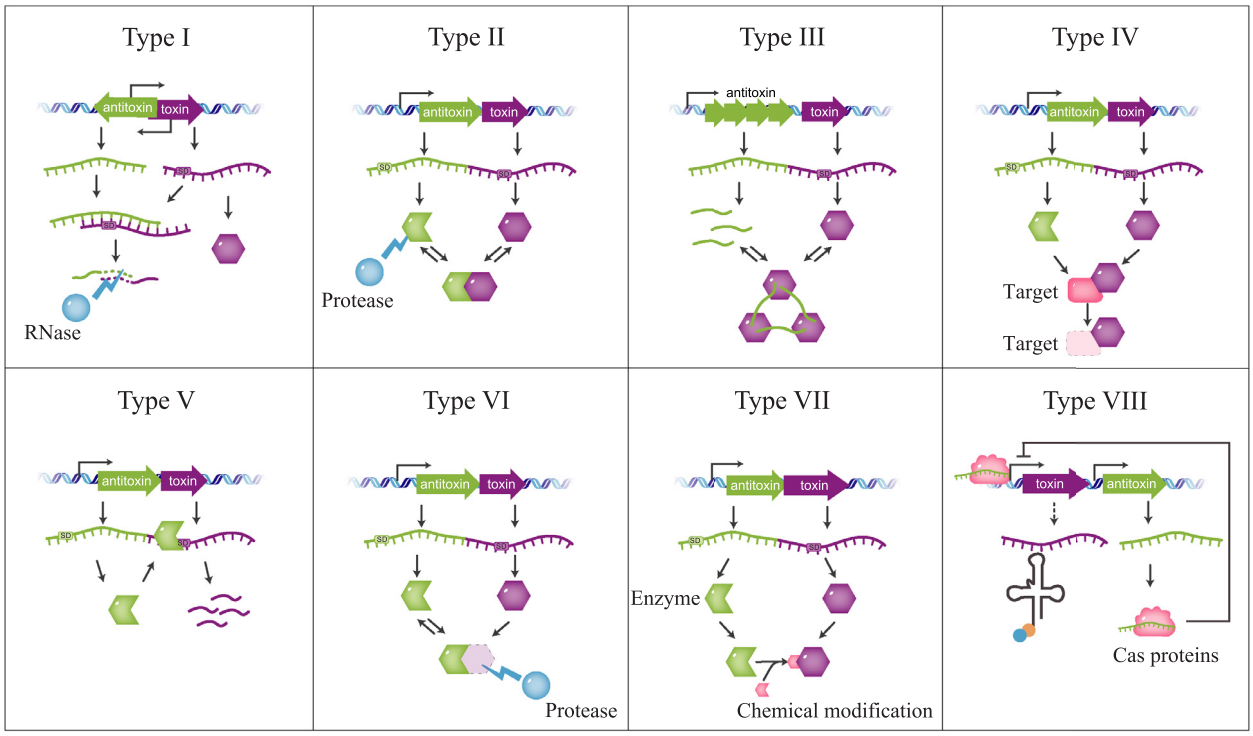

Fig. 1. Current classification of TA systems. The eight major types of TA systems are classified based on the nature of antitoxins (protein or RNA) as well as the way that the antitoxins neutralize the toxicity of the cognate toxins.

those in mobile genetic element stabilization and DNA cloning, as potential phage defense elements and as new drugs.

