ISSN 0003-6838, Applied Biochemistry and Microbiology, 2017, Vol. 53, No. 5, pp. 494–505. © Pleiades Publishing, Inc., 2017.
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Bacterial Toxin–Antitoxin Systems: Properties, Functional Significance, and Possibility of Use (Review)

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Received March 15, 2017

Abstract—Toxin–antitoxin systems are genetic modules usually consisting of two genes encoding a stable toxin and labile antidote (antitoxin). These systems are localized on plasmids, phages, and chromosomes and are widespread in bacteria and archaea. The review summarizes recent data regarding the classifications of toxin–antitoxin systems, their mechanisms of action and toxin targets, as well as their functional significance for bacterial cells and possibility of use.

Keywords: toxin–antitoxin systems, toxin targets, persistence, biofilms, bacterial pathogenicity

DOI: 10.1134/S0003683817050076

INTRODUCTION

Toxin–antitoxin systems (TA systems) are genetic modules consisting of two or, more rarely, three genes. The toxins of all known TA systems are stable proteins, whereas antitoxins are either unstable proteins or non-coding small RNA (sRNA). Under normal conditions toxin activity is neutralized by antitoxin, usually by direct interaction and the formation of the toxin–antitoxin complex. Change in the external conditions leads to a change in the rates of transcription/translation of the module, which results in a relative increase in the toxin content due to the difference in the stability of toxin and antitoxin. This leads either to cell death or to entry into a dormant state in which cells remain viable but are not capable of reproduction, i.e., they enter into a persistent state.

The first TA systems were characterized in 1980 as genetic systems that encode a plasmid and provide its stability during replication. The first TA system was detected on the F plasmid of *Escherichia coli* and was a system controlling cell death (**Ccd**) [1]. This system provided stabilization of F factor and caused the death of cells that did not receive the plasmid [2]. The system includes two genes, *ccd*A and *ccd*B, which are organized in the operon. CcdB protein is a toxin inhibiting DNA gyrase, whereas CcdA is antitoxin preventing lethal action of CcdB by direct binding to the toxin [3]. If the plasmid was not inherited during cell division, short-lived antitoxin CcdA was not replenished by de novo synthesis, whereas the more stable toxin CcdB remained in the cytoplasm causing cell death [4]. This phenomenon was revealed in case of other plasmids and called "post-segregational killing" (**PSK**) [5, 6].

TA systems localized in chromosomes were later found. Novel bioinformatics approaches, including a homology search for nucleotide and amino acid sequences in the databases, made it possible to reveal a large number (more than 10000) [7] and diversity of putative TA systems in bacteria and archaea [8–11], whereas TA systems were not detected in eukaryotes. However, separate homologs of toxin genes were revealed in fungi [12]. Bacterial genomes may contains dozens of genes of TA systems. About 80 putative TA systems were found in *Mycobacterium tuberculosis*, whereas cyanobacteria genomes contain more than 70 TA systems. Up to 2.5% of identified open reading frames in the genomes of proteobacteria may be TA systems [10, 13, 14]. In the same species, plasmids and chromosomes usually contain different TA systems [15]. Bioinformatics analysis suggests that the number of chromosomal TA systems is almost an order of magnitude greater than plasmid systems [16]. At the same time, the functions of TA systems on plasmids are obvious, whereas the biological significance of chromosomal systems was unclear for a long time and only some of their putative functions have been recently determined experimentally.

The data obtained in the study of TA systems greatly expanded the understanding of regulation of bacterial gene activity, bacterial stress response, persistent state of bacterial cells and apoptosis. TA modules are considered promising "targets" for antibacterial preparations, and it is proposed to use their potential against viral infections and oncological diseases. The number of publications regarding TA systems, which includes many reviews, reaches hundreds of articles annually [7, 12, 16–23]. There are informational resources on TA

systems: Rasta-Bacteria is a website providing the identification of TA systems in genomes (http://genoweb1.irisa.fr/duals/RASTA-Bacteria/) [24]; TADB is a database of loci of type II toxin–antitoxin systems from bacteria and archaea http://202.120.12.135/TADB2/index.php [25]; BtoxDB is a database on the structure of proteins of TA systems (http://www.gurupi.uft.edu.br/btoxdb/) [26].

TA SYSTEMS: GENERAL CHARACTERISTICS AND CLASSIFICATION

TA classification is based on the nature of the antitoxin and the mechanisms of its inhibitory action. TA modules are divided into six main types [12]. The systems belonging to types I and II were discovered long ago [1, 27], whereas systems belonging to the other types were identified later. In systems of types I and III, antitoxin is sRNA, whereas antitoxins of the other classes are small proteins. The types of TA systems significantly differ in the mechanism of action of toxins, number, and evolution. The types are divided into families based on the homology of nucleotide and amino acid sequences of TA systems genes/proteins. More than 50 families exist.

Type I Toxin-Antitoxin Systems

The first known systems of this type was revealed for the first time on plasmids and their activity was associated with PSK [27]. Type I is a system in which the toxin is a hydrophobic protein, whereas the antitoxin is an sRNA (50–200 nucleotides) that inhibits toxin expression [28].

