RNA Biology

ISSN: 1547-6286 (Print) 1555-8584 (Online) Journal homepage: https://www.tandfonline.com/loi/krnb20

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To cite this article: Sabine Brantl (2012) Bacterial type I toxin-antitoxin systems, RNA Biology, 9:12, 1488-1490, DOI: 10.4161/rna.23045

To link to this article: https://doi.org/10.4161/rna.23045

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Published online: 01 Dec 2012.

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RNA Biology 9:12, 1488–1490; December 2012; © 2012 Landes Bioscience

Bacterial type I toxin-antitoxin systems

Sabine Brantl

Friedrich-Schiller-Universität Jena; Biologisch-Pharmazeutische Fakultät; AG Bakteriengenetik; Jena, Germany

Keywords: toxin-antitoxin system, antisense RNA, post-segregational killing, persister

Toxin-antitoxin (TA) loci encode two-component systems that consist of a stable "toxin" whose ectopic overexpression either kills cells or confers growth stasis, and an unstable "antitoxin". TA systems have been initially discovered on plasmids, where they confer stability of maintenance through post-segregational killing (PSK). Plasmid loss results in rapid decrease of antitoxin levels, which allows the stable toxin to kill the plasmid-free cell. Later, TA systems were also found on bacterial and archaeal chromosomes, sometimes in staggering numbers.[1,2] They are classified into five types depending on the nature and action of the antitoxin. In type I systems, the antitoxin is a small antisense RNA that base-pairs with the toxin encoding mRNA. By contrast, in type II systems, the antitoxin is a protein that interacts post-translationally with the toxin protein. The antitoxin in type III systems is a pseudoknot containing RNA that directly binds the toxin protein.[3,4] In the recently proposed type IV systems, the protein antitoxin interferes with binding of the toxin to its target rather than inhibiting the toxin directly by binding,[5] whereas the antitoxin protein in type V systems cleaves the toxin-encoding mRNA.[6]

Type I TA systems have been discovered in Gram-negative and Gram-positive bacteria and are arranged either as overlapping, convergently transcribed genes pairs (e.g., *hok*/Sok, *bsrG*/SR4) or as divergently transcribed gene pairs located apart (e.g., *tisB*/IstR1, *shoB*/OhsC). In the first case, the antitoxin is a cis-encoded antisense RNA, in the latter case, it is a trans-encoded sRNA. Chromosomally encoded systems are often present in multiple copies. As Fozo concludes from a comparison of "wild" and laboratory strains of *E. coli*, the number of copies seems to be connected to the ecological niche that is occupied by the bacterial host.[7]

With the exception of SymE, all type I toxins are small hydrophobic proteins (less than 60 aa) containing a potential transmembrane domain. They seem to act similar to phage holins by inducing pores into cell membranes, which impairs ATP synthesis. Consequently, replication, transcription and translation may be inhibited, which leads to cell death. Recently, the TisB toxin was shown to produce clusters of narrow anion-selective pores in lipid bilayers.[8] Ldr from γ-proteobacteria and Fst restricted to firmicutes cause nucleoid condensation.[9,10] Thereby, Ldr affects purin metabolism and decreases intracellular cAMP levels.[9] Many toxins are not bacteriocidal, but interfere with phage propagation, modulate the cell membrane or prevent mature particle formation.[9] In some cases, only overexpression of toxin genes shows a toxic effect.[7]

The first and—at the same time—best studied type I system is *hok*/Sok of *E. coli* plasmid R1, which was discovered by Kenn Gerdes in 1986 and subsequently found to be also encoded in the genomes of several enterobacteriaceae (refs. 11, 12 and reviewed in ref. 9). Later on, other type I systems were found in *E. coli* like *ldr*/Rdl, *tisB*/IstR1, *ibs*/Sib, *shoB*/OhsC and *symE*/SymR, which are reviewed in this Special focus.[7,9,13]

In Gram-positive bacteria, the first type I TA system—*fst*/RNAII—was identified in 1989 on the *Enterococcus faecalis* plasmid pAD, where it acts similar to *E. coli hok*/Sok in post-segregational killing of plasmid free daughter cells. Since then it has been investigated in great detail in Keith Weaver's group.[10] Fst-like systems have been also predicted in plasmids of other Gram-positive bacteria like *Staphylococcus, Lactobacillus* and *Listeria,* and some of them are encoded on chromosomes. By contrast, in *B. subtilis,* only chromosomally encoded type I TA systems are known to date, and the first of them—*txpA*/RatA—was identified in a screen for sRNAs only in 2005 (reviewed in this issue, ref. 14). Meanwhile, four toxin families are known in this species: TxpA/BsrG, BsrH/BsrE, YonT and YheZ.[14]

