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Review

Novel toxins from type II toxin-antitoxin systems with acetyltransferase activity

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ABSTRACT

Type II toxin-antitoxin (TA) systems are widespread in bacterial and archeal genomes. These modules are very dynamic and participate in bacterial genome evolution through horizontal gene transfer. TA systems are commonly composed of a labile antitoxin and a stable toxin. Toxins appear to preferentially inhibit the protein synthesis process. Toxins use a variety of molecular mechanisms and target nearly every step of translation to achieve their inhibitory function. This review focuses on a recently identified TA family that includes acetyltransferase toxins. The AtaT and TacT toxins are the best-characterized to date in this family. AtaT and TacT both inhibit translation by acetylating the amino acid charged on tRNAs. However, the specificities of these 2 toxins are different as AtaT inhibits translation initiation by acetylation of the initiator tRNA whereas TacT acetylates elongator tRNAs. The molecular mechanisms of these toxins are discussed, as well as the functions and possible evolutionary origins of this diverse toxin family.

1. Introduction

Bacterial toxin-antitoxin (TA) systems are small modules composed of a stable toxic protein and an unstable antitoxin. The nature and mode of action of the antitoxin are at the basis for TA system classification (for recent reviews, (Goeders and Van Melderen, 2014; Goeders et al., 2016; Hayes and Van Melderen, 2011; Berghoff and Wagner, 2017; Page and Peti, 2016)). Antitoxins can be RNA either inhibiting translation of the cognate toxin (type I) or its activity (type III). Antitoxins can also be proteins and display diverse activities. Type II antitoxins neutralize their cognate toxins by forming a tight antitoxin-toxin complex. Type IV antitoxins protect the target from interactions with the toxin. In type V and VI systems, antitoxins specifically cleave the toxin mRNA or serve as an adaptor to direct toxin to proteolysis, respectively.

This review focuses on type II TA systems. These systems are generally composed of 2 genes organized as an operon, with the antitoxin gene being located upstream the toxin gene. Notable exceptions are the *higBA* and *hipBA* modules in which the gene order is swapped (see below). The antitoxin is generally composed of 2 distinct domains, with a DNA-binding domain (e.g. RHH, HTH, AbrB-like) at the amino-terminus and a toxin-interacting domain at the carboxy-terminus. Antitoxins alone or in complex with cognate toxins have the capacity to bind to palindromic operator sequences in the operon promoter and thereby regulate TA system expression. In some TA modules the toxin is directly involved in the regulatory process acting as a transcription co-repressor or activator depending on the levels of toxin, in a mechanism known as conditional cooperativity (for review, (Loris and Garcia-Pino, 2014)).

With the increase of bacterial genome sequences and data mining, the paradigm for canonical type II TA systems has evolved (Leplae et al., 2011; Anantharaman and Aravind, 2003). It appears that the genetic organization of these systems is more modular than previously thought. Type II TA systems can be organized in 'reverse' order with the toxin gene preceding that of the toxin (Budde et al., 2007; Christensen-Dalsgaard et al., 2010; Jorgensen et al., 2009). In a few examples, a third component can be part of the TA operon. This comes today in 2 flavors – systems in which transcriptional regulation function is ensured by a regulator located upstream the antitoxin and toxin genes (Hallez et al., 2010; de la Hoz et al., 2000) have been described as well as TAC (toxin-antitoxin-chaperon) systems in which the third gene of the operon encodes a SecB chaperone homologue that is needed for antitoxin folding and activity (Sala et al., 2014).

Type II systems were originally discovered on plasmids in the late 80s. The function of these modules is to ensure plasmid maintenance in growing bacterial populations. They act by killing daughter-bacteria devoid of a plasmid copy. This phenomenon was termed post-segregational killing (Gerdes et al., 1986) and later on addiction (Yarmolinski,

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Table 1
Eight super-families of type II toxins: activity and process inhibition.

Toxin super-family	Toxin	Activity	Inhibition	References
ParE/RelE	RelE	Ribosome-dependent mRNA cleavage	Translation elongation	(Pedersen et al., 2003)
	ParE	DNA-gyrase inhibition	Replication, SOS induction	(Jiang et al., 2002)
CcdB/MazF	CcdB	DNA-gyrase inhibition	Replication, SOS induction	(Bernard and Couturier, 1992)
	MazF	Free mRNAs cleavage	Translation initiation	(Zhang et al., 2003)
	MazF*mt6*M. tb	Helix/loop 70 of domain IV of 23S rRNA	Translation elongation	(Schifano et al., 2013)
	MazF*mt9*M. tb	Anticodon and D loop of elongator tRNAs	Translation elongation	(Schifano et al., 2016)
Fic	Doc	Phosphorylation of EF-Tu elongation factor	Translation elongation	(Castro-Roa et al., 2013)
	Fic	DNA-gyrase and TopoIV adenylylation	DNA topology	(Harms et al., 2015)
PIN	Enteric VapC, Leptospiral VapC	Anticodon stem-loop of initiator tRNA	Translation initiation	(Winther and Gerdes, 2011; Lopes et al., 2014)
	M.tb. VapC20, VapC26	cleavage	Translation elongation	
	Other mycobacterial VapCs	Sarcin–Ricin loop of 23S rRNA	Translation elongation	(Winther et al., 2013; Winther et al., 2016)
		Anticodon stem-loops of elongation tRNAs		(Winther et al., 2016)
HipA	HipA	Phosphorylation of Glutamyl-tRNA-synthetase	Translation elongation	(Germain et al., 2013; Kaspy et al., 2013)
YafO	YafO	Ribosome-dependent mRNA cleavage	Translation elongation	(Zhang et al., 2009)
Zeta	Zeta	Phosphorylation of UDP-Glc-NAc	Peptidoglycan synthesis	(Mutschler et al., 2011)
AtaT/TacT	AtaT	Acetylation of initiator tRNA	Translation initiation	(Jurenas et al., 2017)
	TacT	Acetylation of elongation tRNAs	Translation elongation	(Cheverton et al., 2016)

M.tb.: *Mycobacterium tuberculosis*.