In type I TA systems, the interaction between toxin and antitoxin occurs by complementary base pairing of toxin mRNA and antitoxin sRNA, which are localized on opposite DNA strands [29]. Toxin and antitoxin genes usually overlap one another.

An overlapping fragment can involve Shine-Dalgarno sequence (SD-sequence) (for example, SymE/SymR in *E. coli*) [30] or 3' ends of the genes (for example, TxpA/RatA in *Bacillus sibtilis*) [31]. More rarely, toxin and antitoxin genes are located at a distance from each other (for example, TisB/IstRI in *E. coli*) [32]. Antitoxin RNA inhibits translation or promotes the destruction of toxin mRNA (Fig. 1a).

Type I systems can include a third component. The TA system Hok-Sok of *E. coli* RI plasmid contain a third gene, called *mok*, in addition to the genes of the toxin (*hok*) and antitoxin (*sok*). The open reading frame (ORF) of the *mok* gene overlaps the ORF of the *hok* gene and is required for its translation [6]. The antitoxin Sok regulates Hok translation indirectly by inhibition of Mok protein translation [28].

Homologs of type I TA systems were detected in silico in the genomes of many gram-positive and gram-negative bacteria [9] and 18 families of type I TA

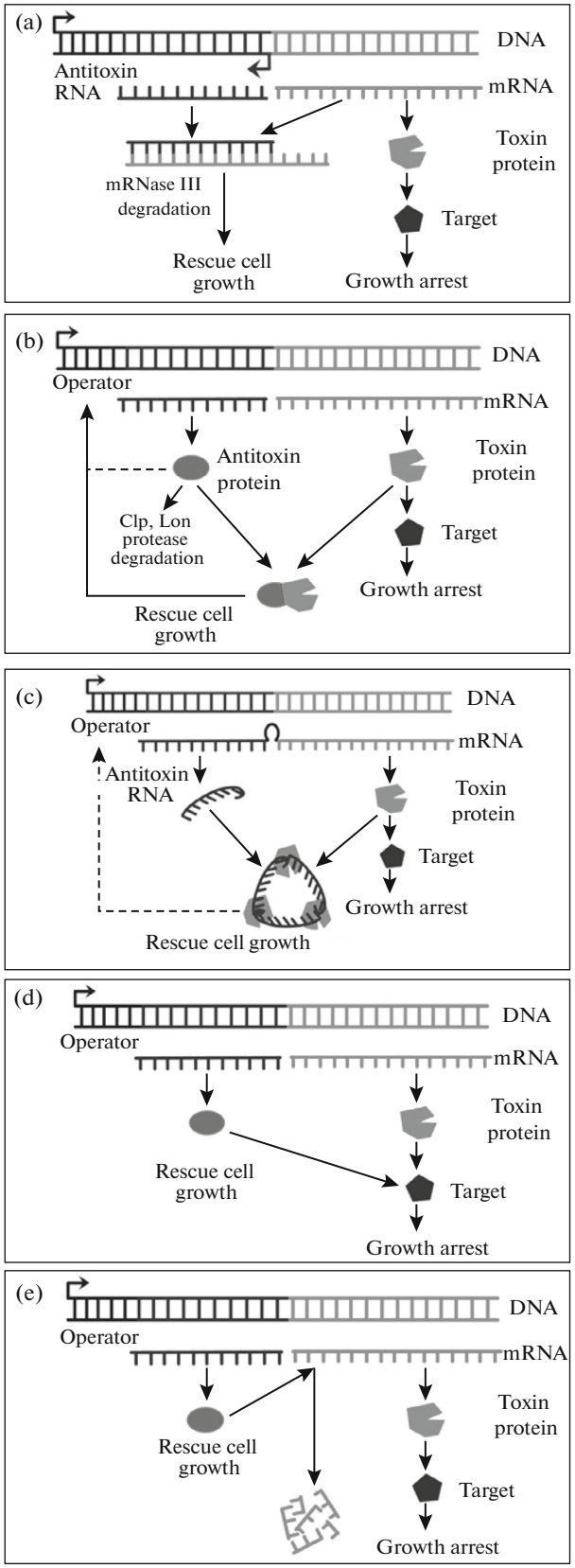

Fig. 1. Organization of types I (a), II (b), III (c), IV (d), and V (e) TA Systems [70].

systems were identified [22]. Chromosomes can contain many copies of type I TA systems. For example, the *E. coli* chromosome contains from 4 to 15 copies of the *hok* gene. The evolution of type I TA systems probably occurred by duplication but not horizontal transfer [28].

