Persister Awakening

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In this issue of *Molecular Cell*, Cheverton et al. (2016) report that *Samonella* toxin TacT contributes to persister formation by acetylating tRNA, a novel mechanism of toxin action. Hydrolyzing corrupted tRNA resuscitates persisters.

Persisters are dormant variants that form a subpopulation of cells tolerant to antibiotics (Lewis, 2010). Persisters are largely responsible for recalcitrance of chronic infections. Knowing the molecular mechanism of persister formation is important for developing treatments that can kill persisters. In this issue of *Molecular Cell*, Cheverton et al. (2016) reported a novel molecular mechanism of persister formation as well as resuscitation.

Most of what we know about the mechanism of persister formation comes from the study of *E. coli*, and toxin-antitoxin (TA) modules have been linked to persister formation. Isolated persisters express high levels of interferases—mRNA-degrading toxins and their associated antitoxins. Ectopic expression of toxins causes multidrug tolerance (Keren et al., 2004). In *E. coli*, deleting single interferases does not produce a phenotype, but the level of persisters was reported to be decreased in a strain deleted in 10 TA interferases (Δ10TA) (Maisonneuve et al., 2013). Single deletion of a toxin may affect persister formation during stresses. One example is TisB, a toxin that is induced during DNA damage and forms an ion channel in the cytoplasmic membrane, decreasing the proton motive force and ATP, leading to drug tolerance (Dörr et al., 2010). Gain-of-function mutations in another toxin, *hipA*, which inhibits protein synthesis by phosphorylating glu-tRNA synthetase (Germain et al., 2013; Kaspy et al., 2013), produces elevated levels of persisters in vitro. The same mutants are present in patients with relapsing urinary tract infections (UTIs), showing that toxins play an important role in the recalcitrance of chronic infections to antibiotics (Schumacher et al., 2015).

In a previous study, the same group reported that persister levels dramatically increase when *Salmonella* enters macrophages. Interestingly, deletion of individual toxins then sharply decreased persister levels in macrophages. One of these toxins is TacT, whose mechanism of action was unknown (Helaine et al., 2014). Here, Cheverton et al. (2016) decipher the molecular mechanism of TacT and provide an important insight into persister resuscitation.

Overexpression of *tacT* in exponential phase had no effect on *Salmonella* growth. However, early overexpression of *tacT* extends the lag phase. To identify the target of TacT, the effect of overexpressing the toxin on label incorporation into DNA, RNA, or protein was tested. Only protein synthesis was inhibited.

TacT has homology to known acetyltransferases, pointing to its possible mechanism of action. Mutations in either the predicted acetyl-CoA binding site or acceptor transfer site completely abolished toxicity of TacT, and deletion of deacetylase CobB lead to toxicity of TacT in exponential phase.

To further pinpoint the target, purified TacT was introduced into an in vitro translation system and was found to acetylate aminoacyl-tRNA but not peptidyl-tRNA. A structural study then revealed that tRNA binds to the TacT dimer by electrostatic interactions, and TacT acetylates the primary amine group of the amino acid on the charged tRNA to prevent the formation of the peptide bond. This is the first report of a toxin that acts by acetylation of charged tRNA (Figure 1).

To better understand growth resumption of persisters, the authors conducted a screen for genes that suppress toxicity of TacT. They found that overexpression of peptidyl-tRNA hydrolase Pth counteracts the toxicity of TacT as well as TacT-induced persister formation, and an in vitro study revealed that Pth hydrolyzes acetylated aminoacyl-tRNA and releases uncharged tRNA. This shows that Pth has enzymatic activity against not only peptidyl-tRNA but also

