An enterococcal phage protein inhibits type IV restriction enzymes involved in antiphage defense

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Presenter: Yu-Chieh Kung **Date/Time:** 2025/06/05 15:20-16:10

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Background: The arms race between bacteria and bacteriophages is an ongoing evolutionary event. Selective pressure on both sides drives the development of bacterial defense systems and corresponding phage counter-defense mechanisms. One key bacterial defense is the restriction-modification (R-M) system, which degrades foreign DNA while protecting self-DNA through specific modifications. In response, phages have evolved their own DNA modification systems to evade cleavage by host restriction enzymes. To overcome these phage adaptations, bacteria have developed type IV restriction enzymes, which specialize in recognizing and cleaving modified foreign DNA. However, the battle continues: in this study, a phage-encoded protein named **type IV restriction inhibiting factor A (TifA)** was identified as an anti-defense factor capable of inhibiting these bacterial enzymes. This discovery illustrates the ongoing and dynamic nature of the evolutionary arms race under natural conditions.

Objective/Hypothesis: This study investigates whether point mutations in the phage-encoded **TifA** protein enable selective inhibition of different type IV restriction enzymes. Screening revealed that specific TifA mutations enhance inhibition of the GmrSD-like enzyme **EF_B0059**, supporting the hypothesis that such mutations increase functional flexibility. Protein interaction and in vivo inhibition assays were used to validate this hypothesis.

Result: Co-immunoprecipitation assays revealed that the mutant TifA displayed higher binding affinity to EF_B0059 compared to the wild-type protein. Correspondingly, in vivo experiments showed stronger inhibition of EF_B0059 activity by the mutant TifA. However, the opposite trend was observed with another type IV restriction enzyme, **BrxU**, where wild-type TifA exhibited stronger inhibition. These findings indicate that TifA's inhibitory capacity correlates with its binding affinity and that point mutations can alter its target specificity. This highlights the evolutionary adaptability of phage anti-defense strategies against diverse bacterial restriction systems.