2. Classification of TA systems

It is noteworthy that the vast majority of toxins are proteins (except for the newly discovered type VIII systems in which the toxins are RNA molecules), and antitoxins can be either proteins or RNAs. Currently, TA systems are grouped into eight main types based on the nature of the antitoxins as well as the way in which the antitoxins interact with toxins to block toxicity (Fig. 1) [29,30]. Notably, the antitoxins of type I, type III and type VIII TA systems consist of RNA. For the remaining systems, the antitoxins consist of proteins. In type I TA systems (the first described is Hok/Sok [31]), the noncoding small RNA antitoxin acts as an antisense RNA that binds to toxin-encoding mRNA and inhibits its translation. Type II TA systems are the most extensively studied among the various types of TA systems. In type II TA systems (the first described is CcdB/CcdA [6]), the antitoxin neutralizes the cognate toxin toxicity via a direct protein-protein interaction, resulting in TA complex formation. In type III TA systems (the first described is ToxN/ToxI [32]), the RNA antitoxins directly bind and counteract toxin proteins to inhibit their toxicity. In type IV TA systems (the first described is CbtA/CbeA [33]), the antitoxin and the toxin do not have a direct interaction. Instead, the antitoxin can neutralize the toxin's activity by interacting with the toxin's target. In the type V TA system, GhoT/GhoS [34], the antitoxin GhoS acts as an RNase that specifically degrades toxin mRNA. In the type VI TA system, the SocB/SocA TA system [35], the antitoxin protein functions as a proteolytic adapter and stimulates degradation of the toxin SocA. Recently, we described a new type of TA system, HepT/MntA, in which the antitoxin functions as an adenylyltransferase enzyme to antagonize the toxin by polyadenylylating the toxin [25]. Then, we proposed and categorized it with Hha/TomB and TglT/TakA as type VII TA systems, all of which have the same neutralization mechanism in which the antitoxin neutralizes toxin proteins by chemical modification [29]. Intriguingly, in the most recently discovered type VIII TA system, both the toxin and antitoxin consist of RNA. The small RNA toxin CreT sequesters $tRNA^{UCU,}$ and antitoxin CreA that resembles crRNA guides the Cas (CRISPR-associated) proteins to transcriptionally inhibit the toxin CreT [36].

3. Biochemical functions of toxins and antitoxins

Toxins in TA systems target numerous cellular processes, including replication, translation and others, ultimately leading to cell death or inhibiting cell proliferation. A wide variety of the molecular activities of toxins and their bacterial hosts are listed in Table 1. Toxins targeting DNA replication function by changing the topological structures of DNA by cleaving DNA or modifying DNA. We have shown that the RalR toxin in the type I TA system, RalRA, is a nonspecific endonuclease and cleaves DNA substrates but not RNA substrates [37]. Many type II toxins are endoribonucleases. Some of them (*e.g.*, RelE and HigB) nonspecifically cleave mRNA in ribosomes in a translation-dependent manner. Remarkably, some toxins, such as MazF and MqsR in *E. coli*, cleave RNA independently of ribosomes with sequence specificity with preferences at ACA and GCN *in vitro*, respectively [38–40]. Additionally, diverse specific recognition and cleavage motifs are reported for MazF homologues in different strains. For example, MazF toxin from *Nitrospira* strain ND1 specifically cleaves the AACU, AACG, and AAUU motifs [41], and MazF toxin from *Clostridium difficile* cleaves mRNA at the consensus UACAU sequences [42] as well as MazF from *Deinococcus radiodurans* strictly recognizes and cleaves the UACA sequences [43].

Accumulating evidence has shown that antitoxins can function as global regulators. We summarized the antitoxins that regulate host metabolic pathways by binding to DNA or RNA sequences similar to those of TA operons or by directly binding to host proteins (Table 2). Among the eight different types of TA systems, type II antitoxins have DNA-binding abilities, and many of them are produced in greater amounts than toxins. The ability of these antitoxins to regulate other host genes that have similar binding motifs is expected and has now been demonstrated in various *E. coli* and *Pseudomonas* strains. Impor-

Table 1
Targeted cellular processes and mechanisms of toxins in different types of TA systems.