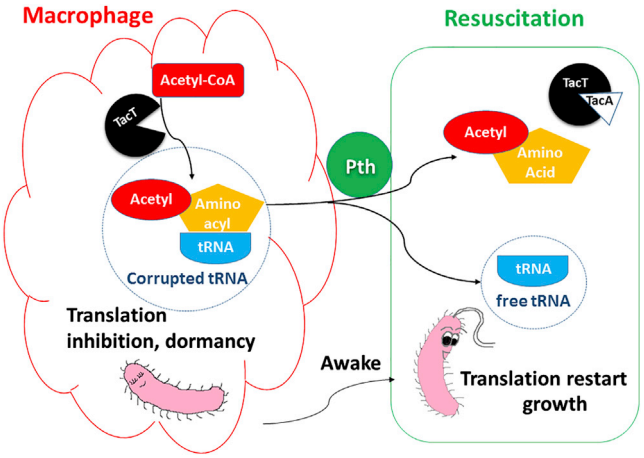

Figure 1. A Novel Molecular Mechanism of *Samonella* Persister Formation and Resuscitation
Samonella toxin TacT acetylates aminoacyl-tRNA to inhibit translation. The action of the toxin may explain the increase in persisters in *Samonella* upon entry into macrophages. The hydrolase Pth cleaves off the acetylated aminoacyl-releasing free tRNA, translation resumes, and persisters wake up.

acetylated aminoacyl-tRNA and may function as a mechanism for persister resuscitation.

This study is the first step toward understanding the physiological roles of this unusually interesting toxin and many important questions remain to be answered: Why does TacT only exhibit toxicity in lag phase—do exponentially growing cells have high deacetylase or hydrolase activity? But then why does a knockout of TacT fail to form persisters when *S. typhimurium* infects macrophages?

It is notable that although TacT is the only known toxin that acetylates charged tRNA, the translation process seems to be a target of choice for many unrelated toxins. For example, mRNA interferases inhibit translation by depleting cellular mRNA pool, and HipA inhibits translation by phosphorylating glu-tRNA synthesase. Interestingly, this mirrors the well-known preference of antibiotics—dozens of unrelated compounds target the highly conserved translation machinery. Bacteriostatic inhibitors of translation, chloramphenicol, or erythromycin cause tolerance to other antibiotics, emulating persister formation. Washing away antibiotics restores growth and susceptibility, but how persisters resuscitate from dormancy caused by toxins has been puzzling. Indeed, if a toxin inhibits translation, it would be difficult for a cell to counter its action by synthesizing an antitoxin. This problem is removed in the case of TacT, where its target can be deacetylated by an existing enzyme such that the detoxification process does not require protein synthesis. Thanks to the study of *Cheverton* and co-authors, we now have the first example of solving the puzzle of persister resuscitation.

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Length Matters: MINDY Is a New Deubiquitinase Family that Preferentially Cleaves Long Polyubiquitin Chains

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Abdul Rehman and colleagues identify a sixth family of deubiquitinase enzymes that are highly conserved throughout eukaryotes and show a remarkable selectivity for cleaving extended Lys-48-linked polyubiquitin chains.

The ubiquitin system orchestrates protein degradation and signal transduction and, as such, is essential for maintaining cellular homeostasis. Ubiquitination describes the covalent ligation of the 76-aa protein ubiquitin to substrates and is reminiscent of glycosylation, in that both chain length and the linkage type dictate signal readout. For example, lysine-48 (K48)-linked ubiquitin chains commonly tag substrates for proteasomal degradation, whereas K63-linked chains may serve as molecular scaffolds to enhance signal transduction (Komander and Rape, 2012). Ubiquitin ligases are the arbiters of selective substrate ubiquitination, whereas deubiquitinases reverse this process. Approximately 100 deubiquitinases have been identified in the human genome and are classified into five families based on the architecture of their catalytic domains: ubiquitin-specific proteases (termed USPs), ovarian tumor proteases (OTUs), ubiquitin carboxy-terminal hydrolases (UCHs), the Machado-Joseph disease proteases (MJDs), and zinc-dependent metalloproteases (JAMMs). Deubiquitinases may contain additional domains that facilitate substrate binding, cofactor association, and otherwise