



Minireview

Transfer RNA-Derived Small Non-Coding RNA: Dual Regulator of Protein Synthesis

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Transfer RNA-derived small RNAs (tsRNAs) play a role in various cellular processes. Accumulating evidence has revealed that tsRNAs are deeply implicated in human diseases, such as various cancers and neurological disorders, suggesting that tsRNAs should be investigated to develop novel therapeutic intervention. tsRNAs provide more complexity to the physiological role of transfer RNAs by repressing or activating protein synthesis with distinct mechanisms. Here, we highlight the detailed mechanism of tsRNA-mediated dual regulation in protein synthesis and discuss the necessity of novel sequencing technology to learn more about tsRNAs.

Keywords: tRF, tRNA, tsRNA, translation

INTRODUCTION

Transfer RNAs (tRNAs) were initially identified to decode genetic information by delivering amino acids to growing polypeptide chains in ribosomes. A growing body of evidence shows that tRNAs have diverse noncanonical functions implicated in cell proliferation, tumor metastasis, and neuronal homeostasis. tRNA fragments also contribute to the noncanonical functions of tRNAs (Schimmel, 2017).

tRNA cleavage products had been considered as degradation products for more than three decades. Recently, they were identified as functional non-coding RNAs and were called tsRNAs (tRNA-derived small RNAs), tRFs (tRNA-derived

RNA fragments), or tiRNAs (tRNA-derived stress-induced RNAs) (Haussecker et al., 2010; Lee et al., 2009; Yamasaki et al., 2009). tsRNAs are produced by cleavage at specific sites in tRNAs or pre-tRNAs, and their expression does not correspond to cognate tRNA levels (Kumar et al., 2014a), demonstrating that tsRNAs are not degradation products but precisely regulated non-coding RNAs.

Several types of tsRNAs have been identified and found to play physiological roles in many cellular processes, including protein synthesis, inheritance, oncogene regulation, transposon element regulation, and chromosome remodeling; however, their detailed mechanisms have not been fully determined (Kumar et al., 2016). They are also implicated in various human diseases, such as cancers and neuronal disorders, suggesting that tsRNAs can be developed as biomarkers or therapeutic targets.

Interestingly, tsRNAs are more deeply involved in protein synthesis compared with other non-coding RNAs. They inhibit global translation by displacing translation initiation factors, displacing mRNA from the translation initiation complex, or binding to aminoacyl-tRNA synthetases (Gebetsberger et al., 2017; Ivanov et al., 2011; 2014; Lyons et al., 2017; Mleczko et al., 2018). They also enhance mRNA translation by unfolding the mRNA secondary structure during translation or interacting with polysomes or the human multisynthetase complex (Fricker et al., 2019; Keam et al., 2017; Kim et al., 2017).

Here, we review the classification of tsRNAs and the detailed mechanisms by which tsRNAs regulate protein synthe-

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sis and discuss the necessity of a novel sequencing method for RNAs bearing modified nucleotides.

tsRNA TYPES

tsRNAs are processed by cleavage at distinct positions in mature tRNAs or pre-tRNAs; thus, they are classified according to their cleavage site. tsRNAs are largely categorized into 5' tsRNAs (tRF-5) and 3' tsRNAs (tRF-3) (Fig. 1) (Haussecker et al., 2010; Lee et al., 2009). In human kidney cells, 5' tsRNA begins with the 5' end of mature tRNA and ends in various positions between D-loop and the anti-codon loop of mature tRNAs, producing 14-16 nucleotide (nt), 22-24 nt, or 28-35 nt form (Kumar et al., 2014a). Different populations of 5' tsRNAs were reported from prostate cancer and *Haloferax volcanii*, indicating that the complexity of 5' tsRNAs might depend on the cell type and species. 3' tsRNAs have relatively uniform length. Their 5' ends are in the T-loop (18 or 22 nt) or anti-codon loop (30-35 nt), and their 3' ends correspond

to the 3' termini of mature tRNA (Kumar et al., 2014a).

In particular, tsRNAs of 30-35 nt in length are referred to as tRNA halves because their length is nearly half of the length of mature tRNAs (Fig. 1) (Lee and Collins, 2005; Yamasaki et al., 2009). They are also called tiRNAs because they are induced under various stress conditions, such as amino acid/glucose starvation, heat shock, hypoxia, or UV irradiation in many species (Elkordy et al., 2018; Fu et al., 2009; Goodarzi et al., 2015; Haiser et al., 2008; Jöchl et al., 2008; Lee and Collins, 2005; Levitz et al., 1990; Thompson et al., 2008).