While the plasmid-encoded systems ensure segregational stability, the biological role of the numerous chromosomally encoded TA systems remained enigmatic for a long time. The RNA cleaving toxin SymE from *E. coli* has been proposed to recycle damaged mRNAs produced under SOS stress conditions or to prevent infection with RNA phages.[9] In *B. subtilis*, many toxin genes (e.g., *txpA, bsrG* and *yonT*) are located on prophages and were suggested to be required for the maintenance of these phages,[14] which resembles the function of plasmid-encoded PSK systems. Some TA systems are induced under certain conditions like oxygen limitation or glucose exhaustion, and the function of these toxins could be to cause bacteriostasis to limit oxygen or glucose consumption, respectively.[14] Kawano suggests that bacteria benefit from TA systems in their defense against invasion factors like bacteriophages.[9] As Wagner and Unoson emphasize in this issue,[13] it has been found only recently that chromosomal TA systems play a role in persister formation.[15,16] Persister cells are a subset of a bacterial population that has stochastically entered a dormant state and thus becomes refractory to the action of antibiotics, whereas their isogenic, rapidly growing siblings are sensitive. The mechanism of persister formation is still unknown. The deletion of *tisAB* led to a sharp decrease of the level of persister cells resistant to the antibiotic ciprofloxacin, whereas SOS-dependent induction of *tisB* expression increased the persister

Correspondence to: Sabine Brantl; Email: Sabine.Brantl@uni-jena.de
Submitted: 11/14/12; Accepted: 11/14/12
http://dx.doi.org/10.4161/rna.23045

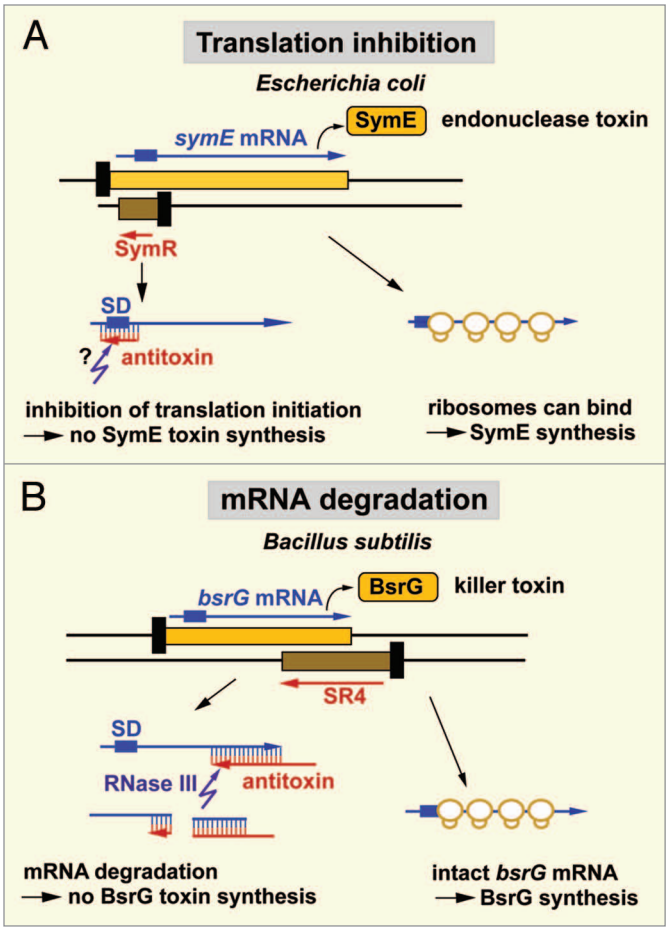

Figure 1. Two regulatory mechanisms employed by type I toxin/antitoxin systems. Antitoxin RNAs are drawn in red, toxin RNAs in blue. Black rectangles denote promoters, and yellow and brown boxes toxin and antitoxin genes, respectively. Open yellow symbols indicate ribosomes. Violet arrows denote RNase III cleavage. ? cleavage not demonstrated experimentally. (**A**) Translation inhibition. The antitoxin RNA is complementary to the SD sequence of the toxin mRNA. Interaction between both molecules prevents ribosome binding, and, hence, translation initiation. The toxin/antitoxin RNA duplex is most likely degraded by RNase III. (**B**) Promotion of mRNA degradation. Toxin and antitoxin RNA are complementary at their 3' ends. Interaction between both RNAs promotes toxin mRNA degradation, and, consequently, does not allow toxin synthesis.

levels significantly.[15] This can be brought in line with the effect of TisB on membrane integrity, decreasing intracellular ATP levels and thus macromolecular synthesis rates. By creating a dormant state, TisB causes a shutdown of antibiotic targets that results in multidrug resistance. The same effect—decrease of persister levels—was observed previously upon deletion of type II TA loci in *E. coli* (reviewed in ref. 16). These studies demonstrated unambiguously an important biological function of chromosomally encoded TA systems in *E. coli*.

