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EDITED BY

Sonia Zuñiga,
Spanish National Research Council
(CSIC), Spain

REVIEWED BY

Phuong Cao Thi Ngoc,
Lund University, Sweden
Kunqi Chen,
Fujian Medical University, China

*CORRESPONDENCE

Juana Díez
juana.diez@upf.edu

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The host tRNA epitranscriptome: A new player in RNA virus infections

Marc Talló-Parra, Elena Muscolino and Juana Díez*

Molecular Virology group, Department of Medicine and Life Sciences, Universitat Pompeu Fabra,
Barcelona, Spain

Viruses completely depend on the host translation machineries to express the viral proteins. Recent data reveal an unprecedented interaction of positive strand RNA ((+)RNA) viruses with the host tRNA epitranscriptome to favor viral protein expression *via* a specific reprogramming of codon optimality that ultimately favors decoding of the viral codons. We propose that this feature is shared by multiple RNA viruses and that the involved tRNA modifying enzymes represent promising novel targets for the development of broad-spectrum antivirals.

KEYWORDS

tRNA, tRNA modifications, tRNA epitranscriptome, (+)RNA viruses, CHIKV, KIAA1456, viruses, codon usage

Introduction

All steps of viral life cycles require factors from the host they infect. However, unlike replication and transcription where viral proteins direct these functions, viruses completely depend on the host translation machinery to express the viral proteins. This dependence is particularly apparent for positive strand RNA ((+)RNA) viruses. Since they do not carry their polymerases in the virions, upon release of their genomes into the cytoplasm, (+)RNA viruses first need to translate their genomes to express the viral replicases (1). To maximize viral protein expression, they display different strategies to take control of the host translation machinery. In turn, the host reprograms this machinery in an attempt to repress virus infection in an ever-evolving race to gain control of it (2). The translation process requires three major steps: initiation, in which the ribosomes are recruited to the initiation codon; elongation, in which the amino acids are brought to the ribosomes by transfer RNAs (tRNAs) and linked together to form a chain; and termination, in which the finished polypeptide is released. Most of our knowledge on the strategies used by (+)RNA viruses to efficiently translate their genomes relate to events affecting the translation initiation step. The involvement of the other steps of the translation process has been almost unexplored. We have recently uncovered an unprecedented interplay of (+)RNA viruses with the host tRNA epitranscriptome to

it down because the cognate tRNAs are lowly abundant in comparison to those tRNAs used for optimal codons (10–12). Modifications at the wobble positions affect their base pairing with the third position of the codon ultimately expanding or restricting the decoding efficiency of certain synonymous codons. Well-studied examples include the mcm^5 and mcm^5s^2 modifications at uridine 34 (U34) of cytoplasmic tRNAs. In the tRNA anticodon, position 35 and 36 follow the base pairing rules defined by Watson and Crick. However, when an unmodified U is located at position 34 (wobble U34), U recognizes both adenine (A) and guanine (G) in the third position of the

codon. Modifications at this position might favor base pairing with A (A-ending codons) or G (G-ending codons). For example, the Glutamic acid (Glu) amino acid is encoded by two synonymous codons GAA and GAG. When the U34 position of the cognate tRNA is not modified, the tRNA decodes both GAG and GAA codons (Figure 2A). However, when U34 is mcm^5/mcm^5s^2 modified, the tRNA favors decoding of the GAA codon (13, 14) (Figure 2A). The fact that U34 is modified in practically all organisms implies a strong evolutionary pressure and a major role in translation regulation (15). Indeed, it is now established that tRNA

