Phage tRNAs evade tRNA-targeting host defenses through anticodon loop mutations

Daan F. van den Berg
Department of Bionanoscience, Delft University of Technology, Delft, Netherlands
https://orcid.org/0000-0002-2217-4074

Baltus A. van der Steen
Department of Bionanoscience, Delft University of Technology, Delft, Netherlands
https://orcid.org/0000-0001-8193-4814

Ana Rita Costa
Department of Bionanoscience, Delft University of Technology, Delft, Netherlands
https://orcid.org/0000-0001-6749-6408

Stan J. J. Brouns (stanbrouns@gmail.com)
Department of Bionanoscience, Delft University of Technology, Delft, Netherlands
https://orcid.org/0000-0002-9573-1724

Short Report

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Abstract

tRNAs in bacteriophage genomes are widespread across bacterial genera, but their exact function has remained unclear for more than 50 years. Multiple hypotheses have been proposed, with the most established being codon compensation, where codons more rarely used by the host but necessary for the phage are supplemented by tRNAs encoded by the phage. Here, we combine several observations and propose a new hypothesis that phage-encoded tRNAs are a means to counteract the tRNA-depleting strategies of the host to defend from viral infection. Based on mutational patterns of tRNA anticodon loops, we predict that phage tRNAs are insensitive to the host tRNAses. For tRNAs targeted in the anticodon itself, we observe phage counter-selection of targeted isoacceptor tRNAs, further supporting the hypothesis that phage tRNAs are selected to be insensitive to host anticodon nucleases.

Importance

The presence of tRNAs in phages was discovered more than 50 years ago and their function has been debated ever since. Here, we propose that phage tRNAs counteract the tRNAse activities of the host, which may represent a depletion strategy of essential cellular components to stop translation and thereby phage infection.

Background

Transfer RNAs (tRNAs) were first discovered in the 1950s (Kresge et al., 2005) and have since been found to play a vital role in the central dogma of molecular biology in all living systems (Crick, 1980). During the 1960s, tRNAs were also reported in viruses of bacteria (phages) (Weiss et al., 1968). We now know that phage-encoded tRNAs are widespread (Bailley-Bechet *et al.*, 2007). Multiple hypotheses have been proposed for the role of these phage-encoded tRNAs. The most established being codon compensation, where codons rarely used by the host but necessary to the phage are supplemented by the tRNAs carried by the phage (Bailly-Bechet et al., 2007). Why phages are pushed towards these alternative codons is generally believed to be a side effect of differences in the GC content of phage and host (Bailly-Bechet et al., 2007; Lucks et al., 2008; Limor-Waisberg et al., 2011). A recent study by Yang et al. (2021) may have hinted at an additional factor: phage tRNAs represent a means to counteract the depletion of host tRNAs that occurs as a general response to phage infection (Thompson & Parker, 2009; Yang et al., 2021; Jain et al., 2021; Amitsur et al., 1989). However, it remains unclear how phage tRNAs avoid being degraded by the same mechanism that results in host tRNA depletion during phage infection.

Hypothesis

We hypothesize that the tRNAs encoded by phages are insensitive to tRNA anticodon nuclease activity, preventing depletion of the tRNA pool during phage infection. To investigate this hypothesis, we analyzed the tRNAs encoded by a large and well-characterized dataset of tRNA-rich bacteriophages (33 tRNAs per phage on average) that infect mycobacteria: mycobacteriophage cluster C1 (Russell & Hatfull, 2017)

