A prophage encoded ribosomal RNA methyltransferase regulates the virulence of Shiga-toxin-producing *Escherichia coli* (STEC)

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Presenter : Fang-Yu Liu **Date/Time** : 2025/05/08 15:20-16:10

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Background

Shiga toxin-producing *Escherichia coli* (STEC) strains are foodborne pathogens that cause severe human diseases. Some patients infected with STEC may develop hemolytic uremic syndrome (HUS), a condition associated with a high mortality rate and permanent sequelae. The main pathogenic mechanism of STEC is the production of Shiga toxin (Stx), which is encoded on a temperate bacteriophage. Treatment of STEC-infected patients with certain antibiotics can induce the lytic cycle of Stx-encoding prophages, resulting in increased production and release of Stx. In addition to antibiotic-induced lytic activation, spontaneous induction of Stx-encoding prophages also contributes to the pathogenicity of STEC. However, different Stx-encoding prophages exhibit variations in virulence, and the underlying mechanisms regulating prophage activation are not yet fully understood.

Objective

To identify the factors that regulate the differences in virulence between two closely related Stx-encoding prophages, φPA2 and φPA8.

Results

First, the authors found that STEC strains harboring φPA8 exhibited significantly higher virulence compared to those containing φPA2. To identify the potential regulators responsible for this difference, they compared the gene sequences of the two prophages. They identified a unique gene in φPA8, encoding M.EcoPA8orf6770P, which is annotated as an adenine DNA methyltransferase. Structural analysis predicted that this protein belongs to the MT-A70 RNA methyltransferase superfamily. Functional assays revealed that M.EcoPA8orf6770P alone exhibits weak methyltransferase activity in vitro. However, co-expression with a neighboring gene encoding a putative nucleic acid binding protein called PNB-2 significantly enhanced its methyltransferase activity. Furthermore, when in complex with PNB-2, the holoenzyme exhibited strong RNA methyltransferase activity, preferentially targeting 16S rRNA. Taken together, these findings suggest that M.EcoPA8orf6770P requires interaction with PNB-2 to acquire substrate specificity and full methyltransferase activity.

Conclusion

This study reveals that the prophage-encoded RNA methyltransferase M.EcoPA8orf6770P functions with PNB-2 to form an holoenzyme. This complex enhances STEC virulence, potentially by promoting prophage induction through RNA modification, specifically targeting 16S rRNA.