How a multifunctional bacteriophage protein counteracts bacterial defense: Structural basis of inhibition of *E. coli* Type I RM system by T3 SAM lyase

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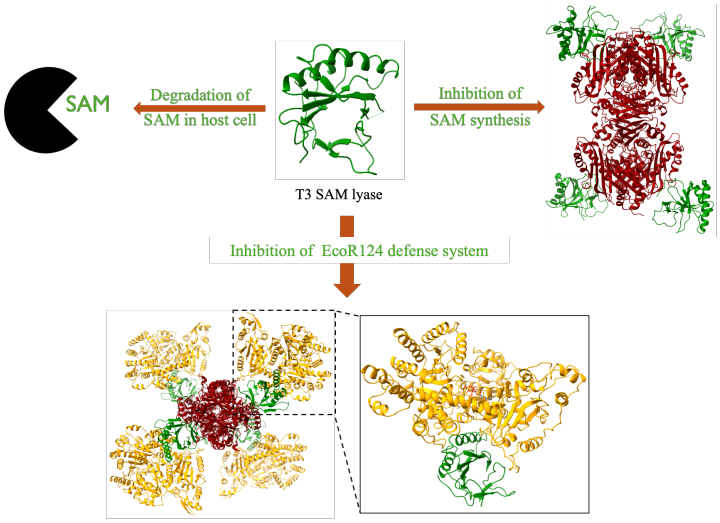
Bacteriophage T3 encodes an S-adenosyl-methionine (SAM) lyase (T3S) which both degrades SAM, and by direct binding inhibits SAM synthesis by MetK[1], leading to inhibition of the SAM-dependent *E. coli* Type I restriction-modification (RM) and BREX defense systems. In addition, *in vitro* and *in vivo* studies demonstrated that T3S inhibits the SAM-independent EcoR124 system, and that catalytically deficient T3S mutants retain this anti-restriction activity. Here we used single particle cryo-EM to clarify how T3S inhibits EcoR124 through direct binding to the HsdR subunit (R) of EcoR124. We purified T3S:HsdR complexes in a pull-down assay and subjected them to single-particle cryo-EM. From one sample, we identified two distinct protein complexes - a small heterodimeric (137 kDa, 3.10 Å) complex of T3S and HsdR, and a large complex (716 kDa, 3.08 Å) where four T3S-HsdR units are bound to a central MetK tetramer. The structures showed that T3S binds to HsdR on the side opposite to the DNA-binding groove, thereby contacting the endonuclease, helicase and helical domains of HsdR. Comparison with previous structures of EcoR124I in the active R_2M_2S complex (composed of HsdR, HsdM and HsdS) revealed that T3S binding would sterically clash with one of the HsdR-HsdM interfaces and induce confirmational changes in HsdR in the second interface, thereby preventing formation of the functional R_2M_2S complex.



T3S counteracts *E. coli* defense systems through three distinct mechanisms.

Reference:

1. Andriianov A, Trigüis S, Drobiazko A, *et al.* (2023) Phage T3 Overcomes the BREX Defense through SAM Cleavage and Inhibition of SAM Synthesis by SAM Lyase. *Cell Reports*, *42* (8): 112972.