

 BACTERIAL TOXINS

# An antidote to persistence

Toxin–antitoxin modules that are composed of a stable toxin and a labile antitoxin can regulate bacterial growth, with growth toxicity mediated by the toxin and countered by the antitoxin. Of 14 putative toxin–antitoxin modules that have been identified in the genome of the enteric pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium, three are predicted to encode an *N*-acetyltransferase as a new class of toxin. Now, Cheverton *et al.* show that one of these toxins, TacT, acetylates aminoacyl-tRNAs to inhibit translation, slow growth and increase persister formation, and that TacT toxicity can be reversed by an antitoxin-independent mechanism that enables *S. Typhimurium* to resume growth.

Growth assays of *S. Typhimurium* mutants that overexpressed TacT and/or its antitoxin confirmed that they form a bona fide toxin–antitoxin module. Furthermore, *in vitro* acetylation assays and cell-free expression assays showed that TacT acetylates the amino acid of aminoacyl-tRNAs to inhibit translation, using acetyl-CoA as a cofactor. X-ray crystallography of TacT with acetyl-CoA in the active site revealed a dimeric atypical acetyltransferase that is characterized by two positively charged surface regions, which molecular modelling suggested are binding sites, on opposing monomers, for the negatively charged phosphate groups of the acceptor stem of the tRNA molecule and the remainder of the tRNA molecule, respectively.

TacT did not impair growth at exponential phase but instead delayed the exit from lag phase by several hours. Infections with *S. Typhimurium* are often characterized by persistence, in which transiently slow or non-growing cells, such as those in lag phase, survive antibiotic treatment. Consistent with a role for TacT in persister formation, expression of the toxin increased the number of persister cells when *S. Typhimurium* was exposed to bactericidal concentrations of three different classes of antibiotic *in vitro*.

These growth and persister formation phenotypes could be prevented by expression of the antitoxin; however, as TacT inhibits translation, *S. Typhimurium* is probably not able to produce the antitoxin once TacT toxicity has occurred. To see whether alternative mechanisms might instead be responsible for detoxification, the authors designed a screen for genes that suppress TacT toxicity, which identified peptidyl-tRNA hydrolase (Pth). The ability of Pth to deacetylate acetylated aminoacyl-tRNAs, suppress TacT-induced growth defects and prevent the increase in persister formation that is associated with TacT toxicity was confirmed *in vitro* by acetylation assays, growth assays and antibiotic-exposure assays, respectively. Furthermore, the identification of Pth as a detoxifying factor suggested that TacT acetylates the primary amine of aminoacyl-tRNAs, which molecular modelling indicated is located in close proximity to acetyl-CoA in the TacT active site. Finally,



CobB, which is the only non-specific deacetylase known to be encoded by the *S. Typhimurium* genome, may further contribute to detoxification, as deletion of *cobB* enhanced the extension of the lag phase produced by TacT and even resulted in a new phenotype of slow growth during exponential phase. However, whether CobB directly deacetylates aminoacyl-tRNAs has not yet been determined.

Together, the findings of the study show that translational inhibition, and ultimately persister formation, mediated by TacT depends not only on the relative abundances of the toxin and antitoxin, but also on the balance of acetylation by TacT and deacetylation by Pth (and possibly CobB), which determines the acetylation state of aminoacyl-tRNAs. Thus, *S. Typhimurium* uses an antitoxin-independent detoxification mechanism to resume growth following TacT toxicity.

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**ORIGINAL ARTICLE** Cheverton, A. M. *et al.*  
A *Salmonella* toxin promotes persister formation through acetylation of tRNA. *Mol. Cell* <http://dx.doi.org/10.1016/j.molcel.2016.05.002> (2016)

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