TA pair	TA type	Toxin	Mechanism/Targeted cellular process	Organism	Source
			DNA replication		
RalRA	I	RalR	DNase	*E. coli*	[37]
FicTA	II	FicT	Adenylylation of DNA gyrase and topoisomerase IV	*Bartonella schoenbuchensis*	[44]
ParDE	II	ParE	Inhibition of DNA gyrase	*E. coli, Vibrio cholerae*	[45,46]
CcdBA	II	CcdB	DNA topoisomerase II poison	*E. coli*	[47]
DarTG	IV	DarT	ADP-ribosylation	*Thermus aquaticus*	[48]
SocAB	VI	SocB	Direct interaction with DnaN	*Caulobacter crescentus*	[35]
			Translation		
SymE/SymR	I	SymE	mRNA cleavage	*E. coli*	[49]
MazEF	II	MazF	Ribonuclease independent of ribosomes	*E. coli*	[38]
HicAB	II	HicA	mRNA cleavage	*E. coli*	[20]
HipBA	II	HipA	Phosphorylation of the glutamyl-tRNA-synthetase	*E. coli*	[50]
VapBC	II	VapC	Cleavage of initiator tRNA	*Shigella flexneri, Salmonella enterica, Mycobacterium tuberculosis*	[51–53]
TacAT	II	TacT	Acetylation of tRNA	*Salmonella enterica*	[54]
AtaRT	II	AtaT	N-acetylation of the initiator $tRNA^{fMet}$	*E. coli*	[55]
RatA/RatB	II	RatA	Inhibition of the formation of 70S ribosomes	*E. coli*	[56]
RelBE	II	RelE	Cleavage of ribosome-independent mRNA and tmRNA	*E. coli*	[22,57]
ToxIN	III	ToxN	Cleavage of mRNA	*Erwinia carotovora*	[32]
HepT/MntA	VII	HepT	Cleavage of mRNA	*Shewanella oneidensis*	[25]
HEPN/MNT	VII	HEPN	Cleaving 4 nt from the 3′ end of tRNA	*Aphanizomenon flos-aquae*	[58]
CreTA	VIII	CreT	Sequestering $tRNA^{UCU}$	*Haloarcula hispanica*	[36]
			Others		
HoK/Sok	I	HoK	Depolarizing the bacterial membrane	*E. coli*	[31,59]
Doc/Phd	II	Doc	Phosphorylation of EF-Tu	*E. coli*	[60]
GhoT/GhoS	V	GhoT	Disrupting the cell membrane	*E. coli, Shigella*	[34]
Retron	/	RcaT	Hydrolyze nucleosides/nucleotides	*Salmonella enterica*	[61]

tantly, the cellular targets of type II antitoxins are often the master regulators of the bacterial stress response. In *E. coli*, the stationary phase sigma factor RpoS controls up to 500 genes. We found that the antitoxin MqsA of type II TA MqsRA directly regulates RpoS by binding to an MqsA-specific palindrome [62]. Evidence has demonstrated that the type II antitoxin HigA in the HigBA TA system in *Pseudomonas aeruginosa* binds to the promoter of the virulence-related sigma factor MvfR to regulate virulence [63,64]. Moreover, our recent study found that the antitoxin PrpA in the PrpTA system directly binds to iterons in the plasmid origin, which could hinder binding of the Rep protein to the iterons, leading to a reduction in the plasmid copy number [12]. Furthermore, we have recently proven that antitoxin CrlA by itself can inhibit phage infection, and potential binding sites were observed in phage genomes [65]. Collectively, antitoxins can serve as flexible regulators in regulating gene expression by recognizing specific sequences in their promoter regions.