1995). As mentioned above, antitoxins are unstable as compared to toxins. In plasmid-free daughter cells, since antitoxin is not replenished, the toxin will be liberated from the antitoxin-toxin complex. The free toxin will then act on its target to inhibit cell growth and eventually cause cell death. This will lead to apparent plasmid stabilization by reducing the frequency of plasmid-free cells. Another hypothesis proposes that these systems increase fitness of their host replicons by outcompeting competitor plasmids from the same incompatibility group (Cooper and Heinemann, 2000). Moreover, mathematical models developed on restriction-modification systems, but transposable to TA systems, predict that the addiction phenomenon allows propagation of these modules in bacterial populations independently of their original frequencies (Mochizuki et al., 2006). This might provide a rationale explanation for the high prevalence of these systems. Multiple studies have emphasized the abundance of type II TA systems in bacterial chromosomes (see e.g. (Leplae et al., 2011; Anantharaman and Aravind, 2003; Guglielmini and Van Melderen, 2011; Pandey and Gerdes, 2005; Ramisetty and Santhosh, 2016; Coray et al., 2017)). It is commonly accepted that these systems move through horizontal gene transfer and integrate into bacterial chromosomes as part of genomic islands (phages, transposons) or constitute genomic islets on their own. The role of these systems when located in chromosomes remains an interesting debate in the field (for reviews, (Diaz-Orejas et al., 2017; Kedzierska and Hayes, 2016; Lobato-Marquez et al., 2016)). The role in phages, transposons and genomic islands is most likely reminiscent of the addiction function: they serve to maintain these integrated mobile elements (Wozniak and Waldor, 2009; Huguet et al., 2016; Yao et al., 2015). This is best exemplified by TA systems encoded by superintegron gene cassettes (Escudero et al., 2015). These addiction cassettes might prevent the loss of silent cassettes and favor the formation of large integron arrays. Other systems, whether they are located in chromosomes or on plasmids, might serve as defense mechanisms against invasion by phages (Abortive infection, Abi) (Koga et al., 2011) or against plasmid establishment (anti-addiction) (Saavedra De Bast et al., 2008). On a more controversial side, type II systems have been recently associated to persistence and antibiotic tolerance in *E. coli* (Maisonneuve et al., 2011). The basis of the mainstream persistence model relies on TA-induced dormancy, a physiological state that allows bacteria to tolerate stress such as antibiotics or intracellular conditions. Activation of toxins inhibiting translation leads to such a dormant state. However, contradictory data questioning this model have been published recently (Ramisetty et al., 2016; Van Melderen and Wood, 2017; Shan et al., 2017). Nevertheless, TA systems have been shown to be involved in *Salmonella typhimurium* intracellular persistence in macrophages, the natural cell type infected by this pathogen (Helaine et al., 2014) as well as in fibroblast and epithelial cells (Lobato-Marquez et al., 2015). In line with this finding, TA systems were reported to be associated with virulence in other bacterial pathogens (for recent reviews, (Kedzierska and Hayes, 2016; Lobato-Marquez et al., 2016)). However, the precise molecular mechanisms of activation of TA systems in the different in vivo models remain to be elucidated.

2. Diversity of molecular mechanisms of type II toxins

Remarkably, activity of type II TA toxins appears to be mainly directed towards translation (for reviews, (Goeders and Van Melderen, 2014; Page and Peti, 2016; Guglielmini and Van Melderen, 2011)). Toxins targeting nearly every step of the translation process and using diverse molecular mechanisms have been described. In the context of TA-induced dormancy, inhibition of translation is certainly sufficient to halt cell metabolism and growth and does not preclude returning to a normal physiological state upon toxin neutralization. In addition, inhibition of protein synthesis is less harmful than other type of activities such as inhibition of replication or peptidoglycan synthesis. Thus, toxins inhibiting translation might have been selected preferentially during evolution rather than other more ‘toxic’ activities (Guglielmini and Van Melderen, 2011).

Seven main super-families of toxins have been defined based on amino acid sequence similarity and three-dimensional structures (Table 1). Our group, as well as others, recently identified and characterized a novel toxin super-family, presenting novel toxin-fold and activity (see below) (Cheverton et al., 2016; Jurenas et al., 2017).

Commonly, toxins from the same super-family share very low sequence similarity indicating functional divergence in the time of evolution (Leplae et al., 2011). Accordingly, different members, although sharing a similar fold, show distinct activities and targets. In the CcdB/MazF super-family, CcdB-type of toxins are DNA-gyrase inhibitors leading to replication inhibition and SOS induction while MazF toxins are endoribonucleases cleaving free mRNAs with relaxed specificity, elongator tRNAs or the 23S rRNA (Bernard and Couturier, 1992; Zhang et al., 2003; Schifano et al., 2013). Divergence is also observed within the RelE/ParE super-family. While RelE toxins are endoribonuclease cleaving mRNAs in a ribosome-dependent manner, also with relaxed specificity (Goeders et al., 2013; Feng et al., 2013), ParE toxins are thought to inhibit DNA-gyrase (Jiang et al., 2002). This leads to replication inhibition and SOS system induction as observed in the case of CcdB, although the precise molecular mechanism is likely to be different since mutants resistant to CcdB are sensitive to some of the ParE toxins (Yuan et al., 2010).

Toxins with the Fic-fold also display different activities and targets.