There are two additional types of tsRNAs; however, their involvement in the regulation of protein synthesis remains unclear. One is derived from the internal body of mature tRNA straddling the anti-codon region (i-tRF or tRF-2), which has a heterogeneous length (Kumar et al., 2016; Telonis et al., 2015). The other is derived from the 3'-trailer sequence of pre-tRNA (Haussecker et al., 2010; Lee et al., 2009). The following websites provide information on the identified ts-

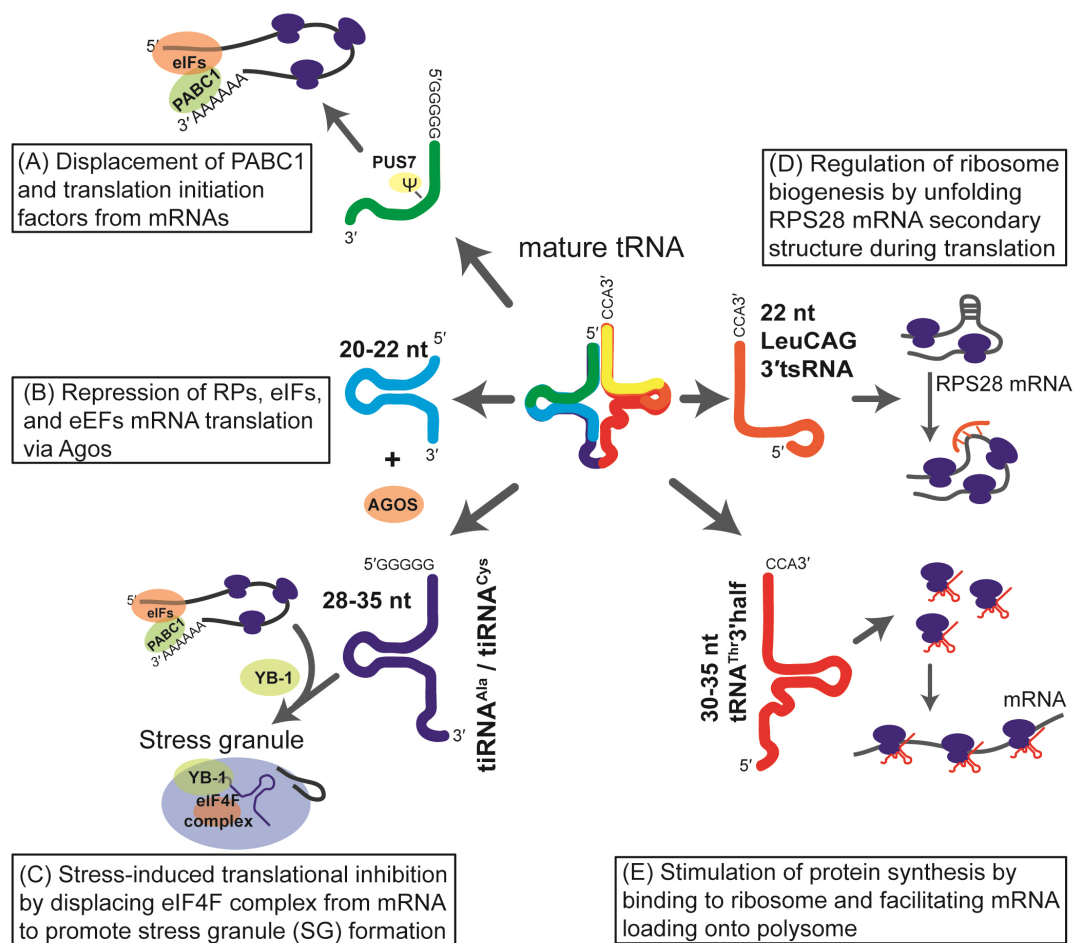


Fig. 1. tsRNA regulates mRNA translation with distinct mechanisms. (A) 18-nt TOG-contained 5' tsRNA binds to PABPC1 to displace PABPC1 and translation initiation factors from mRNA. (B) 20- to 22-nt 5' tsRNA binds to RPs, eIFs, and eEFs mRNAs and repress their translations. (C) TOG contained 5' tiRNAs are induced under various stresses and inhibit global translation by displacing eIF4F complex from mRNA and promoting SG formation. (D) 22-nt LeuCAG 3' tsRNA is required for ribosome biogenesis. It enhances *RPS28* mRNA translation by unfolding the secondary structure of target sites in *RPS28* mRNA during translation. (E) tRNA^{Thr} 3' half stimulates protein synthesis by binding to ribosome and facilitating mRNA loading onto polysomes.