4. Applications of TA systems

4.1. TA systems in mobile genetic element stabilization

TA systems have been shown to be involved in stabilizing different mobile genetic elements. TA systems were originally discovered as plasmid addiction systems, and their function is to prevent the formation of plasmid-free progeny, as plasmids exhibit a metabolic burden and are easily lost [6,8,80]. A plethora of findings have demonstrated that TA systems contribute to the stability of plasmids. For instance, the PrcA/PrcT system in the pCAR1 plasmid, a typical member of the RES-Xre family, which was cloned into the unstable plasmid pSEVA644 has been demonstrated to enhance pSEVA644 plasmid stability in *P. resinovorans* and *E. coli* strains [81]. The $ParDE^{I}$ TA system in Enterobacteriaceae, a member of the ParDE superfamily, has been found to function in plasmid maintenance with additional functions in promoting persister cell formation and providing antibiotic tolerance [82]. Additionally, the type II TA system, PumAB, which is encoded by the IncP-1 plasmid pUM505 in *P. aeruginosa*, was reported to maintain the stability of the pJET plasmid under nonselective conditions in *E. coli* [83]. We recently also described three TA systems, including the VapC/VapB, YoeB/YefM and Orf2769/Orf2770 TA systems in deep-sea *Streptomyces* sp. SCSIO 02,999 significantly increased pCA24N plasmid maintenance in *E. coli* [84,85].

Plasmid instability is a major problem for the large-scale industrial production of proteins in bacterial hosts. Applications of TA systems in large-scale industrial fermentation consist of maintaining the existence and replication of plasmids in enzyme production. The *hok/sok* TA system has been well demonstrated to stabilize the highly unstable pUC derivative pMJR1750 in large-scale industrial fermentation [86]. The most obvious advantage indicated by this research is that there is no need to sustainably add antibiotics to maintain the existence of plasmids in bacterial culture processes, which reduces pollution from antibiotics to the environment and saves production costs.

TA systems also stabilize other mobile genetic elements. $ParE_{SO}/CopA_{SO}$, a type II TA system, is critically important in stabilizing circular CP4So prophages after their excision in *Shewanella onidensis* [15]. Likewise, we also found that the PfiT toxin in the type II PfiT/PfiA system contributes to phage stabilization [87]. Deletion of the PfiT toxin activated the expression of the replication initiation factor gene PA0727 and greatly increased Pf4 phage production. Additionally, a widely distributed TA system, the SgiAT system, is encoded by an integrative mobilizable element called *Salmonella* Genomic Island 1 (GI1) and plays a vital role in stabilizing the GI [17]. Similarly, the HipAB TA system encoded by GI21 can stabilize the composite GI48 in *Shewanella putrefaciens* CN-32 [88]. In addition, an integrative and conjugative element (ICE), SXT, has been found that was maintained by a newly identified TA pair MosAT, conferring maintenance of antibiotic resistance [16]. Overall, TA systems are potential tools especially for stabilizing plasmids and other mobile genetic elements in synthetic biology.

4.2. TA systems in DNA cloning

Currently, plasmid-encoded TA systems have been shown to be crucial and promising tools for positive selection in bacterial DNA cloning and protein expression in synthetic biology and clinical trials. The CcdB toxin of CcdB/CcdA from the F-plasmid was inserted into vectors for positive selection (Fig. 2A) [89,90]. Clones with inserted foreign DNA that lead to toxin inactivation have been proven to be a successful strategy in DNA cloning.

Table 2
Antitoxins regulate host genes by binding to DNA or RNA sequences similar to those of TA pairs or by directly binding to host proteins.

