Contribution ID: **32** Type: **Poster only**

Poster #32: Mechanism of activation of the defense-associated reverse transcriptase DRT4 by a phage single-stranded DNA-binding protein

Monday, 5 October 2026 20:49 (1 minute)

DRTs are widespread anti-phage defense systems, yet the molecular mechanisms underlying their activation remain poorly understood. Here, we investigate the mechanism of the type 4 defense-associated reverse transcriptase (DRT4), a member of the UG/Abi class 1 systems. Screening against the BASEL bacteriophage collection revealed that DRT4 provides broad protection against diverse phage families. Using phage escape mutants, we identified the Bas17-encoded single-stranded DNA-binding protein (SSB) as an activator of DRT4. Bacterial survival assays demonstrated that gp44 is sufficient to trigger DRT4-dependent growth arrest, indicating that gp44 directly activates the defense response.

CryoEM revealed that DRT4 forms a homohexameric complex and synthesizes covalently linked single-stranded DNA through a protein-priming mechanism. Biochemical and mass spectrometric analyses showed that the synthesized ssDNA is composed predominantly of adenine and guanine nucleotides, with the SSB altering its length distribution in vitro. AlphaFold3 redicted a direct interaction between SSB and the flexible C-terminal domain (CTD) of DRT4, which was supported by mutagenesis, phage infection assays, in vivo transformation assays, and in vitro reconstitution. Disruption of the predicted interaction interface or deletion of the CTD abolished anti-phage defense without impairing ssDNA synthesis, demonstrating that the CTD is required for productive activation rather than polymerase activity. Furthermore, the CTD-interaction was necessary for SSB-dependent stimulation of DRT4 RNase activity.

Together, these findings establish a mechanism in which a phage SSB protein activates DRT4 through interactions with its flexible CTD and ssDNA product, providing new mechanistic insight into reverse transcriptase-mediated bacterial immunity and expanding our understanding of UG/Abi defense systems.

Reference #1

Reference #2

Reference #3

Authors: JASNAUSKAITE, Monika (Vilnius University); CIBULSKIS, Augustas (Vilnius University); KAZLAUSKAITE, Kristina (Vilnius University); SKORUPSKAITE, Aiste (Vilnius University); JUOZAPAITIS, Jonas (Vilnius University); MIKSYS, Algirdas (Vilnius University); RUKSENAITE, Audrone (Vilnius University); TOMKUVIENE, Migle (Vilnius University); MALINAUSKAITE, Lina (Vilnius University); PAUSCH, Patrick (Vilnius University)

Presenter: PAUSCH, Patrick (Vilnius University)

Session Classification: Poster-Session