Table 1. Publicly available tsRNA database

Website	Website address	Reference
tRFdb	http://genome.bioch.virginia.edu/trfdb	(Kumar et al., 2014b)
tRF2Cancer	http://rna.sysu.edu.cn/tRFfinder	(Zheng et al., 2016)
MINTbase v2.0	https://cm.jefferson.edu/MINTbase/	(Pliatsika et al., 2018)
PtRFdb	http://223.31.159.8/PtRFdb/index.php	(Gupta et al., 2018)

RNA population from multiple high-throughput sequencing data (Table 1) (Gupta et al., 2018; Kumar et al., 2014b; Pliatsika et al., 2018; Zheng et al., 2016).

NEGATIVE REGULATORS

Global translation inhibition under stress conditions

Cytoplasmic stress granules (SGs) are formed under stress conditions and reprogram cellular translation, which alleviate energy-consuming cellular processes including transcription and translation. Two different pathways generate SGs. One is dependent upon eukaryotic translation initiation factor (eIF) 2 α phosphorylation. Stress conditions allow eIF2 α to be phosphorylated, which reduce ternary complexes (eIF2 α -GTP-tRNAiMet) and promote the formation of SGs in the cytoplasm (Anderson and Kedersha, 2008). The other SG-generating pathway is independent of eIF2 α phosphorylation (Dang et al., 2006; Farny et al., 2009). This pathway is triggered by two kinds of compounds. One class inhibits the function of the RNA helicase eIF4A, including 15-deoxy- Δ (12,14)-prostaglandin J2, pateramine A, hippuristanol, and silvestrol. The other class interferes with the interaction of eIF4E and eIF4G, containing sodium selenite, hydrogen peroxide, and tiRNAs (Panas et al., 2016).

tRNA cleavage generating tsRNAs (tiRNAs) under stress was initially considered as a cellular process that reduces the abundance of mature tRNA under stress conditions. However, the mature tRNA pool is not significantly altered even though the abundance of tiRNAs is markedly increased under stress conditions (Lee and Collins, 2005; Thompson et al., 2008; Yamasaki et al., 2009), indicating that cleavage events do not actually reduce the level of mature tRNA. Instead, the tiRNA themselves play a role in global translation inhibition under stress, which was originally observed in plant and human cells (Yamasaki et al., 2009; Zhang et al., 2009). In plants, 5' and 3' tiRNAs or bigger tRNA fragments were found to be enriched in the phloem sap (PS) but not in the leaves of pumpkin plants. These fragments inhibited translation in an unspecific manner in an *in vitro* translation assay (Zhang et al., 2009).

tiRNA-mediated global translation inhibition was also observed in human U2OS cells by Anderson's group. They identified the arsenite, heat shock, or ultraviolet irradiation induced tiRNAs and a secreted ribonuclease, angiogenin is a ribonuclease required for producing tiRNAs under stress conditions (Yamasaki et al., 2009). The transfection of angiogenin-induced endogenous tiRNAs was reported to cause phospho-eIF2 α -independent translational suppression (Yamasaki et al., 2009). Angiogenin-induced 5' tiRNAs but not 3' tiRNAs displaced translation initiation factor eIF4A/

F/G from uncapped/capped mRNAs and induced phospho-eIF2 α -independent SG assembly (Emara et al., 2010; Ivanov et al., 2011). In particular, selected 5' tiRNAs (e.g., tiRNA^{Ala} and tiRNA^{Cys}) bearing the terminal oligoguanine (TOG) motif (four to five guanine residues) at their 5'-termini were observed to form intermolecular RNA G-quadruplexes (RG4), which displaced translation initiation factor eIF4A/F/G from mRNAs (Ivanov et al., 2011; Lyons et al., 2017).

In addition, tiRNAs could interact with YB-1 protein to facilitate SG assembly (Emara et al., 2010; Ivanov et al., 2011; 2014). YB-1 (YBX-1) is a Y-Box Binding protein 1, which is known to be a translation initiation repressor by preventing association of the eIF4G with mRNAs (Evdokimova et al., 2001; Nekrasov et al., 2003). For example, tiRNA^{Ala} was found to spontaneously enter motor neurons by forming a G-quadruplex secondary structure and induce a neuroprotective response with YB-1 protein (Ivanov et al., 2014). However, YB-1 protein appears dispensable for displacing translation initiation factors from mRNAs (Lyons et al., 2016), suggestive of the involvement of other factors or mechanisms.

