Contribution ID: **36** Type: **Short-talk only**

Function of Phage Transfer-Messenger RNA in a crAss-like phage phi14:2

Monday, 5 October 2026 15:20 (15 minutes)

CrAss-like phages are a group of viruses that include some of the most abundant phages in the human gut. These phages infect bacteria of the Bacteroidetes phylum. Despite their abundance, their molecular biology remains poorly understood.

In this study, we use phi14:2, a crAss-like phage isolated from the marine microbiome that infects *Cellulophaga baltica*, as a model system to investigate the role of phage-encoded transfer-messenger RNA (tmRNA). In bacteria, tmRNA rescues stalled ribosomes from defective mRNA transcripts lacking a stop codon and directs incomplete proteins for degradation through a process known as trans-translation. The role of phage tmRNA remains unknown.

We show that the phage tmRNA is one of the most abundant transcripts produced during infection. The entire pool of phage tmRNA co-fractionates with host ribosomes and interacts with host SmpB, a protein required for tmRNA activity. We demonstrated that the phage tmRNA-encoded tag peptide is a degradation tag, promoting more efficient degradation than the host one. To reveal the function of the phage tmRNA, we established a reporter system in *C. baltica* to monitor trans-translation.

In parallel, we established in-cell crosslinking mass spectrometry for phage-infected bacteria, enabling detection of phage-host protein interactions. We identified phage protein gp40 interacting with host RNase R, an enzyme responsible for degrading non-stop transcripts in coordination with trans-translation. This suggests that the phage may contribute to the disposal of non-stop transcripts.

Reference #1

Drobysheva, A.V., Panafidina, S.A., Kolesnik, M.V. et al. Structure and function of virion RNA polymerase

Reference #2

Janssen, B.D., Hayes, C.S. The tmRNA ribosome-rescue system. Advances in Protein Chemistry and Structural

Reference #3

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Session Classification: Organisation and regulation of the viral infection cycle

Track Classification: Cellular organisation and regulation of the viral infection cycle