(Fig. 1A,B). The existence of these tRNA-rich phages coincides with a high abundance of tRNA nucleases (tRNAses), including the well-characterized VapCs, MazFs, and RelEs in Mycobacterium (Winther et al., 2016; Chauhan et al., 2022; Cruz et al., 2015; Cintrón et al., 2019; Barth et al., 2021; Pedersen et al., 2003). A subset of these tRNAses target the tRNA anticodon loop and are activated upon a variety of stress responses, including phage infection (Calcuttawala et al., 2022). When activated, these anticodon nucleases cleave specific tRNAs in conserved regions within the anticodon loop to inactivate these tRNAs and thereby regulate protein translation of the host (Winther et al., 2016). The cleavage region within the tRNA anticodon loop is sequence-dependent and highly specific for the type of tRNA. Mutations within the recognition and cleavage site in the anticodon loop have been found to cause insensitivity to these anticodon nucleases (Winther et al., 2016; Cruz et al., 2015). We compared the tRNAs encoded by phages with those of their host and observed all 10 phage-encoded tRNAs that are known to be targeted by anticodon nucleases to contain anticodon loop mutations (Winther et al., 2016; Cruz et al., 2015; Chauhan et al., 2022), reinforcing the idea that phage-encoded tRNAs are likely insensitive to cleavage (Fig. 1C). We hypothesize that these phage tRNAs represent a means to counteract the depletion of tRNAs by anticodon nucleases during phage infection, allowing the phage to translate its proteins and complete its infection cycle (Fig. 1D).

Supporting the selective pressure of the host-encoded tRNA nucleases, we also observed a strong counter-selection for tRNAs that are cleaved in the anticodon itself (Table S1). This is the case for the majority of the serine-coding tRNAs that are cleaved at the GA site within the anticodon: tRNA-Ser(gga), tRNA-Ser(tga), tRNA-Ser(cga), and tRNA-Ser(aga) (Winther et al., 2016). In this instance, the phage encodes an isoacceptor tRNA that is not targeted (tRNA-Ser(gct)) to carry out translation independent from cleaved serine isoacceptor tRNAs. We observed the same counter-selection for the UAN anticodons, which are known targets of RelE in *E. coli* (Pedersen et al., 2003). Interestingly, we observed that phage genes do not avoid codons of cleaved tRNAs, nor do they have a preference for codons with nuclease insensitivity (Welch Two Sample t-test, $t = 0.53848$, $df = 41.583$, p-value > 0.05), suggesting that the selection of phage tRNAs is only determined by their insensitivity to tRNAses and not by codon usage. Altogether, our observations support the hypothesis that phage tRNAs are selected to be insensitive to anticodon nucleases to counteract tRNA-depletion strategies of the host that limit phage propagation. We expect that our hypothesis may be extended outside of Mycobacteria as phage tRNAs and host tRNAses are widespread (Ogawa et al., 2006; Covard & Lazdunski, 1979; Jones et al., 2017).

Implications

We argue that phage-encoded tRNAs escape targeting by host tRNAses, which can be helpful in selecting or engineering bacteriophages capable of infecting hosts containing anticodon nucleases.

Method

All C1 cluster mycobacteriophage genomes were downloaded from https://phagesdb.org/ on the 1st of September 2022. tRNAs were annotated using Aragorn (Laslett & Canback, 2004) and tRNAscan-SE

(Chan *et al.*, 2019), and were further analyzed using MXfold2 (Sato et al., 2021). Cusp was used to calculate the codon frequency (Rice et al., 2000).

Declarations

Competing interests:

The authors declare no competing interests.