Type II Toxin-Antitoxin Systems

Type II TA systems are the most studied and are the most numerous. The toxin and antitoxin are proteins. Their genes are organized into an operon and have a common promoter. The operon of a type II TA system is characterized by a small size both of genes (80–630 bp) and include a small fragment, which either separates these genes or forms the region where the genes can overlap (from –20 to +30 nucleotides) [8]. The first gene in the operon usually encodes antitoxin; however, exceptions to the standard operon organization are known. For example, TA module HigB/HigA of *Proteus vulgaris* Rts1 plasmid, the *higB* toxin gene is located upstream of the *higA* antitoxin gene [33]. Interaction between the toxin and antitoxin lead to the formation of an inactive toxin–antitoxin complex (protein–protein) in which the antitoxin adopts a compact structure [34] (Fig. 1b). The antitoxin and toxin-antitoxin complex is responsible for negative self-regulation of the operon [20]. The TA operon can contain several promoters. For example, two promoters of different strengths were found at the beginning of *mazEF* operon in *E. coli* [35] and *yefM-yoeB*$_{Spn}$ operon in *Streptococcus pneumoniae* [36]; the *axe-txe* operon of pRUM plasmid in *Enterococcus faecium* contains an additional promoter in the toxin gene [37], whereas the *yefM-yoeB*$_{Lrh}$ operon in *Lactobacillus rhamnosus* contains additional transcription start sites in the antitoxin gene and sRNA, which presumably regulates the activity of the operon [38]. Perhaps, different promoters are activated and regulate expression of TA genes under different conditions.

Changes in the conditions of the existence of the cell (for example, stress conditions, such as low or high temperatures, antibiotics, thymine starvation, changes in pH and salinity, and phage infection) lead to antitoxin destruction by cell proteases (Lon and Clp), the activation of operon transcription, the accumulation of free toxin, and inhibition of growth and death of the cell [39–41].

Type II antitoxins are small unstable proteins consisting of two domains: DNA-binding N-terminal domain and C-terminal domain binding toxin [42, 43]. On the contrary, DNA-binding and toxin-binding domains of MqsA antitoxin are located in the C-terminus and N-terminus of the protein, respectively [44]. In most cases, the structure of the DNA-binding domain can belong to one of three types: helix-turn-helix (HTH), ribbon-helix-helix (RHH), and SpoVT/AbrB-like domain [23]. PaaA antitoxin of *E. coli* O157:H7 and epsilon antitoxin of *Streptococcus pyogenes* pSM19035 plasmid do not possess DNA-binding domain. These TA systems contain a third component, transcriptional regulator, in addition to the toxin and antitoxin. The TA module of pSM19035 plasmid consists of three components, ω-ε-ζ. In contrast to other type II TA systems, neither ζ toxin nor ε antitoxin or the ζ2ε2 complex regulate operon expression. The promoter activity of the Pω operon is regulated by ω2 dimer, a global transcriptional regulator [45]. The first gene of the *paaR-paaA-paaE* operon is required for the control of TA module transcription in *E. coli* O157:H7. However, in contrast to ω-ε-ζ, TA complex PaaE-PaaA is also involved in the regulation of its own transcription, although its effect is much weaker than that of ParR. In the PasA/PasB/PasC system of the pTF-FC2 plasmid of *Thiobacillus ferrooxidans*, a third component, PasC, is not involved in the regulation of operon expression but provides a toxin–antitoxin complex [46]. In *Mycobacterium tuberculosis*, the TA operon HigA1/HigB1 (Rv1955-Rv1956) contains the gene of the chaperone Rv1957. The chaperone interacts with the antitoxin, which prevents its destruction by the proteases and facilitates its folding and interaction with the toxin [47].

In contrast to other type II toxins, the toxin PhoH2 of *M. tuberculosis* and *M. smegmatis* has two domains: N-terminal PIN-domain typical of VapC toxins and C-terminal PhoH RNA-helicase domain. The molecular weight of the protein is about 50 kDa and exceeds the mass of other toxins of TA systems [48]. A novel type of TA system organization was described for the EzeT system of *E.coli*. The system was represented by a single protein, the C-terminal domain of which determines toxicity and is homologous to zeta toxin, whereas its N-terminal domain inhibits toxin activity and acts as an antitoxin [49].

Interaction between the toxin and antitoxin is usually highly specific, i.e., the toxin interacts only with its related antitoxin. This specificity can be violated by a single mutation. For example, the replacement of one amino acid residue in the Txe protein of the *Enterococcus faecium* toxin makes possible its interaction with an unrelated YefM antitoxin [50]. Cross-interactions between the components of different TA modules RelBE, MazEF, and MazE-VapC in *M. tuberculosis* were described as an exception [51–52]. The type II toxin SpoIISA of *Bacillus cereus* and *B. subtilis* can be neutralized by two different antitoxins, SpoIISB and SpoIISC. Each of the three genes possesses its own promoter and is transcribed under different conditions [53].

