MS03-2-2 Mechanism of regulation and neutralization of tRNA-phosphorylating small RelA-SpoT Homologue toxins
#MS03-2-2

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Abstract
RelA-SpoT Homologue (RSH) enzymes control bacterial physiology in order to overcome and survive to stressful conditions through synthesis and degradation of a nucleotide second messenger, the guanosine penta and tetraphosphate (also known as a (p)ppGpp). Small alarmone synthetases (SASs) are single-domain RSH enzymes that contains the (p)ppGpp synthesis (SYNTH) domain, but absence of the hydrolysis (HD) domain and regulatory C-terminal domains characteristic from long RSHs family members such as RelA and SpoT. These catalytic domains are found as part of different subfamilies and can be functionally divided into small pyrophosphotransferases including the well-studied RelP, RelQ, TAS1 alarmone synthetases (SASs) that catalyze ATP hydrolysis and pyrophosphate transfer to nucleotides or uncharged tRNA, and small alarmone hydrolases that catalyze the cleavage of phospho-ribose nucleotides.
The faRel2-atFaRel2 operon from Coprobacillus sp. D7 was recently identified as a type II toxin-antitoxin (TA) module in which the toxin FaRel2 that targeted protein synthesis by pyrophosphorylating uncharged tRNAs at the free 3'OH of the CAA end is neutralised by the antitoxin AtFaRel2[1]. The antitoxin from the faRel2-atFaRel2 system has a novel fold that has not been observed associated with toxins from other TA families. While the activity of the aforementioned tRNA-phosphorylating toxins can be counteracted by bacterial SAHs, the natural regulation of these type II TAs toxins by their cognate antitoxin remains unknown. Tas1, the effector enzyme from Pseudomonas aeruginosa PAO1 type VI secretion system (T6SS) is a remote homologue of FaRel2. Once processed and secreted into the target cell, Tas1 produces (p)ppApp, mounting a strong toxic response via depletion of the ATP pool and inhibition of purine biosynthesis [2]. The action of Tas1 is counteracted by the immunity factor Tis1 via blocking of the acceptor nucleotide binding site. However, sequence analysis could not detect meaningful similarity between Tis1 and AtFaRel2 suggesting that tRNA-modifying small RSH toxins may be neutralised via a different mechanism representing a new way of control RSH enzymes.

References
[1] Kurata, T. et al. RelA-SpoT Homolog toxins pyrophosphorylate the CCA end of tRNA to inhibit protein synthesis. Mol. Cell. P3160-3170 (2021)
[2] Ahmad, S. et al. An interbacterial toxin inhibits target cell growth by synthesizing (p)ppApp. Nature 575, 674–678 (2019)