What is known about the mechanism of action of the RNA antitoxins? In the majority of cases (*hok*/Sok, *tisB*/IstR1, *fst*/RNAII, *ibs*/Sib), the antitoxins inhibit translation of the toxin mRNAs, whereas in others, they promote toxin-mRNA degradation (**Fig. 1**). Translation can be prevented by different means: In *hok*/Sok, the Sok RNA binds to the SD sequence of *mok*, which is translationally coupled to *hok*, and thus, indirectly blocks *hok*-mRNA translation (reviewed in refs. 9, 12). Similarly, in *ldrD*/RdlD, a *mok*-like ORF termed *ldrX* overlaps *ldrD, as the ldrD* mRNA has also no capacity to bind ribosomes directly, and RdlD obstructs translation of *ldrX*.[9] In *tisB*/IstR1, the *tisB* SD is sequestered by intramolecular base-pairing. The IstR1 RNA binds 100 nt upstream of the *tisB* translation initiation region (TIR) at an unstructured ribosome standby-site, thereby restraining ribosomes from binding there and sliding into the TIR when the stem-loop region opens up occasionally.[13] Interestingly, the antitoxin RNA II of the *E. faecalis* plasmid pAD combines features of cis- and trans-encoded sRNAs: Toxin and antitoxin RNA overlap by a bidirectional terminator (similar to cis-encoded antisense RNAs) and, additionally, contain a set of direct repeat sequences located far apart (like trans-encoded antisense RNAs). RNAII/*fst* RNA binding yields a partial duplex, which is sufficient to block *fst* translation.[10] The Sib antitoxins also interact with two regions of their *ibs* toxin RNAs, one located in the SD sequence, the other within the *ibs* ORF, to block toxin translation. Thereby, interaction with the ORF region is critical for specificity, i.e., discrimination between different target toxins.[7]

In all *B. subtilis* type I TA systems (e.g., *txpA*/RatA, *bsrG*/SR4, *yonT*/As-yonT), the antitoxin RNA binds at the 3' end of the toxin-mRNA and promotes its degradation.[14] Durand et al. propose that this type of regulation is due to differences in the RNA degradation machinery between Gram-positive and Gram-negative bacteria.[14] However, as in some of these cases the SD sequence is located in a double-stranded region (*txpA, bsrG*) and, therefore, barely accessible, it cannot be entirely excluded that the antitoxin RNA has an additional effect on toxin translation.[14]

Complementary base-pairing of antitoxin and toxin RNAs results in double-stranded RNA regions, which are substrates for endoribonuclease III that initiates toxin RNA degradation. In some cases, RNase III is essential for the action of the antitoxin (*txpA*/RatA, *yonT*/As-yonT), whereas in others (*hok*/Sok, *symE*/SymR, *ibs*/Sib, *tisB*/IstRI and *bsrG*/SR4) it is dispensable.[7,9,13,14]

Although toxin inhibition by the RNA antitoxin is the major regulatory principle, various additional strategies are employed by type I TA systems to guarantee tight regulation of toxin expression or to keep toxin levels low unless they are required. In many cases, the toxin SD sequence is sequestered by intramolecular base-pairing (*tisB, shoB, bsrG, txpA*) that obstructs ribosome binding, and the start codon of *yonT* is GUG, which also likely reduces translation initiation.[14] *txpA* and *yonT* mRNAs have "perfect SD sequences" (≥ 11 bp complementarity to anti-SD in 16S rRNA), which are predicted to efficiently recruite, but slowly release ribosomes (reviewed in ref. 14). Often, translation competent molecules are produced only after 3' or 5' processing of the toxin mRNA (*hok, tisB, shoB*): The 3' end of *hok* mRNA folds back and base-pairs with the 5' end that contains an efficient translation initiation region (*tac*). Gradual processing of *hok* mRNA from the 3' end results in two truncated species, the shortest of which is translation competent.[9] Similarly, only

one of three *tisB* mRNA variants derived from 5' endonucleolytic processing is translated.[13] Sometimes, the toxin gene is only transcribed under certain conditions: *tisB* and *symE* are under SOS control, i.e., regulated by the Lex repressor. Proteolytic digestion by Lon protease affects SymE levels.[9] The stability of *bsrG* mRNA is reduced 3- to 4-fold at high temperatures.[14]

In spite of the data accumulated over the past years, our knowledge on type I TA systems is still limited. It seems that all bacteria that do not live as intracellular parasites encode numerous TA modules in their genomes. Notwithstanding the variation between the type I systems discovered so far, they seem to constitute a conserved family with similar regulatory properties. Although a connection between certain type I and II TA systems and persister formation has been established, for a large number of chromosomally encoded type I TA systems no biological function is known so far. It remains to be seen if other hypotheses (see above) for the acquisition of TA systems will receive experimental support.

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