TA pair	TA type	Antitoxins	Regulated genes and sequences	Organisms	Source
MqsRA	II	MqsA	*mqsRA*, TAACCTTTAGGTTA and ACCTTTTAGGT	*E. coli*	[66,67]
			rpoS, AACCTTGCAGGTT		[62]
			csgD, AACCTTAAGGTT		[68]
			binds to *cspD, bssR, spy, mcbR* promoter regions		[69]
MqsRA	II	MqsA	*mqsRA*, TTAACCTGGATCACAGC	*Pseudomonas putida*	[70]
			algU(*rpoE*), ACCTGCCAGGT		[70]
			PP_3288, ACCTCAGAGGT		[70]
			nadB, ACCTGGCAGGT		[70]
HigBA	II	HigA	*higBA*, TTAACGTTAA	*Pseudomonas aeruginosa*	[63, 71-73]
			mvfR, TTAACGTTAA		[63]
			pelA, TTGACGTTAA		[63,72]
			clpP2, TTAACTGTTAA		[63]
			cysC, GTTAACTTAAC		[63]
			chtA, TAACGTTA		[72]
			nadA, TTAACGTTAA		[72]
			fpvG, TACCGTTA		[72]
			hexR, TGACGTTA		[72]
			dctA, TAACGCTA		[72]
			pslO, TACCGTTA		[72]
			cntO, TAGCGTTA		[72]
			pa2440, TAGCGTTA		[72]
YafQ/DinJ	II	DinJ	*dinJ-yafQ*, CTGAATAAATATACAG		[74]
			cspE, TACTG$(TA)_5$CAGTA		[75]
PrpTA	II	PrpA	*prpAT*, GTCATGGTAGTTTGTAATGATATGTCTTAT *ori*, TGTAAGTATTTGAAATATATAGA and CGTGTAGGTTTGTAATACGCTAT	*Pseudoalteromonas rubra*	[12]
ParEso/CopAso	II	CopAso	*parEso-copAso*, GTATTACCTAGTAGTAC		[15]
			pemKso-pemIso GTATTACAATGTAATAC		[15]
RalRA	I	RalA	*ralR*, AAGUGAAAAAGAAGCA	*E. coli*	[37]
			rnb, CACAGUGAAAAAGAACGUGAAUC and AAUCUGGAAAAAGAAGCACCAGA		[37]
			rnE, ACCGAAUUAAAAGAAGCACUGGC		[37]
			recE, GCGUCCGUAAAAGAAGCACCAAU		[37]
			ygcH, CCGUUAAUAAAAGAAGCAGAACA		[37]
SprG1/ SprF1	I	SprF1	binds ribosomes to attenuate translation and promotes persister cell formation	*Staphylococcus aureus*	[76]
CbtA(YeeV)/ CbeA(YeeU)	IV	CbeA	enhances the bundling of cytoskeletal polymers of MreB and FtsZ via direct protein-protein interaction	*E. coli*	[33]
CptA (YgfX)/CptB(YgfY)	IV	CptB	enhances the bundling of cytoskeletal polymers of MreB and FtsZ via direct protein-protein interaction	*E. coli*	[77]
YkfI/YafW	IV	YafW	YkfI binds to FtsZ	*E. coli*	[78]
YpjF/YfjZ	IV	YfjZ	YpjF binds to FtsZ		[78]
ToxSAS/antiToxSAS	IV	antiToxSAS	degrades the molecular product ppGpp and ppApp of ToxSAS toxin	*Cellulomonas marina*	[79]