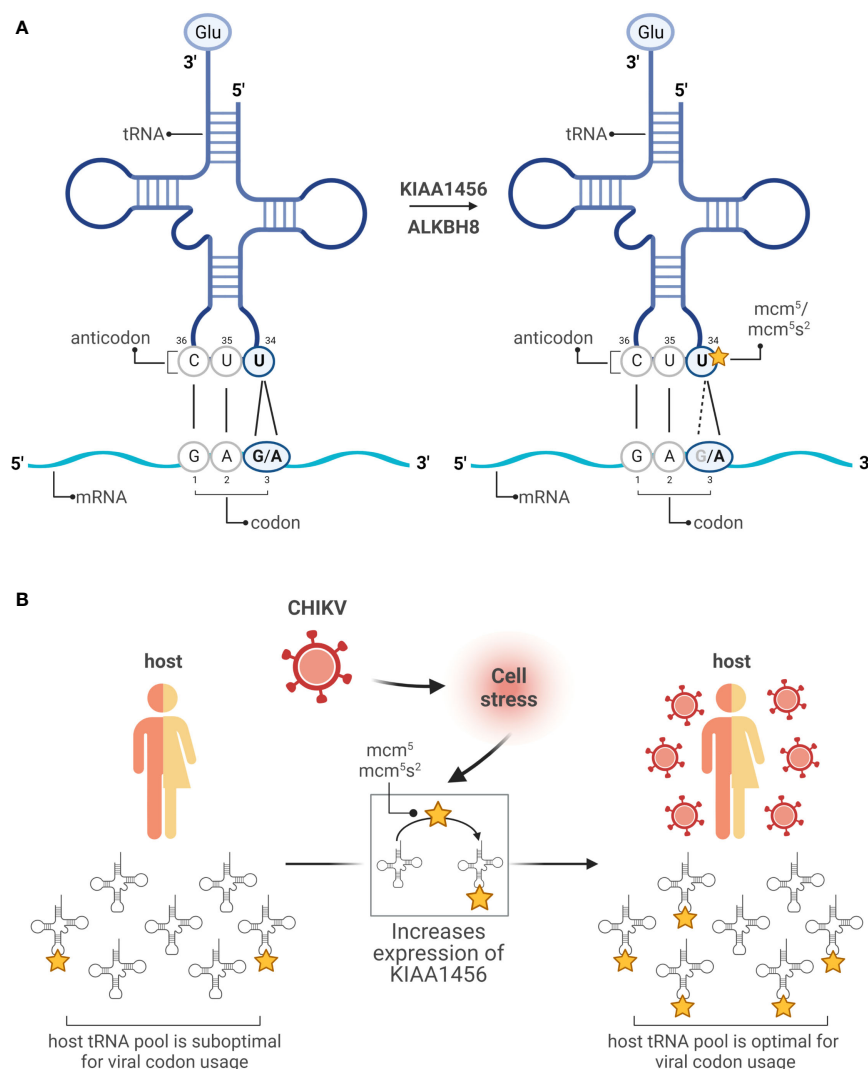


FIGURE 2

Modifications in the wobble uridine changes the reading. **(A)** In the anticodon of a tRNA coding for Glu (GAG/A), unmodified U34 base pairs with G or A in the third position of the mRNA codon (left). Following modifications promoted by KIAA1456 or ALKBH8 enzymes, U34 is favored to pair with an A (right). **(B)** Working model: the host tRNA pool is enriched toward host mRNAs containing optimal codons, while the CHIKV genome is enriched in suboptimal codons. CHIKV infection triggers a DNA damage response that promotes the expression of the tRNA-modifying enzyme KIAA1456. This ultimately results in a reprogramming of codon optimality that favors translation of suboptimal codons, enriched in both the host mRNAs encoding DNA damage response genes and the CHIKV RNAs. Created with [BioRender.com](https://www.biorender.com).

modifications are highly dynamic and their prevalence varies in response to levels of cellular metabolite and environmental stresses (4, 16). Moreover, global analyses have demonstrated a coordinated interplay between changes in the modifications of the wobble position, including U34, and selective translation of stress-dependent gene expression enriched in synonymous codons which decoding is favored by the induced modifications [reviewed in (16)]. Together, this indicates a coordinated interplay between the tRNA epitranscriptome and the codon bias to adapt translation during stress responses (17).

The genomes of numerous and diverse (+)RNA viruses, including SARS-CoV-2, dengue (DENV), zika (ZIKV) or chikungunya (CHIKV) viruses, are enriched in rare codons. While highly expressed human mRNAs are enriched in G/C-ending codons, the genome of multiple (+)RNA viruses is enriched in A/U-ending codons (17). How (+)RNA genomes are able to express their proteins so efficiently in spite of their unfavorable codon usage? To address this fundamental question, we use CHIKV as a model system because its genome is translated to extremely high levels. Genome-wide transcriptome and translome analyses together with fractionation studies showed that CHIKV infection dramatically alters the host mRNA translome at the endoplasmic reticulum, the translation compartment where CHIKV efficiently translates, while few significant changes were observed at the cytosol, the other major cellular translational compartment (3). The set of translationally repressed host mRNAs were enriched for genes related to mitochondria, ribosomes and RNA translation. The set of translationally activated host mRNAs were enriched for genes related to DNA damage response, cell cycle and RNA transport. Importantly, these translationally activated mRNA, as the CHIKV RNA, displayed a poor codon usage, mostly enriched in GAA (Glu), AAA (Lys), CAA (Gln), AGA (Arg) and GGA (Gly) codons. In contrast the translationally repressed mRNAs displayed an optimal one enriched in the corresponding G-ending synonymous codons (3). This indicates a virus-induced reprogramming of codon optimality that favors translation of viral RNAs. Mechanistically, it involves CHIKV-induced translational activation of the KIAA1456 enzyme, a paralog of the ALKBH8 enzyme involved in the mcm^5/mcm^5s^2 modifications (described above) that favor decoding of the GAA (Glu), AAA (Lys), CAA (Gln), AGA (Arg) and GGA (Gly) codons over the G-ending ones (Figure 2B) (18, 19). Interestingly, the KIAA1456 mRNA is itself enriched in these five codons, suggesting a positive feedback loop. Importantly, DENV, whose genome is enriched in these codons, also induces in the infected cell overexpression of the KIAA1456 enzyme and a corresponding increase in mcm^5 tRNA modification levels while hepatitis C virus, whose genome is not enriched, does not (3). Notably, the use of KIAA1456-enzymes by viruses seems to be conserved beyond human viruses as in *Escherichia coli* the tRNA^{Lys} U34 modification is required for the phage lambda to express gpG and gpGT, two viral replication proteins. However, in this case the tRNA modification modulates

a specific ribosomal frameshift essential for proper expression of the gpG:gpGT ratio instead of affecting global reprogramming of codon usage (20).

