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Review

Structural overview of toxin–antitoxin systems in infectious bacteria: A target for developing antimicrobial agents

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ABSTRACT

The bacterial toxin–antitoxin (TA) system is a module that may play a role in cell survival under stress conditions. Generally, toxin molecules act as negative regulators in cell survival and antitoxin molecules as positive regulators. Thus, the expression levels and interactions between toxins and antitoxins should be systematically harmonized so that bacteria can escape such harmful conditions. Since TA systems are able to control the fate of bacteria, they are considered potent targets for the development of new antimicrobial agents. TA systems are widely prevalent with a variety of systems existing in bacteria: there are three types of bacterial TA systems depending on the property of the antitoxin which binds either the protein toxin or mRNA coding the toxin protein. Moreover, the multiplicity of TA genes has been observed even in species of bacteria. Therefore, knowledge on TA systems such as the individual characteristics of TA systems, integrative working mechanisms of various TA systems in bacteria, interactions between toxin molecules and cellular targets, and so on is currently limited due to their complexity. In this regard, it would be helpful to know the structural characteristics of TA modules for understanding TA systems in bacteria. Until now, 85 out of the total structures deposited in PDB have been bacterial TA system proteins including TA complexes or isolated toxins/antitoxins. Here, we summarized the structural information of TA systems and analyzed the structural characteristics of known TA modules from several bacteria, especially focusing on the TA modules of several infectious bacteria.

1. Introduction

1.1. Definition of TA systems

The bacterial toxin–antitoxin (TA) system is a module that may play a role in cell survival under stress conditions such as amino-acid starvation, antibiotics treatment, temperature change and oxidative stress even though TA systems are not essential for normal cell growth [1,2]. In TA systems, the toxin molecules (proteins) are regarded as harmful molecules to cells due to the inhibition of cell-growth and thereby conferring growth stasis in stress environments. The toxin molecules have a variety of actions such as cleavage of DNA [3–5] or RNA [6–8], transfer of phosphate groups [9], phosphorylation of proteins [10,11], inhibition of ATP synthesis [12] and so on. Based on the activities of toxins, the actions of toxins are mainly focused on regulating cellular replication and translation processes. Through these regulatory functions, the toxins lead bacterial cells to cell death similar to the programmed cell-death [13] and control their population. The antitoxin molecules are usually binding partners of toxins either on a gene or protein level and regulate the activity of the toxin molecules. Unlike toxin molecules, the known antitoxin molecules consist of antisense RNAs as well as proteins [14,15]. The life times of both toxin and antitoxin molecules are quite different in cells: toxin molecules tend to be stable whereas antitoxin molecules tend to be unstable. Thus, to prevent the toxic effects of toxin molecules, antitoxin molecules tend to be produced continuously [16,17].

2. General classification of TA system

TA systems are widely prevalent in bacteria as well as archaea and are usually categorized into three types depending on the nature of the antitoxin and the composition of TA operon (reviewed in 1, Fig. 1). Briefly, the type I system controls toxin gene expression with an antisense RNA (antitoxin) reversely transcribed from the toxin gene or near the toxin gene [15]. The mRNA of the toxin forms a complex with the antisense RNA (antitoxin) and the resultant RNA complex, which is incapable to bind to ribosome, may be degraded. In *Escherichia coli*, several type I TA systems exist, such as SymR–SymE (antitoxin–toxin) and RdlD–LdrA [18,19]. The type II system is organized in an operon that consists of two overlapping genes. Usually, the antitoxin genes are

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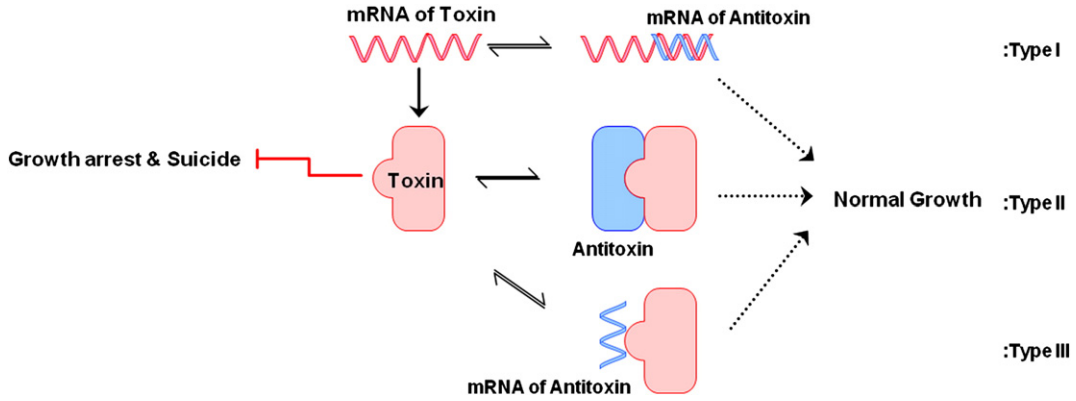

Fig. 1. Working models of TA systems. Toxin and antitoxin (antisense RNA) are transcribed separately in a Type I TA system. The RNA-antitoxin makes a complex with the mRNA of toxin, inhibiting translation of the toxin molecule. In a Type II TA system, the mRNAs of the antitoxin and toxin are synthesized within an operon and both are translated into proteins. The antitoxins bind toxins and block the active site of the toxins. Antitoxins are usually subjected to cleavage under stress conditions by an ATP-dependent protease. The released toxin from the TA complex attacks its cellular target. An RNA antitoxin forms a complex with a toxin protein in a Type III TA system. The free toxins in every TA system lead to bacterial cell growth arrest and eventual cell death.

located upstream of the toxin genes. Under normal conditions, the expressed antitoxin and toxin proteins form a stable complex so that the function of the toxin is inhibited. *E. coli* MazE–MazF and RelE–RelB are examples of a type II TA system [17,20]. The type III system looks like an intermediate of the type I and the type II. This type was recently identified, compared to the type I and the type II systems. The representative type III system is ToxI–ToxN [21,22]. The nature of the type III system is that the toxin protein makes a complex with the antitoxin RNA of which gene exists upstream of the toxin gene with a tandem array of nucleotide repeats [22].

2.1. TA systems in bacteria

Bacterial TA systems have three types depending on the property of the antitoxin with the toxin always being a protein [1,2]. A small RNA molecule is the antitoxin of type I showing complementarity to the toxin mRNA and regulating toxin expression by inhibiting the toxin's translation [15,23]. The antitoxin of type II is a small, unstable protein that sequesters the toxin through the formation of a complex regulating the level of their gene transcription [14]. The type II system has been investigated more, especially for their biological roles. In the type III system, a RNA antitoxin molecule has to form a complex with its cognate toxin for the toxin function to be completely inhibited [6,22].

2.1.1. Type I toxin–antitoxin

The small RNAs that have been characterized act by two general mechanisms. A small number of small RNAs bind to proteins and modify their activities. The other small RNAs function by base pairing with target mRNAs. Base pairing can lead to changes in gene expression by altering the stability and translation of the target. The majority of the characterized chromosomally encoded small RNAs act by base pairing with targets that have limited complementarity. In contrast, most of the small RNAs carried on plasmids are encoded on the antisense strand relative to their targets and have extensive complementarity with the mRNA. Until now, only a limited number of chromosomally encoded small RNAs with potential for extensive base pairing to their target mRNAs were known, but an increasing number of small RNAs are being discovered. Intriguingly, most of these small RNAs are expressed at high levels. These mRNA–small RNA pairs have been classified as the type I toxin–antitoxin system [24]. Some type I TA pairs are found on both plasmids and chromosomes, while some are exclusively plasmid or chromosomally encoded. In most cases, the protein toxin and RNA antitoxin are encoded on opposite strands, with the overlap occurring at either the 5′ end or the 3′ end of the mRNA transcript. For two pairs, the mRNA and small RNA are encoded divergently in the same intergenic region but share 19 and 23 nucleotides of contiguous complementarity [23]. While all chromosomally encoded type I toxin–antitoxin pairs have been characterized in the model organisms *E. coli* and *Bacilus subtilis*, homologs of all of the type I toxins can be found in related bacteria. No global searches for type I toxin–antitoxins have been reported, but it is predicted that they could be as broadly distributed like the type II toxin–antitoxins. A possible reason for the paucity of identified type I loci is that type I toxins are smaller than the type II toxins and in some cases, consist of only a short transmembrane helix, making reliable prediction difficult. In addition, the hydrophobic properties of the toxins can be maintained even with substantial sequence divergence. The Hok–Sok system of R1 plasmids was the first type I toxin–antitoxin pair to be discovered through the characterization of a locus that stabilized various plasmids in gram-negative bacteria and homologs of Hok–Sok including SrnB–SrnC and PndA–PndB are found on other plasmids [25–31]. RNAI (Fst)–RNAII encoded on the pAD1 plasmid of *Enterococcus faecalis*, the first type I toxin–antitoxin pair found for a gram-positive bacterium, was identified on the basis of a postsegregational killing phenotype [32]. Two families of chromosomally encoded type I toxin–antitoxin pairs were initially identified as genomic repeat sequences. The long direct repeat (LDR) sequences are approximately 530 nucleotides in length, and each encodes an Ldr-Rdl toxin–antitoxin [19]. Genomic repeat sequences of approximately 165 nucleotides were initially denoted as the QUAD repeats in *E. coli* K-12 but were subsequently renamed as SIB (short, intergenic, abundant) sequences when it became clear that a fifth repeat was present [33,34]. The SIB repeats are in three locations on the chromosome, with expansion or contraction in the number of repeats at each locus. The toxin genes encoded by each of the repeat sequences have been named *ibs* (induction brings stasis), and the antitoxin genes have been named *sib* for the Ibs-Sib toxin–antitoxin system. Four toxin–antitoxin pairs were discovered more recently in global searches for small RNAs including TisB–IstR-1 (toxicity-induced by SOS), ShoB–OhsC (short hydrophobic open reading frame), SymE–SymR, and TxpA (toxic peptide)–RatA (RNA antitoxin) [18,34–38]. The type I toxin–antitoxin modules may not have any function in the cell but may be examples of “selfish DNA” especially since several of the type I toxin–antitoxin pairs have been deleted from bacterial chromosomes without observable consequences to the bacteria. Like transposons and other mobile DNA elements, these RNA-regulated toxins could be collected by bacterial species as a result of horizontal transfer or duplication. At least some of the type I toxin–antitoxin pairs may confer a selective advantage to cells, since no point mutations are present in either the coding sequences or the ribosome binding sites of all the copies of the *ldr* or *ibs* genes identified to date. The type I TA loci appear to be less prevalent