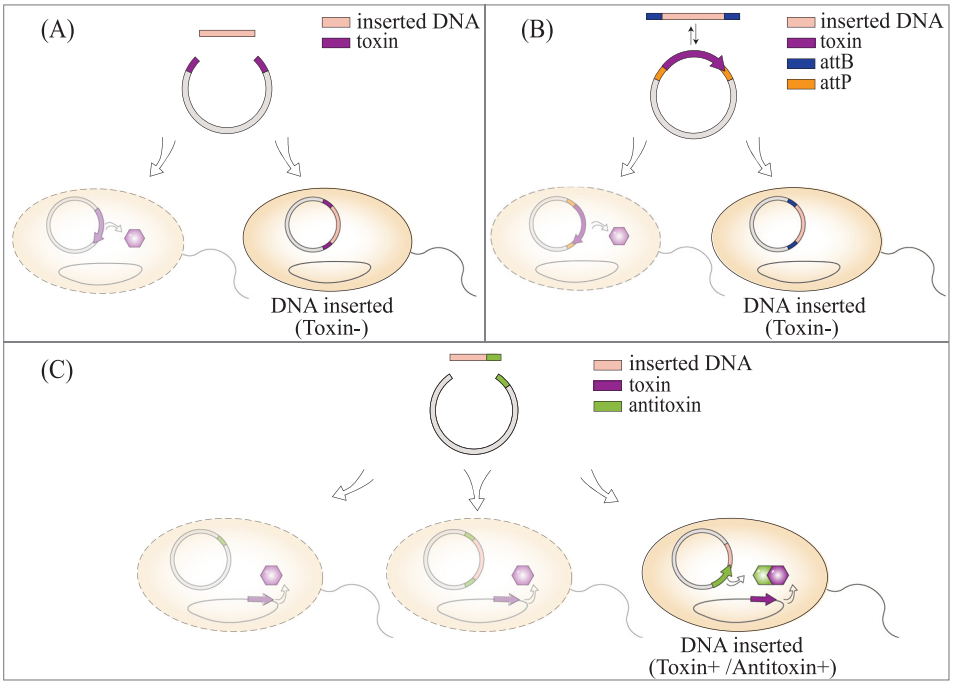

Fig. 2. Bioengineering applications of TA systems in DNA cloning. (A)Toxin disruption-based strategy for positive selection. Type II toxin is inserted into cloning vectors, using for positive selection. (B) Toxin replacement-based strategy for positive selection. Type II toxin is used *in vitro* recombination cloning such as Gateway™ system, using for positive selection. (C) Toxin neutralization-based strategy for positive selection. Toxin is inserted in host chromosome and antitoxin is inserted in the cloning vector, using for positive selection.

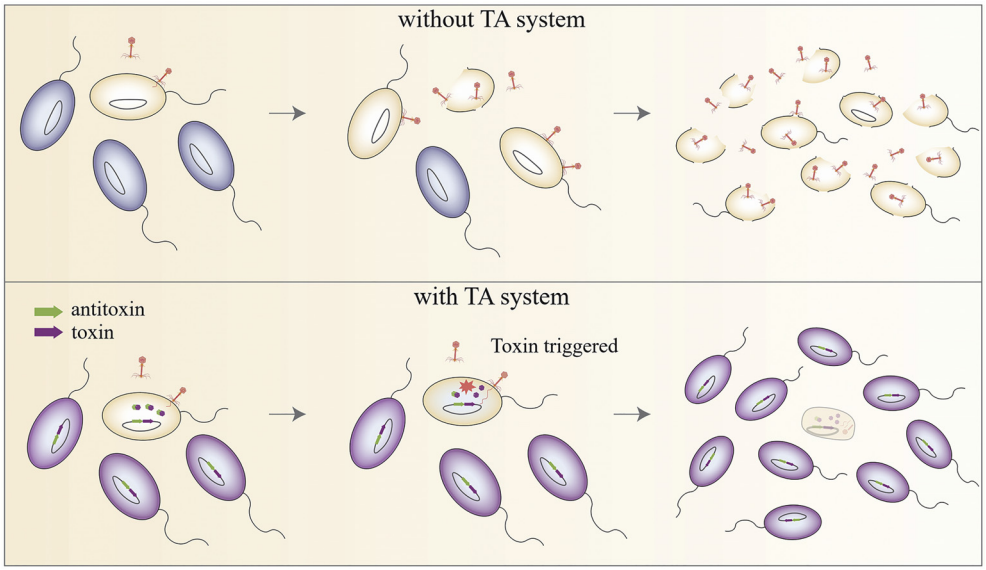

Fig. 3. Integration of TA systems into industrial engineering bacterial chromosomes to construct phage-resistant cells. Host cells without TA system are quickly lysed upon phage infection **(Upper panel)**. Individual cells with TA system integrated can activate toxin production when sensing phage attack to inhibit phage propagation, providing anti-phage protection at the population level **(Lower panel)**.

The second DNA cloning technology, the Gateway™ system, based on the phage λ recombinant system and TA system, was also proposed for use in the positive selection of plasmids (Fig. 2B) [90]. Basically, the constructed plasmid contains the *ccdB* toxin gene between the *attP1* and *attP2* sites. The target gene flanked by *attB1* and *attB2* was then introduced into the constructed plasmid through *in vitro* recombination, and this reaction contains integration host factor and λ integrase from *E. coli*. Cells harboring the plasmid in which the *ccdB* gene was successfully replaced by inserted foreign DNA formed colonies.