The DNA damage response is a regulatory network that detects DNA damage and mediates its repair (21). Interestingly, in yeast, alkylation stress that cause DNA damage result in an increase of (i) the expression of TRM9, the yeast homolog of human ALKBH8/KIAA1456, (ii) the levels of mcm^5/mcm^5s^2 tRNA modification and (iii) the expression of genes related to DNA damage and cell cycle control, essential to survive alkylation stress. These genes are enriched in GAA and AGA codons. The similarities of these findings with the CHIKV observations strongly suggest that CHIKV has adapted its genome to the host translation environment under infection conditions. CHIKV infection triggers a DNA damage response that results in a reprogramming of codon optimality that ultimately would favor CHIKV RNA genomes enriched in the same codons as the DNA damage response genes. We propose that this mechanism is shared by other (+)RNA viruses beyond DENV and that the DNA damage response triggered by viral infection might have played a role in the shaping of the (+)RNA virus codon usage. This proposal is based on the next observations. First, as CHIKV and DENV, many other (+) RNA viruses are enriched in GAA, AAA, CAA, AGA and GGA codons (unpublished observation). Of note, as the genomes of some viruses outside the (+)RNA viral group are also enriched in these codons (unpublished observation), this mechanisms might be conserved across viral groups. Second, growing evidence indicates a complex interplay between RNA viruses and DNA damage responses. The activation and manipulation of the DNA-damage response by DNA viruses and retroviruses to optimize their replication processes is well-studied (22–24). This activation is triggered by the incoming viral DNA, the integration of retroviruses or in response to the aberrant DNA structures generated upon replication of DNA viruses among other factors. Although at present, only a few RNA viruses have been reported to interact with the DNA damage response, growing evidence indicates that RNA viruses also can manipulate this pathway to their benefit (22, 24, 25). Two examples include ZIKV and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Two main components of the DNA-damage response regulatory network are the ataxia–telangiectasia mutated (ATM) and ATM Rad3-related (ATR) kinases (26). In ZIKV infection, the induction of the ATM signaling and corresponding S-phase arrest increases viral replication (27). Moreover, both ATM and ATR kinases have been described to be activated by PRRSV infection, co-localize with the viral replication complexes and promote replication (28). How (+)RNA viruses that replicate at the cytosol induce the DNA-damage network remains unclear. Putative mechanisms would include promoting inappropriate S phase entry or direct modification or components of the DNA damage response.

Conclusions and remarks

Novel emerging evidence highlights the roles of tRNA modifications in viral infections. Besides the described GAA, AAA, CAA, AGA and GGA codons CHIKV-induced translationally activated host mRNAs and CHIKV RNAs were enriched in other A/U ending codons whose recognition would depend on modifications at the U34 position still to be identified. Thus, it is much likely that besides KIAA1456, other tRNA modifying enzymes play major roles in CHIKV infection. We propose that this feature is shared by multiple RNA viruses and that the involved tRNA modifying enzymes represent promising novel targets for the development of broad-spectrum antivirals. Excitingly, recent work demonstrates that the host immune system also relies on tRNA modifications. A specific tRNA methylation (m1A58) selectively enhances protein translation in a codon-usage manner of a set of proteins that ultimately drive T cell proliferation (29). Together, these findings represent the starting study point of a previously unappreciated tRNA epitranscriptome-virus-immune system regulatory network that is awaiting to be deciphered and will provide novel opportunities for therapeutic interventions.

The tRNA epitranscriptome is a growing field whose complexity presents major challenges. First, to elucidate the tRNA expression patterns in the different tissues and cell types. The human genome contains around 400 genes, including isodecoders and isoacceptors (tRNA genes that share the same anticodon but have different body sequences and vice versa, respectively) that are not all transcribed in the same cell and whose expression is cell-specific. Although major advances have been made in the tRNA sequencing methods (30–32), to obtain and analyze a complete tRNA profile remains challenging in comparison with RNAseq. Second, to complete the mapping of tRNA modifications and the assignment of their roles. The diversity, dynamic nature and low abundance of some modifications are major challenges for their identification. Next-generation sequence (NGS), mass-spectrometry and nanopore-based detection are the main techniques towards the characterization of RNA modifications. However, the current methods have many limitations (31, 33). Moreover, only around 50% of tRNA modifying enzymes have been experimentally validated. This hampers the study of the function of many tRNA modifications. Thus, there is much fundamental biology of the tRNA epitranscriptome still to be learnt. Viruses are powerful tools to uncover cellular processes. Indeed, virus

research has identified fundamental translation features such as capping, polyadenylation or splicing. We thus expect that the encounter of the virology and the tRNA epitranscriptome fields will help revealing new insights not only into viral infections and their control but also into tRNA biology.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization and writing of the perspective: MT-P, EM, JD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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