Modification-dependent translational regulation

Bellodi's group reported that 18 nt TOG-containing 5' tsRNAs with pseudouridine at the 8th uridine position could inhibit global translation, whereas normal uridine-bearing 18 nt TOG-containing 5' tsRNAs could not (Guzzi et al., 2018). These tsRNAs were observed to bind to polyadenylate-binding protein 1 (PABPC1) and lead to the displacement of PABPC1 and translation initiation factors from mRNAs. Pseudouridine was converted from uridine by pseudouridine synthetase (PUS7) and regulated translation and cell growth in embryonic and hematopoietic stem cells (Guzzi et al., 2018). This finding suggested that tsRNAs' functions might be differentially regulated by modifications of their bodies.

Sequence-specific translation inhibition

Lu's group showed that 5' tsRNAs could regulate global translation by suppressing the mRNA translation of ribosomal proteins (RPs), eukaryotic translation initiation factors (eIFs), or eukaryotic translation elongation factors (eEFs) in *Drosophila melanogaster* (Luo et al., 2018). They found that 20-22 nt tsRNAs occupied 30-50% of shorter than 30 nt tsRNAs and preferentially bound to Ago-1 and -2 proteins, which are essential for microRNA-mediated gene silencing activity, based on the analysis of 495 public small RNA libraries. In contrast, 23-29 nt tsRNAs were associated with Ago-3, AUB, and PIWI proteins, suggesting that tsRNA functions might differ based on their length and interacting proteins (Luo et al., 2018).

Transfection and polysome experiments with 20-22 nt tsRNAs beginning with the 4th-6th nt and ending with the

23th-25th nt of mature tRNAs in S2 cells showed that these tsRNAs could decrease polysome levels, suggestive of global translation inhibition. Ribosome profile data demonstrated that these tsRNAs more efficiently suppressed the translation of mRNAs containing more target sites. Unlike microRNAs, the target sites of mRNAs are distributed through all regions of mRNAs, including the 5'-UTR (untranslated region), CDS (coding sequence), and 3'-UTR. In particular, mRNAs of RPs, eIFs, and eEFs have more target sites than other mRNAs. Ago-2 knockdown derepressed their translation rather than that of other mRNAs, demonstrating that Ago-2 is required for the tsRNA-mediated translational suppression. Although their mechanism of inhibiting translation differs from that of tiRNAs, these tsRNAs are also induced under serum starvation and inhibit global translation by suppressing the translation of RPs and translation initiation and elongation factors (Luo et al., 2018).

Translation inhibition by interacting with ribosomes or aminoacyl-tRNA synthetases

Polacek's group identified 14 different 5' tsRNAs of 20-44 nt in length as stress-dependent ribosome-associated non-coding RNAs in *Haloflex volcanii* based on deep sequencing. Val^{GAC}5' tsRNAs interacted with the 30S subunit and polysome under high pH stress and inhibited global mRNA translation by interfering with peptide bond formation in an *in vitro* assay (Gebetsberger et al., 2012). In addition, Val^{GAC}5' tsRNAs competed with mRNAs for ribosome binding and displaced mRNAs from the translation initiation complex, leading to global translation inhibition *in vivo* and *in vitro* (Gebetsberger et al., 2017).

In yeast, tsRNAs were reported to bind to ribosome-associated aminoacyl-tRNA synthetases and inhibit *in vitro* translation by regulating tRNA aminoacylation with unknown mechanism (Mleczek et al., 2018).

POSITIVE REGULATORS

Sequence- and structure-dependent translational activation

Kay's group reported that the 22 nt LeuCAG3' tsRNA enhanced the mRNA translation required for ribosome biogenesis (Kim et al., 2017). They evaluated a number of 3' tsRNAs and found that the 22 nt LeuCAG3' tsRNA was essential for human cancer cell viability based on the antisense-mediated inhibition and transfection of the tsRNA mimics. Unlike microRNAs, this tsRNA was not associated with any Ago proteins and did not exhibit gene-silencing activity. Instead, it optimized ribosomal protein S28 (*RPS28*) translation by binding to two target sites in *RPS28* mRNA. One is in the CDS and the other is in the 3'-UTR in *RPS28* mRNA. The CDS target site forms a double strand by itself, and the 3'-UTR target site forms a secondary structure with the region including the translation initiation site, implying that this tsRNA might bind the duplexed target sites in mRNA and unwind them during translation. The *RPS28* mutant (in which the target sites were altered) and icSHAPE (*in vivo* click selective 2'-hydroxyl acylation and profiling experiment) demonstrated that the LeuCAG3' tsRNA bound to double-stranded target sites in

RPS28 mRNA and unfolded their secondary structure during translation in human cancer cells (Kim et al., 2017).