In addition, the CcdA/CcdB module was applied to the StabyCloning™ system introduced by Delphi Genetics (Fig. 2C) [90,91]. In this technology, host cells contain the *ccdB* toxin gene in their genomes, and a truncated inactive CcdA antitoxin is contained in the linearized vector. When a 14 bp sequence that is attached to the 5′-end of the DNA fragment is correctly inserted into the vector, the truncated antitoxin restores an active CcdA antitoxin to neutralize the toxin. Consequently, this system will positively select recombinant plasmids that are free of antibiotic resistance genes, and only clones containing a vector with the target gene in the correct orientation can survive. Notably, this technology also significantly enhances the stability of the plasmid. Therefore, due to the particularly efficient stabilization and avoidance of using antibiotics, this technology has a promising future in DNA cloning. Recently, this method has been successfully utilized in clinical trials to produce safe and efficacious DNA vaccines without antibiotic resistance genes against pseudorabies [92]. Taken together, the above three techniques can be utilized in DNA cloning and have wide prospects in the gene therapy field.

4.3. TA systems as phage defense elements in the fermentation industry

Phage contamination is a frequent and persistent threat in industrial fermentation processes and leads to low-quality products and even causes failure of the entire fermentation process [93]. Several studies have reported that phage infections are prevalent in industrial fermentations, which include the production of cheese [94], milk [95], cucumber by lactic acid bacteria (LAB) [96], and acetone-butanol-ethanol by *Clostridium saccharoperbutylacetonicum* [97]. Currently, the commonly used methods to eliminate phage contamination in the fermentation industry consist of rotating bacterial strains and adding phage inhibitors.

Engineering bacterial strains with antiphage elements can provide a different line of defense against phage infection. The primary role of TA systems is to provide antiphage defenses [26,98]. The mechanism of most TA system defenses against phage infection is known as abortive infection (Abi) [99]. When phages infect bacteria, the toxins of TA systems are triggered and then induce cell death after infection but before the phage replication cycle is complete, which thereby prevents phages from spreading to nearby cells and protects uninfected bacteria. Several TA systems have been found to protect cells by preventing phage infection, including Hok/Sok [100], MazEF [101], RnlAB [102], ToxIN [103], DarTG [104], retron-based TA systems [105], CapRel^{SJ46} fused TA systems [106] and kinase-kinase-phosphatase (KKP)-based TA systems [107], and most of them have been shown to provide phage defense via Abi.

A recent study engineered *E. coli* K-12 strains in which the SspBCDE phage defense system was integrated into the genome, which can confer high levels of phage resistance during industrial fermentation processes [108]. Based on above evidences, it is an effective way to integrate and design high-efficiency TA antiphage elements into bacterial chromosomes by using synthetic biology to construct phage-resistant industrial engineering bacteria (Fig. 3). However, evolution of escaper phage mutants that overcome individual phage defense systems is common, and the antiphage efficiencies of the engineered strains will decrease during the fermentation process. A feasible way to solve this problem is to combine TA systems with other phage defense systems that prevent different types of phages, such as CRISPR–Cas [109], restriction-modification (R-M) systems [110] and a series of recently identified antiphage elements into the genomes of industrial bacteria [111–113], which can theoretically exert broad-spectrum antiphage effects to resist phages that are ubiquitous in production environments.

4.4. TA systems as new drugs

Since TA systems can lead to cell death when the toxin is activated, they can be utilized in the development of new antimicrobial agents [90,114,115]. The strategy of using TA systems as novel antimicrobial agents relies on tunable toxin activation, involving TA complex disruption, activation of cellular proteases that degrade antitoxins, or translation inhibition of antitoxin expressions [116]. Previous studies have shown that designed peptides that mimic binding of the TA complex are designed to disrupt the TA complex and thus release toxins [117–120]. Another alternative strategy is to induce the expressions of proteases that can degrade antitoxins, *e.g.*, overproducing Lon protease can cause cell death by degrading YefM and releasing YoeB toxin to degrade mRNAs for the YoeB/YefM TA pair [121]. Another different strategy is to design antisense RNA that can bind to antitoxin mRNA to hinder antitoxin translation [122].