Type II TA systems are divided into 8–14 families based on the similarity in the amino acid sequence of toxins and antitoxins. The MazE/MazF, RelB/RelE, HipA/HipB, VapB/VapC, omega-epsilon-zeta, and ParE/ParD systems are the best known. Related families are combined into superfamilies. For example,

CcdB/CcdA, MazE/MazF, and Kid/Kis are combined into the same superfamily [8, 54]. Initially, it was assumed that the toxin in each family interacts with a specific antitoxin. However, hybrid systems, in which the TA locus contains toxin and antitoxin from different families, have been discovered [55–57]. Therefore, type II TA systems are also divided into 13 superfamilies based on similarities in amino acid sequences and the tertiary structure of the proteins and into 20 superfamilies based on antitoxins, in addition to the division into families [10, 19]. Four superfamilies of single toxins, which inhibit *E. coli* growth, were also identified. Nevertheless, antitoxins blocking their action have not been revealed [10]. The homology of nucleotide/amino acid sequences does not always determine the mechanisms of their functional activity. For example, no similarities were found between type II systems CcdAB and ParDE belonging to different superfamilies. Nevertheless, these systems have the same target, DNA gyrase. The toxins of the systems CcdAB and Kis/Kid are very similar in the structure; however, the first toxin inhibits activity of DNA gyrase, whereas the second is mRNA interferase [58]. Similarities between toxins/antitoxins of different types of TA systems were also found. For example, the type II MazF toxin is similar to the type III ToxN toxin of the ToxI/ToxN system in their 3D structure [59].

Type II systems are numerous and diverse; however, their number in each bacterial species can vary. It can be assumed that *M. tuberculosis* possesses about 80 TA systems. More than 60 systems have been tested to inhibit bacterial growth when toxins were expressed and restore the growth after neutralization of the toxins by the antitoxins. Functional activity was demonstrated for 37 systems. Most of the systems belonged to VapBC (50 systems) and MazEF (10 systems) families, whereas others belonged to YefM/YoeB, RelBE, HigBA, and ParDE families [14]. Ten putative TA systems were revealed in silico in the genomes of *Streptococcus pneumonie*, and four of them were shown to be active [60]. Five type II TA systems were identified in *Staphylococcus aureus,* and four of them were studied [61]. Six TA systems were found in *L. rhamnosus,* and three of them were shown to be active [57]. *Bifidobacterium longum* was detected seven TA systems [62]. It should be noted that, if the functional activity of TA systems have not been detected under the considered experimental conditions, it was not proof that this TA system does not function.

It is assumed the evolution of type II systems occurred mainly by horizontal transfer [10, 16].

Type III Toxin-Antitoxin Systems

The first known type III system, ToxI/ToxN, was found in the pECA1039 plasmid of the plant pathogen *Pectobacterium atrosepticum*. It was initially described as a bacterial defense system against bacteriophage infection [63]. Similar to those of type I, type II antitoxins are sRNA; however, interaction with the toxins occurs by different mechanisms. A motif consisting of 5.5 direct tandem repeats of a 36 nucleotide motif, a short palindromic sequence, and the gene of the toxin *tox*N are located downstream of the promoter of the operon. Tandem direct repeats are the *tox*I gene. An inverted repeat is the transcriptional terminator and regulates the relative amount of the transcripts of sRNA antitoxin and mRNA toxin mRNA. Toxin protein is neutralized by sRNA antitoxin by direct contact and the formation of an RNA-protein complex. Study of the crystalline structure of the ToxIN complex revealed a heterohexamer formation consisting of three ToxN protein molecules and three ToxI RNA molecules (Fig. 1c) [64]. In addition to ToxI/ToxN, another type III TA system, AbiQ, whih is located on the plasmid of *Lactococcus*, was studied [65]. Both TA systems provide resistance to phages and are components of the modules of abortive phage infection.

Phylogenetic analysis of 125 type III systems identified in silico revealed the existence of three independent families ToxIN, CptIN, and TenpIN. Most of these putative type III systems are located on chromosomes, and about 15% of ToxIN and TenpIN systems are located on the plasmids, whereas one ToxIN system was found in the prophage genome. The functioning of some systems was studied in *E. coli* by evaluation of the toxicity of putative toxicin proteins and the capability of related antitoxin repeats to inhibit lethal effect [59]. The evolution and distribution of type III systems occurred by horizontal transfer [16, 59].

Type IV Toxin–Antitoxin systems

TA systems of this type are not numerous. The *E. coli yee*U/*yee*V module (also designated as *cbt*A/*cbt*B) belonged to type IV TA systems. In other TA types, toxins and antitoxins interact at the RNA or protein levels, whereas type IV toxin and antitoxin do not interact directly (Fig. 1d). YeeU antitoxin counteracts YeeV and stabilizes toxin targets, MreB and FtsZ polymers [66]. A similar model of interaction was demonstrated for *cpt*A/*cpt*B (*ygf*X/*ygf*Y), another TA module in *E. coli* [67].

Type V Toxin-Antitoxin systems

Only one system, GhoS/GhoT detected in *E. coli,* belonged to type V TA systems. GhoS antitoxin interacts with the mRNA of the toxin but not with the protein. GhoS antitoxin possesses specific endoribonuclease activity and cleaves the mRNA of GhoT toxin, preventing its translation. GhoS antitoxin is a stable protein unable to bind the promoter of its operon (Fig. 1e) [68].