Since *RPS28* is required for 18S rRNA (ribosomal RNA) biogenesis and is a component of the 40S ribosomal subunit (Robledo et al., 2008), the inhibition of LeuCAG3' tsRNA could impair ribosome biogenesis and in turn resulted in a decrease of cell viability, leading to apoptosis in cancer cells, such as HeLa and HCT-116 (human cervical and colon cancer cell lines, respectively) (Kim et al., 2017). The LeuCAG3' tsRNA was observed to be highly expressed in mouse hepatocellular carcinoma cells compared with normal mouse liver cells, suggesting the possibility that this tsRNA could be used as a therapeutic target for hepatocellular carcinoma treatment. The authors inhibited the LeuCAG3' tsRNA in patient-derived orthotopic hepatocellular carcinoma in mouse xenograft model using a modified antisense oligonucleotide and observed that patient-derived tumors in which the LeuCAG3' tsRNA was inhibited underwent apoptosis and showed suppressed tumor growth (Kim et al., 2017). This finding provided an additional layer to complex translational regulation and demonstrated that the LeuCAG3' tsRNA could be novel potential therapeutic target for cancer treatment.

The authors claimed that the LeuCAG3' tsRNA selected target sites based on favorable minimal free energy and their double-stranded nature. However, more efforts are needed to elucidate the detailed mechanism by which the LeuCAG3' tsRNA selects target sites and unfolds the secondary structure of target mRNAs.

Translational activation by interacting with ribosomes or aminoacyl-tRNA synthetases

Polacek's group also identified the translational activating function of tsRNAs from *Trypanosoma brucei* (Fricker et al., 2019). They analyzed small non-coding RNA interactomes of ribosomes during different growth conditions and life stages of *T. brucei* and found that tRNA^{Thr} 3' half was highly accumulated under nutrient deprivation conditions and the stationary phase of procyclic *T. brucei*. In contrast, tRNA^{Ala}5' half showed different expression pattern, which accumulated under normal growth conditions but disappeared under starved conditions in procyclic and bloodstream *T. brucei*. Polysome profiles with sucrose gradients demonstrated that translation was globally suppressed and that 3' tRNA half bound to ribosomes and polysomes under starved conditions. *In vivo* and *in vitro* translation experiments demonstrated that the ribosome-bound tRNA halves stimulated protein synthesis by facilitating mRNA loading onto actively translating ribosomes (Fricker et al., 2019). This is the first evidence that tiRNA can activate global translation.

Hutvagner's group also reported the translational activation of tsRNAs. They performed immunoprecipitation and a mass spectrometry using SILAC (stable isotope labeling with amino acids in cell culture) and demonstrated that the 19 nt 5' tRF Gln19 was associated with the human multisynthetase complex and increased ribosomal and poly(A)-binding protein translation (Keam et al., 2017). However, the detailed mechanism needs to be determined.

CONCLUSION AND FUTURE PERSPECTIVES

tsRNAs are classified into various types and exert diverse functions in the regulation of gene expression and maintenance of cellular homeostasis. Especially, tsRNAs can suppress or activate mRNA translation with distinct mechanisms dependent on or independent of the target sequence/structure, whereas microRNAs and piRNAs suppress mRNA levels at the transcriptional or posttranscriptional levels. The understanding of the detailed mechanism by which tsRNAs regulate translation is critically important for the therapeutic application of tsRNAs. However, the modifications of tsRNAs may impede the investigation of tsRNAs. tRNAs have 92 different modified nucleotides and an average of 11-13 modifications per molecule (Phizicky and Hopper, 2010), which can interfere with cDNA synthesis during tsRNA library preparation, resulting in incorrect sequencing results (Cozen et al., 2015; Kim et al., 2017; Zheng et al., 2015). Most of the sequencing data of tsRNAs are obtained from conventional sequencing methods, indicating that current sequencing data may mislead tsRNA-research direction. Only sequencing data of tsRNAs generated from the 3'-trailer sequence of the pre-tRNA might be reliable because 3'-trailer sequence are unmodified or very little modified.

Thus far, there are two sequencing methods for overcoming methylation of tRNAs. Lowe's and Pan's groups separately developed demethylase sequencing methods that can remove methylation on tRNAs (Cozen et al., 2015; Zheng et al., 2015). However, as tsRNAs are subjected to various modifications as well as methylation, novel sequencing methods must be developed to accurately analyze tsRNA sequence and its expression. All of these efforts would contribute to the development of novel diagnostic markers and therapeutic targets.

Disclosure

The author has no potential conflicts of interest to disclose.

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