Although TA systems are mostly found in prokaryotes, considering the biochemical function of toxins such as RNA cleavages, toxins may also be used to selectively eliminate target cells in eukaryotes. Encouragingly, some type II TA systems have been shown to trigger apoptosis in human cells and are being considered for applications in molecular biology and medicine [123,124]. For instance, bacterial TA systems MazF/MazE TA can be designed to eliminate cells that are infected by human immunodeficiency virus (HIV-1) and hepatitis C virus (HCV) based on the endoribonuclease activity of MazF in sequence-specific manners. The main strategy of MazF/MazE system as an antiviral tool is based on the principle that MazF fused with a C-terminal unstructured peptide from MazE by using a viral protease-specific cleavage site as a linker. The linker would be effectively cleaved when HIV-1 and HCV infected cells, resulting in release of the MazF toxin to kill HIV-1- and HCV-infected cells [125,126]. Another alternative strategy is to construct MazF under the control of the HIV-1 LTR (long terminal repeat) promoter and transduce it by self-inactivating retroviral vectors in the genome of CD4+ T lymphocytes. Upon HIV-1 infection, MazF was induced to degrade the infecting HIV-1 mRNA and ultimately completely suppressed HIV-1 proliferation but did not inhibit cell growth [127]. Studies also indicated that the MazF ribonuclease in the MazF/MazE TA system was specific and effective in inhibiting tumor growth *in vivo* and induced selective cell death of lung, colorectal and pancreatic cancer cells based on the adenovirus delivery system [128].

Future uses of TA systems to treat pathogens are promising; however, challenges remain due to the strong interactions of toxins and antitoxins and the possible attenuation of toxin activity after interference. In recent years, the role of TA system in phage defense has been elucidated, and several groups found that phage protein can trigger the toxicity of the TA systems. For instance, the newly synthesized phage major capsid protein directly binds to the antitoxin of the fused TA system CapRel^{SJ46} which liberates toxin component [106], and the phage protein YopM binds to the antitoxin of the MazF/MazE TA system which can also activate toxin activity [129]. Although many studies have shown that TA systems play a vital role in eukaryotic disease-related therapy, one limitation is the failure of the adenovirus delivery system in the above strategies since a majority of people have antibodies against adenoviruses. Therefore, developing novel delivery methods for TA systems in disease-related therapy is needed. These new findings provide feasible strategies to activate the toxin of the TA systems in a controllable manner, leading to cell death in prokaryotic and eukaryotic cells.

5. Future perspective

In this review, we summarized that different TA systems present diverse biological functions and applications in mobile genetic element maintenance, DNA cloning, phage resistance, and as new drugs. However, most TA system applications in synthetic biology are immature and are still under development.

Extensive sequencing and comparative analysis comparisons reveal a very broad presence of TA systems in both bacteria and archaea. TA functions in archaea are mostly uncharted. Recent studies have shown that TA systems in prokaryotes act as ancient antiviral immunity elements, and several toxins, such as HepT toxins, harbor active domains that are also conserved in eukaryotic cells. These findings are not only expected to stimulate research in archaea that could shed light on diverse cellular processes regulated by TA systems across different life domains but could also provide important insights into TA applications in various fields.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given her role as Editorial Board Member, Dr. Xiaoxue Wang, had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Dr. Qunxin She.

Acknowledgments

This work was supported by the National Science Foundation of China (31970037, 42188102, 91951203, 31625001, 32100030), Science & Technology Fundamental Resources Investigation Program (2022FY100600), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2019BT02Y262), Guangdong Major Project of Basic and Applied Basic Research (2019B030302004), Guangdong Basic and Applied Basic Research Foundation (2022A1515010702) and the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0407).

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