Type VI Toxin-Antitoxin Systems

Only type I–III systems are widespread and numerous; type IV systems are not numerous, and

Table 1. Mechanisms of action and targets of TA system toxins

Toxin	Antitoxin	Type of TA system	Toxin mechanism of action	Target process in cell	Reference
Hok TisB	Sok/RNA IstR-1/RNA	I	Integration into the intracellular membrane	ATP synthesis	[27, 71]
SymE	SymR/RNA	I	mRNA cleavage	Translation	[30]
RalR	RalA/RNA	I	DNA cleavage	Transcription? DNA replication?	[72]
MazF	MazE/Protein	II	Cleavage of ribosome-independent mRNA as well as 23S and 16S rRNA	Translation	[73–75]
Kid HicA	Kis/Protein HicB/Protein	II	Cleavage of ribosome-independent mRNA	Translation	[76, 77]
RelE	RelB/Protein	II	Cleavage of ribosome-independent mRNA	Translation	[78]
VapC	VapB/Protein	II	Site-specific cleavage of mRNA, tRNA, and rRNA	Translation	[79, 80, 96]
PhoH2	PhoAT	II	RNA cleavage, RNA-helicase and ATPase activities	Translation	[48]
RatA	RatB/Protein	II	Binding to 50S ribosomal subunit and inhibition of ribosome formation	Translation	[81]
HipA	HipB/Protein	II	Phosphorilation of glutamyl-tRNA synthetase	Translation	[82]
Doc	Phd/Protein	II	Phosphorilation of elongation factor EF-Tu	Translation	[83]
CcdB ParE	CcdA/Protein ParD/Protein	II	Inhibition of DNA gyrase	DNA replication	[84, 85]
Zeta	Epsilon/Protein	II	Phosphorilation of uridine diphosphate-N-acetylglucosamine	Peptidoglycan sythesis	[86]
ToxN, AbiQ	ToxI/RNA RNA	III	Cleavage of RNA	Translation; abortive phage infection	[64, 65]
YeeV CptA	YeeU/Protein CptB/Protein	IV	Inhibition of FtsZ and MreB polymerization	Cytoskeleton formation	[87, 67]
GhoT	GhoS/Protein	V	Integration into the intracellular membrane	ATP synthesis	[68]
SocB	SocA/protein	VI	Inhibition of DnaN activity	DNA replication	[88]

type V system is unique. With the expansion of research, new systems with unique properties are found. For example, SocAB system, in which antitoxin protein acts as an adaptor for ClpXP protease degrading SocB toxin, was found in *Caulobacter crescentus*. The system belonged to the novel type VI [69]. However, this type of TA system is not yet commonly accepted due to its uniqueness.

MECHANISMS OF ACTION AND TARGETS OF TA SYSTEMS

A description of the cell target affected by toxins is essential for an understanding of the significance of TA systems for bacteria. The main targets of the toxins are listed in Table 1.

Most type I systems encode a small hydrophobic protein, which acts as phage holin, forming the pores in cell membranes [22, 28]; this leads to the loss of membrane potential and inhibition of ATP synthesis. The toxins SymE (RNAse) and RalR (nonspecific endonuclease cleaving methylated and unmethylated DNA) of *E. coli* are the exceptions [30, 72]. Destruction of the membrane and inhibition of ATP synthesis are also caused by GhoT, a type V toxin of the GhoS/GhoT system [68].

Targets of type II toxins are more diverse. More often these toxins are ribonucleases (mRNA inter-

feras) that cleave either free mRNA or mRNA bound to the ribosomes. MazF [73], Kid [76], ChpBK [89], MqsR [90], and HicA [77] are typical examples of toxins cleaving free mRNA. HicA toxin does not have a specific consensus of recognizable motifs, whereas Kid toxin demonstrates a preference for UA(A/C) sites. ChpBK and MqsR specifically cleave at UAC and GCU sequences, respectively. The MazF toxin of *E. coli* cleaves mRNA at ACA sites, whereas MazF toxins of other microorganisms recognize motifs consisting of 3, 5, and 7 nucleotides, which often contain ACA sequence [91]. VapC toxins of *M. tuberculosis* interact specifically with mRNA but are also able to cleave tRNA [79]. RelE is the most fully characterized toxin, cleaving mRNA via a ribozyme-dependent pathway. It interacts with the ribosomal A-site, which leads to mRNA cleavage and inhibition of translation elongation [78]. RelE toxins of other microorganisms act in the same way [92]. YoeB toxin of *E. coli* is another mRNA-dependent interferase that interacts with the 50S ribosomal subunit and blocks translation initiation, cleaving mRNA directly upstream of the initiation codon [93].

ToxN toxin, of type III TA system, also possesses RNAase activity and cleaves toxI/toxN transcript at direct repeats, allowing 36-nucleotide RNA antitoxin to release [64]. Other type III toxins act in the same way. RNA is cleaved at specific sites with a high adenine content [94].

The toxins can also inhibit translation in another way, affecting tRNA and rRNA but not mRNA. For example, VapC toxins of *Salmonella enterica* and pMYSH6000 plasmid of *Shigella flexneri* demonstrate site-specific endoribonuclease activity, interacting with formyl-methionyl-tRNA [96]. VapC-mt4 toxin of M. *tuberculosis*, one of 50 VapC toxins of this bacterial species, cleaves three of 45 tRNA at anticodon loops [95], whereas VapC-mt20 of *M. tuberculosis* cleaves 23S rRNA [96]. MazF-mt6 toxin, one of the MazF toxins of *M. tuberculosis*, cleaves 23S rRNA at the active ribosomal A-site [75]. The MazF-mt3 toxin of *M. tuberculosis* affects two targets: 23S rRNA and anti-SD sequence of 16S rRNA [97]. The MazF-mt9 toxin of *M. tuberculosis* specifically cleaves two tRNA into two parts [98]. The MazF toxin of *E. coli* cuts 43 nucleotides, including the anti-SD sequence from the 3' end of 16S rRNA [74]. Toxins also affect other components of the translational machinery. RatA toxin binds to the 50S ribosomal subunit and prevents its association with the 30S subunit and the formation of 70S ribosome [81]. Doc toxin is a kinase that inactivates elongation factor EF-Tu [83]. HipA toxin is a kinase that phosphorylates and inactivates glutamyl-tRNA synthetase [82].

Toxins can also inhibit DNA replication. ParE and CcdB type II toxins initially found on *E. coli* plasmids and then on the chromosomes of different microorganisms were shown to inhibit the GyrA subunit of DNA gyrase. The mechanisms of action of toxins isolated from different microorganisms differ [84, 85, 99]. SocB toxin belonging to the putative type VI binds to DnaN protein and inhibits elongation of DNA replication [88].

The bacterial cytoskeleton is another target for type II toxins. The zeta toxin of the epsilon-zeta TA system of *S. pneumoniae* possesses kinase activity. Zeta toxin phosphorylates UNAG (UDP-N-acetylglucosamine precursor), and phosphorylated UNAG inhibitis MurA, the enzyme catalyzing the initial step in bacterial peptidoglycan biosynthesis, thereby blocking bacterial cell wall formation [86]. The type IV YeeV toxin of *E. coli* interacts with cytoskeletal proteins FtsZ and MreB, homologs of eukaryotic tubulin and actin. It inhibits GTPase activity and polymerization of FtsZ, as well as ATP-dependent MreB polymerization, which prevents cytoskeleton formation [87]. Another type IV toxin, CptA, possesses similar features [67].

Some antitoxins have targets outside TA operons. For example, the MqsA antitoxin of *E. coli* downregulates expression of the *rpo*S gene, which encodes stress sigma-factor, as well as some other genes, including those involved in biofilm formation [100]. The type II antitoxin DimJ can also regulate the activity of the *rpoS* gene [101]. A bioinformatic search of the palindromes binding to HipB antitoxin suggested that this antitoxin regulates the activity of at least 33 genes possessing various functions [102].

FUNCTIONS OF TA SYSTEMS

The functions of TA systems localized on plasmids are obvious. In most cases, they cause PSK, while they cause a shift into a persistent state in other cases (for example, the CcdA/CcdB TA system of F-plasmid from *E. coli*) [22, 103]. Type III ToxN/ToxI and AbiQ TA systems, which are localized on plasmids, determine resistance to phages (so-called abortive phage infection) [64, 65]. The role of widespread chromosomal TA systems in bacterial cells is more diverse and remains largely unclear.

Separate TA systems localized on chromosomes can perform a function analogous to PSK and provide the resistance of the certain genetic structures. Type I TA systems localized on prophages (for example, TxpA/RatA *B. subtilis*) are required for their preservation on bacterial chromosomes [104]. The introduction of RelBE and ParDE modules from *Vibrio vulnificus* into the *E. coli* chromosome caused stabilization of large DNA fragments [105]. The MosAT TA system on the *V. cholerae* chromosome stabilized an integrative conjugative element [106]. TA systems performing similar functions in the cells of pathogenic microorganisms can stabilize pathogenicity islands and determine microorganism virulence.

Chromosomal TA modules are able to prevent bacteriophage invasion in bacterial cells (for example, RnlAB [107] and MazEF [108] TA systems, which protect *E. coli* fromt T4 phage). Chromosomal TA modules can also prevent PSK caused by homologous TA systems encoded by the plasmid. For example, CcdAB system protects *Erwinia chrysanthemi* from the homologous system of F factor [109].

It was shown that many TA systems are involved in the shift to a persistent state, i.e. in a genetically homogeneous bacterial population, some cells are able to shift into a metabolically inactive state that allows them to survive under stress conditions. This property belongs not to the separate cells but to the whole population, which is divided into two parts (bimodal development) under stress conditions (for example, antibiotics action). One of these parts dies, whereas the other shifts into a persistent state and survives. Superexpression of HipA toxin in *E. coli* leads to an increased number of persistent cells, whereas deletion in *hipAB* operon decreases it drastically [110]. Superexpression of VapC toxin in *Mycobacterium smegmatis* leads to the formation of ovoid nonreplicating cells, whereas superexpression of VapB antitoxin prevents shifting into the "noncultivating" state [111]. The number of persistent cells also decreased due to the deletion of the *mqs*R and *tis*B antitoxins genes in *E. coli* [110, 112]. The SOS-induced type I TA system TisB/IstR-1 of *E. coli* is also involved in the shift into the persistent state [113]. The various TA systems of a bacterial cell make up the total contribution to the persistence state formation [114].

The mechanism by which TA systems are involved in the development of the persistence state has been described recently. It was shown that the MazEF TA system of *E. coli* causes programmed cell death. Short-term sublethal exposure of the dense bacterial suspension ($\geq 10^8$ CFU) to such stress factors as thymine starvation [115], phage infection [116], and treatment with antibiotics [117] led to the death of 90% of the cells, whereas it did not occur in the case of the strains with *maz*EF operon deletion. The cells did not die instantly, and, after the beginning of toxin action, they remained metabolically active. An antitoxin addition was able to stop the process. The cells then reached the "point of no return" [118]. Quorum sensing (QS) peptides and extracellular death factor (EDF), small (5–16 amino acid residues) peptides increasing the endoribonuclease activity of MazF toxin, enhance cell death [119]. MazF toxin does not inhibit protein synthesis completely and about 10% of the proteins are synthesized. These are small (about 20 kDa) proteins that cause the death of the most cells but lead to the survival of some [120].

The mechanisms of selective protein synthesis are associated with the formation of changed translation machinery and stress-ribosomes. MazF toxin simultaneously cuts 43 nucleotides, including the SD-sequence from the 3' end of 16S rRNA and nontranslatable mRNA containing the anti-SD sequence from the 5' ends of selected mRNA at ACA-site [74, 121]. The capacity for canonical mRNA translation is restored by RtcB RNA-ligase [122]. MazF-mt3 toxin from *M. tuberculosis* demonstrated similar activity. It acts as the ribonuclease of 23S rRNA and anti-SD sequence of 16S rRNA [97]. The selective translation caused by MazF toxin is a novel and important mechanism for gene activity regulation. It is involved in the shift into the persistent state [123].

An association of TA systems with biofilm formation was demonstrated [124]; however, the mechanism of this association has not been understood. Biofilms are formed by one or several bacterial species embedded in the extracellular matrix that are attached to biotic or abiotic surfaces. Biofilm is an alternative to the planktonic state of bacterial population. The bacterial cells in biofilms are extremely resistant to various bactericide factors, including antibiotics. Many chronical infections are associated with the ability of pathogenic bacteria (for example, *P. aeruginosa* and *M. tuberculosis*) to form biofilm in the human body. The MqsR/MqsA system of *E. coli* inhibits biofilm formation. Degradation of the MqsA antitoxin results in a drastic increase in the capability of biofilm formation. It can be assumed that MqsA protein represses the activity of the genes involved in the stress response [125]. Decreased biofilm formation in *Shewanella oneidensis* and *E. coli* was caused by inactivation of the toxin *hip*A gene. In this case, it was the result of a decrease in extracellular DNA extraction, which is the basis for adhesion during biofilm formation [126].

An association between the presence of TA systems in pathogenic bacteria and their virulence was repeatedly shown, and a correlation between the number of TA modules and bacterial virulence was demonstrated [127]. For example, it was shown that *M. tuberculosis* contains about 80 putative TA loci [13], whereas the related nonpathogenic bacterium *M. smegmatis* contains only three [8]. The MazEF TA system is widespread on the plasmids of vancomycin-resistant enterococci [128, 129]. Deletion of the VapBC module in *Haemophilus influenza* results in a sharp decrease in virulence, both in cell cultures and experimental animals [130]. In some cases, TA modules stabilize plasmids, resulting in virulence. This was shown for the MvpAT system on the plasmid of *Shigella flexneri* Pmysh6000 [131], the HigBA system on the plasmid of *Proteus vulgaris* Rts1 [132], and the omega-epsilon-zeta system on the pSM19035 plasmid of *S. pyogenes* [133]. Above, we mentioned the association of TA systems with the persistent bacteria state. The persistent state of latent infections, which are resistant to many antibiotics, impedes their treatment. However, toxins can have the opposite effect on virulence. For example, in *S. aureus*, the MazF toxin recognizes a specific 5-nucleotide RNA sequence that is mainly typical to the mRNAs of virulence proteins, including SraP pro-

tein, i.e., activation of the MazF toxin inhibits virulence [134].

As mentioned in the previous section, changes in the transcription and/or translation of antitoxin that result in the destruction of the toxin–antitoxin complex and toxin activation are caused by external stress factors. The transcription of TA systems genes is changed due to external factors (sublethal concentrations of antibiotics, heat shock, and depletion of the nutrient medium) by 1–4 orders of magnitude [135]. The activation of such TA systems as HicAB, HigBA, and YafQ-DinJ suppresses the requirement for alternative sigma factor (σ^E), which affects the stress response in bacterial cells [136]. TA systems belonging to different types can interact with each other. For example, the MazEF system of *E. faecalis* regulates both its own and type I TxpA-RatA system expression [137], *relBE* operon in *E. coli* is activated by the production of MazF, MqsR, HicA, and HipA toxins, and RelE toxin expression in turn activates the *mazEF* operon during amino acid starvation [138]. The MqsRA system controls the activity of the type V GhoST TA system [125]. It can be assumed that the main biological role of the TA system is associated with the general regulatory network, but this process is still poorly understood.

