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Short Conceptual Overview

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Alternative genetic code for amino acids and transfer RNA revisited

Abstract: The genetic code is highly conserved among all organisms and its evolution is thought to be strictly limited. However, an increasing number of studies have reported non-standard codes in prokaryotic and eukaryotic genomes. Most of these deviations from the standard code are attributable to tRNA changes relating to, for example, codon/anticodon base pairing and tRNA/aminoacyl-tRNA synthetase recognition. In this review, we focus on tRNA, a key molecule in the translation of the genetic code, and summarize the most recently published information on the evolutionary divergence of the tRNAs. Surprisingly, although higher eukaryotes, such as the nematode (worm), utilize the standard genetic code, newly identified nematode-specific tRNAs (nev-tRNAs) translate nucleotides in a manner that transgresses the code. Furthermore, a variety of additional functions of tRNAs, beyond their translation of the genetic code, have emerged rapidly. We also review these intriguing new aspects of tRNA, which have potential impacts on translational control, RNA silencing, antibiotic resistance, RNA biosynthesis, and transcriptional regulation.

Keywords: evolution; genetic code; protein synthesis; transfer RNA; tRNA fragment.

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Introduction

The genetic code is a set of essential and fundamental rules for living cells. In general, the genetic information in DNA is transcribed to messenger RNA (mRNA), and proteins are synthesized on the basis of the genetic code using the information in the template mRNA. Because many organisms use the same genetic code and any change would produce widespread changes in the amino acid sequences of proteins, the code was thought to be invariable in all organisms ('frozen accident') (1). However, in 1979, mammalian mitochondria were found to use a code that deviates from the universal genetic code (2), and since then, not only further differences in the mitochondrial code but also in the nuclear code have been discovered (summarized in Table 1). In many cases, stop codons are reassigned to various sense codons, which designate amino acids. For example, the UGA stop codon has been reassigned to either tryptophan (Trp) in a few bacteria (such as *Mycoplasma*) (3–5) and certain ciliated protozoans (6) or cysteine (Cys) in the ciliate *Euplotes* (6). The UAA stop codon has been reassigned to glutamic acid (Glu) in three peritrich species, *Vorticella microstoma*, *Opisthonecta henneguyi*, and *Opisthonecta matiensis* (7). Moreover, many ciliates (6), ulvophycean green algae (8), diplomonads (9), and oxymonads (10) use UAR (UAA and UAG) for glutamine (Gln). Stop codons have also been used to expand the genetic code to include selenocysteine (Sec) (11) and pyrrolysine (Pyl) (12, 13). In all kingdoms of life, the 21st proteinogenic amino acid, Sec, is usually an encoded amino acid in enzymes involved in oxidation-reduction reactions (11). In some methanogenic archaea and bacteria, the 22nd proteinogenic amino acid, Pyl, is present in enzymes involved in methane-producing metabolism (12, 13). In contrast, in several species of the genera *Candida* and *Debaryomyces*, a sense codon, the leucine (Leu)-designating CUG codon, has been reassigned to serine (Ser) (14). This widespread occurrence of deviant codes clearly indicates that the genetic code is not universal.

Transfer RNA (tRNA) is a small non-coding RNA of about 70–85 nucleotides that acts as an adapter molecule between the nucleotide sequences of mRNAs and the amino acid sequences of proteins during translation. In some cases, a single-nucleotide substitution in the tRNA molecule has direct and specific effects on the decoding

Table 1 Summary of nuclear genetic code alternatives.

Codon	Standard to alternative code	Organism	References
UGA	Stop→Trp	Firmicutes *Mycoplasma* spp. *Spiroplasma citri* *Bacillus subtilis* Proteobacteria *Hodgkinia cicadicola* Ciliates *Colpoda inflata* *Blepharisma americanum*	(3–6)
UGA	Stop→Cys	Ciliates *Euplotes* spp.	(6)
UAA	Stop→Glu	Ciliates *Vorticella microstoma* *Opisthonecta henneguyi* *Opisthonecta matiensis*	(7)
UAR	Stop→Gln	Many Ciliates All diplomonads other than *Giardia* Oxymonads *Streblomatrix strix* Green algae *Acetabularia* spp. *Batophora oerstedii*	(6, 8–10)
UGA	Stop→Sec	Many species in three domains	(11)
UGA	Stop→Sec/Cys	Ciliates *Euplotes crassus*	(29)
UAG	Stop→Pyl	Some methanogenic archaea and bacteria	(12, 13)
CUG	Leu→Ser	Fungi Many *Candida* spp. Many Ascomycetes	(14)

process (15); thus, tRNA is considered one of the major factors involved in non-standard codon assignments. These tRNA changes can be categorized into two types. The first involves tRNA/mRNA pairing. For instance, a specific mutation in the tRNA anticodon occurs when a stop codon is reassigned, as described above. In *Mycoplasma capricolum*, besides the common $tRNA^{Trp}$ with a CCA anticodon, a deviant $tRNA^{Trp}$ with a UCA anticodon is also encoded and decodes the UGA stop codon as Trp (16). Similarly, RNA editing of the tRNA anticodon causes limited codon reassignment in several mitochondria. In the kinetoplastid protist *Leishmania tarentolae*, the host $tRNA^{Trp}$ with the UCA anticodon is imported into the mitochondrion and the anticodon is converted to UCA by RNA editing (17). The resulting tRNA decodes both the UGG and UGA codons to Trp only in the mitochondria, and not in the nucleus (17). Additionally, a number of alterations of post-transcriptional base modifications at tRNA anticodons modify the codon/anticodon base-pairing rules ('wobble rule'), resulting in codon reassignments (18). The second type of tRNA change that alters the genetic code affects tRNA/aminoacyl-tRNA synthetase (aaRS) recognition. The attachment of a specific amino acid to the 3′ end of each tRNA is catalyzed by enzymes called aaRSs, through the appropriate tRNA recognition (19). Therefore, mutations of the tRNA identity elements, which are specifically recognized by only one aaRS during aminoacylation, are also known to be one of the leading causes of deviations in the genetic code (18). These findings clearly suggest that the genetic code is still in a state of evolution and that this evolution is closely related to tRNA diversification.

In this review, we revisit the evolution of tRNA and its relevance to the genetic code. We first describe a novel type of tRNA that has specifically diverged in the nematode (worm) lineage and, surprisingly, decodes an alternative genetic code for leucine (20). In addition to the nematode tRNA, recent comprehensive tRNA analyses have identified a variety of deviant tRNA genes, mainly in the Archaea and primitive eukaryotes (21). