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Review

Ribonucleases in bacterial toxin–antitoxin systems ☆

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ABSTRACT

Toxin–antitoxin (TA) systems are widespread in bacteria and archaea and play important roles in a diverse range of cellular activities. TA systems have been broadly classified into 5 types and the targets of the toxins are diverse, but the most frequently used cellular target is mRNA. Toxins that target mRNA to inhibit translation can be classified as ribosome-dependent or ribosome-independent RNA interferases. These RNA interferases are sequence-specific endoribonucleases that cleave RNA at specific sequences. Despite limited sequence similarity, ribosome-independent RNA interferases belong to a limited number of structural classes. The MazF structural family includes MazF, Kid, ParE and CcdB toxins. MazF members cleave mRNA at 3-, 5- or 7-base recognition sequences in different bacteria and have been implicated in controlling cell death (programmed) and cell growth, and cellular responses to nutrient starvation, antibiotics, heat and oxidative stress. VapC endoribonucleases belong to the PIN-domain family and inhibit translation by either cleaving $tRNA^{fMet}$ in the anticodon stem loop, cleaving mRNA at -AUA(U/A)-hairpin-G- sequences or by sequence-specific RNA binding. VapC has been implicated in controlling bacterial growth in the intracellular environment and in microbial adaptation to nutrient limitation (nitrogen, carbon) and heat shock. ToxN shows structural homology to MazF and is also a sequence-specific endoribonuclease. ToxN confers phage resistance by causing cell death upon phage infection by cleaving cellular and phage RNAs, thereby interfering with bacterial and phage growth. Notwithstanding our recent progress in understanding ribonuclease action and function in TA systems, the environmental triggers that cause release of the toxin from its cognate antitoxin and the precise cellular function of these systems in many bacteria remain to be discovered. This article is part of a Special Issue entitled: RNA Decay mechanisms.

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1. Introduction

The genomes of prokaryotic organisms harbor non-essential genes encoding toxins whose expression leads to growth inhibition and, in some cases, cell death. Toxin–antitoxin (TA) systems were first discovered as plasmid maintenance systems and have subsequently been found in plasmids and/or chromosomes of bacteria and archaea. The molecular basis for the almost universal distribution of these bacterial toxins has remained largely unexplained and often it is highly speculative as to their role and function in microbial biology and evolution. The toxin components are co-expressed with a cognate short-lived neutralizing antitoxin and this organization is a hallmark of toxin–antitoxin (TA) modules or TA operons (Fig. 1). TA modules have been classified into three types according to the molecular identity of each component. For Type I modules, the antitoxin is a small antisense RNA molecule that prevents toxin translation. Type II modules encode a protein antitoxin that binds its cognate toxin protein and inhibits its activity. Type III TA modules encode a protein toxin and an RNA antitoxin that interact and form a protein–RNA complex. Recently two novel TA systems were reported and the authors propose that these systems be classified as Type IV and Type V [1,2]. Type IV is characterized by an antitoxin that does not form a complex with its cognate toxin, but acts as an antagonist for toxin toxicity [1]. Type V is characterized by a protein antitoxin inhibiting the toxin by specifically cleaving its mRNA [2]. Bacteria often harbor more than one type of TA module and it remains to be determined if these systems interact to regulate whole cell physiology. This is not trivial to address given the observation that some bacteria contain up to 88 TA systems [3].

The toxin components of the TA modules exert their effects in different ways by targeting an essential cellular function (e.g., DNA replication, mRNA stability, protein synthesis, cell division and peptidoglycan biosynthesis). For the purposes of this review, we will focus on three TA modules with a ribonuclease function that act by cleaving mRNA in a site-specific manner that is independent of ribosome function. These TA modules include MazF, VapC and ToxN. MazEF and VapBC are Type II TA systems and ToxIN is a Type III TA system.

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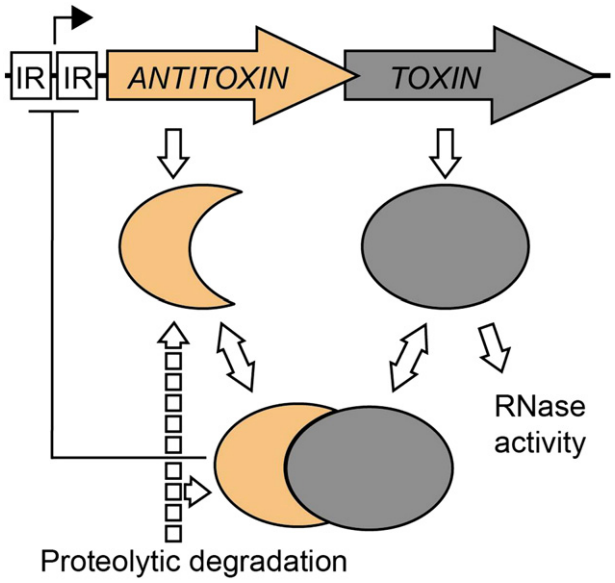

Fig. 1. Organization of a typical Type II toxin–antitoxin system. Antitoxin- and toxin-encoding mRNA is synthesized from the same promoter and translated into the respective products. The toxin is neutralized by the antitoxin by forming a complex, which also autoregulates the expression of the TA operon via binding to inverted repeat elements in the promoter. The antitoxin alone can also bind to the TA promoter to regulate expression. Degradation of the labile antitoxin by ATP-dependent proteases causes release of the toxin, which enables the attack of cellular mRNA, causing inhibition of cell growth and eventually cell death.

2. The MazF–MazE system: a prototype for understanding ribosome-independent ribonuclease function in TA modules

The most common target of TA systems is mRNA and the toxin components that cleave cellular mRNA have been termed mRNA interferases [4]. They have been classified into two groups: ribosome-dependent RNA interferases that cleave RNA only when associated with ribosomes (e.g., RelE) or RNA interferases that cleave RNA in the absence of ribosomes (e.g., MazEF, VapBC). Ribosome-independent RNA interferases are sequence-specific endoribonucleases that target specific RNA sequences.

2.1. The target RNA sequence of MazF varies in different bacterial species

The MazF RNA interferase of *Escherichia coli* cleaves mRNA both in vitro and in vivo and inhibits protein synthesis [5,6]. MazF-mediated cleavage is site-specific at the 5′ end of ACA sequences causing the release of a 2′3′-cyclic phosphate group at one side and a free 5′-hydroxyl group at the other [6,7]. However, MazF cleavage in vivo also occurs at coding regions of *lpp* mRNA and tmRNA, with strong cleavage at the stop codon UAA and sense codon UAC [5]. While the MazF toxins cleave RNA directly, translated mRNA has been shown to be cleaved more efficiently, probably due to removal of secondary structures [6,8]. Proteins associated with mRNA can inhibit cleavage [6,9]. For example, *Staphylococcus aureus* MazF was shown to cleave the mRNA transcripts of *recA*, *gyrB* and *sarA* in vitro, but these transcripts were not cleaved in vivo, due to protection by RNA-binding proteins [9].

MazF is a sequence-specific endoribonuclease, but the RNA sequence cleaved varies in those bacteria that have been studied (reviewed in [10]). The *E. coli* MazF toxin cleaves at ∧ACA or A∧CA 3 base sequences and this cleavage is independent of the ribosome and reading frame [6]. MazF is also able to cleave its own mRNA [6]. *S. aureus* MazF cleaves RNA at U∧ACAU five base sequences [11] and this sequence is common in several genes important for pathogenesis including *sraP*, which is involved in the adhesion of *S. aureus* to human tissues [12]. The chromosome of *Bacillus subtilis* encodes a MazF homolog (EndoA) that cleaves RNA at U∧ACAU sequences [13]. *Bacillus anthracis* contains a PemIK homolog in its chromosome [14] and the PemK toxin has endoribonuclease activity that targets pyrimidines (C/U) [15]. *Mycobacterium tuberculosis* harbors genes for nine MazF homologs and seven (MazF-mt1-7) have been characterized [16]. The target cleavage sites have been identified for mt1, mt3, mt6 and mt7. MazF-mt1 cleaves at U∧AC, MazF-mt6 cleaves in U rich regions (U/C)U∧(U/A)C(U/C), MazF-mt3 cleaves RNA at (U/C)U∧CCU or CU∧CCU and MazF-mt7 cleaves RNA at U∧CGCU [16,17]. *Myxococcus xanthus* harbors a solitary *mazF* gene that lacks a cotranscribed antitoxin gene and recognizes GU∧UGC [18]. The rationale for mRNA cleavage at 3-, 5- or 7-base recognition sequences is unclear, but could reflect the greater specificity of MazF as the sequence becomes longer [10]. Inouye and colleagues [10] have recently reported a 7-base sequence for MazF from the halophilic archaeon *Haloquadratum walsbyi* UU/ACUCA [10]. The variation in sequence size between different MazF proteins predicts that MazF is effective at eliminating the majority of cellular mRNA when it cleaves at ∧ACA or A∧CA sequences and becomes more targeted to a specific group of mRNAs as the sequence is extended. It is also possible that for organisms with a high number of TA systems from the same family, such as *M. tuberculosis*, those toxins might have different sequence targets as demonstrated for MazF leading to control of different physiological functions [16].

2.2. Structure of MazEF reveals a highly effective mechanism for toxin neutralization

Despite the abundance of TA systems in prokaryotic genomes, the structural analysis reveals that toxin proteins belong to a limited number of structural families [19]. Blower and colleagues [19] define six structural classes of toxin based on common protein folds: Kid (e.g., MazF and ToxN), RelE, Doc, VapC, HipA and ζ. The conserved regions of the Kid/MazF/ToxN compact globular fold are the core β-barrel, the β-strands of the large loop and the C-terminal α-helix. ToxN has an embellished 3–4 loop and an extended C-terminal helix. Although the fold is conserved across these toxins they do not share active site residues and thus the same function has been differentially grafted onto the conserved fold.

The crystal structure of the MazF–MazE complex reveals a linear symmetrical heterohexameric (2:4) complex composed of alternating toxin and antitoxin homodimers (viz. $MazF_2$–$MazE_2$–$MazF_2$) (Fig. 2) [20]. One MazE homodimer sits between two symmetrically arrayed MazF homodimers. Interactions between MazE and MazF are mediated by two long unstructured negatively charged C-terminal extensions, each binding to a dimer, primarily the medial MazF protomer, and blocking the active site (Fig. 2). Each MazF monomer consists of a seven-stranded, twisted antiparallel β-sheet with three α-helices and interactions between MazF monomers are via hydrophobic contacts [20]. The MazE homodimer forms an intertwined β-barrel with two extended C terminal segments with a strong negative electrostatic potential (Fig. 2).

Structural information on the MazF–MazE complex was available before it was revealed that this family of toxins was mRNA interferases [6,7]. Consequently, little was understood about the interactions of MazF with RNA substrates and the conformational nature of the RNA-binding site. Inouye and coworkers suggested that MazF functioned in a manner similar to RNase A, akin to an RNA restriction enzyme [7]. NMR experiments using a MazF mutant (E24A) and a 13 base single-stranded DNA (ssDNA) as an mRNA substrate analog revealed that the MazF homodimer contains two symmetrical RNA-binding sites (K_d approximately 10^{-5}–10^{-6} M). Two molecules of ssDNA appear to bind simultaneously to the MazF homodimer and overlap with the two binding sites for the C-terminus of MazE [21]. It is not known whether the MazF homodimer can act as a bivalent enzyme, cleaving two RNA substrates simultaneously or delivers a single cleaved RNA product with a non-cleaved molecule released. These nucleotide-binding sites coincide with the positively charged MazE-binding site in the MazF homodimer implying that the negatively charged ssDNA backbone interacts with this basic region of MazF. The substrate specificity determinant for different MazF toxins is not known.

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