APPLICATION OF TA SYSTEMS

Since TA systems are present in all bacterial genomes and are able to inhibit growth and cause the death of bacterial cells, they were proposed as potential targets and active components for the development of antibacterial drugs. A strategy for the application of TA systems was formulated. Compounds activating toxin by antitoxin complex destruction or prevention of its formation, as well as the activation of cell proteases that cause antitoxin degradation and toxin activation, should possess an antibacterial effect. To provide efficiency, a TA system widespread among pathogenic bacterial strains should be used. Preference should be given to the type II TA systems, since they are the most studied [139, 140]. Although the application of TA systems is theoretically justified, practical works are not numerous. The so-called extracellular death factors (EDFs) encoded by the chromosomes of *E. coli, B. subtilis, Pseudomonas aeruginosa* which activated MazEF TA system were proposed to be used as antibacterial agents [119]. Peptides preventing in vitro formation of the TA complex by the components of the PemIK TA system of *B. anthracis* [141] and the VapBC Ta system of *M. tuberculosis* [142] were synthesized. Induction of the MazF toxin of *E. coli* located on the plasmid and controlled by tetracycline promoter led to a reduction of solid tumor in mice [143]. The gene of the Kis antitoxin of *E. coli* was combined with E6 target of oncogenic human papilloma virus HR-HPV. Introduction of the vectors containing the wild-type *kid* gene and hybrid *kis* gene to the culture of HR-HPV–positive human cells sharply inhibited their proliferation [144].

TA systems can also be used in gene therapy for viral infections. Some toxins possessing endonuclease activity (MazF) cause the death of eukaryotic cells. Retroviral vector containing the *mazF* gene of *E. coli* under the control of the TAR promoter from HIV-1 was designed. The HIV infection cycle begins with the expression of the virulence protein Tat, which binds to the TAR sequence to induce the transcription of the whole HIV-1 genome. Cells containing TAR-*mazF* express MazF in the presence of HIV-1, which in turn effectively cleaves viral mRNA and prevents HIV-1 replication in vivo [145]. MazF toxin was also used against the hepatitis C virus as component of zymoxin. Zymoxin consists of combined proteins of the toxin and corresponding antitoxin and contains the NS3 protease recognition site. Zymoxin injected into a cell infected by the virus is exposed to the specific proteolysis, releasing MazF toxin and causing cell death [146]. The main problems with the design of these antibacterial, antitumor, and antiviral preparations are associated with their delivery to the target cells and minimization of their effect on healthy eukaryotic cells.

Another opportunity for TA application is based on the application of the toxin targets. Peptide analogs of CcdB toxins of *E. coli*, which inhibited DNA gyrase and topoisomerase IV activity in vitro, were synthesized [147]. Zeta toxin from the omega-epsilon-zeta system of pSM19035 plasmid phosphorylates peptidoglycan precursor, UNAG, which inhibits protein MurA and blocks peptidoglycan synthesis. Phosphorylated UNAG-3P was proposed as an antibiotic [133].

The ability of plasmids carrying TA modules to perform postsegregational killing of cells devoid of plasmid is used in genetic engineering to select clones carrying the plasmid vector [148]. The GeneGard system, in which toxin (zeta and Kid toxins) and antitoxin genes are located on the plasmid and chromosome, respectively, was used to develop vectors that are incapable of horizontal transfer and unable to spread among other strains and species in the environment [149].

TA systems are usually species-specific, whereas the intraspecies distribution of TA systems and the single nucleotide polymorphism of TA genes are strain-specific [6, 57, 150, 151]. This made it possible to use type II TA systems as markers for the genotyping of the species and strains of lactobacilli [152, 153] and bifidobacteria [154], as well as *M. tuberculosis* stains [155, 156].

TA systems are optional components of bacterial genomes, i.e., deletion of the genes of one or several TA systems does not cause death of bacterial cell but can change its viability under extreme conditions [114]. One of the early works on in silico identification of type II TA systems belonging to five families (RelBE ParDE, HigBA, VapBC, MazEF, Phd/Doc, and CcdAB) revealed 31 bacterial strains that do not contain TA

systems. They were mainly obligate parasites living under constant environmental conditions. It was assumed that TA systems are typical of free-living bacteria [8]. Since that time the number of known TA systems has increased, and novel TA systems are constantly detected. It is extremely difficult to find a bacterial genome in which all known TA systems are absent. Functions of TA systems are diverse, but involvement in bacterial cell regulatory network is the most important of them. There are a lot of data confirming this fact; however, a general view of TA involvement in bacterial cell functioning has not yet been developed. There is no doubt that the future application of TA systems as drugs is extremely promising.

ACKNOWLEDGMENTS

The work was supported by the Presidium of the Russian Academy of Sciences (program no 0112-2015-0043 "Basic Research for the Development of Biomedical Technologies").

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Translated by A. Bulaev