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Figures

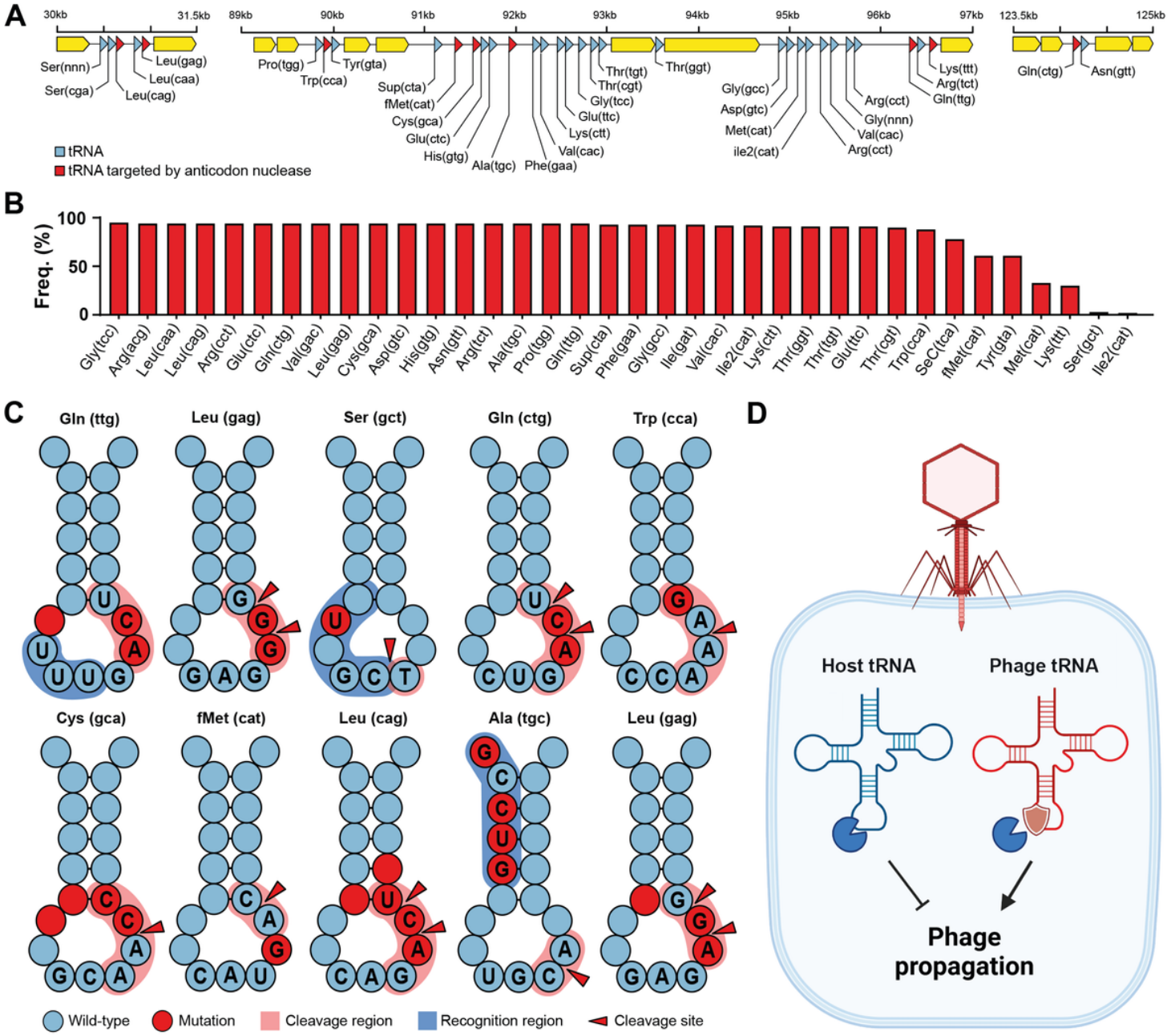

Figure 1

Phage tRNAs are predicted to be anticodon nuclease resistant. (A) The genomic context of the tRNA clusters containing 36 tRNAs present in C1 mycobacteriophage Rizal (Russell & Hatfull, 2017). (B) Prevalence of individual phage-encoded tRNAs in the C1 mycobacteriophage cluster, composed of 161 phages. (C) Mutations in the anticodon-loop of phage tRNAs in comparison to host tRNAs, located in the cleavage site of anticodon nucleases. (D) Proposed mechanism of action of phage tRNAs. During phage infection, tRNAses are activated and deplete the host tRNA pool via tRNA cleavage to prevent phage propagation. Phage tRNAs are insensitive to cleavage and refill the tRNA pool allowing the phage to propagate.

Supplementary Files

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- TableS1.xlsx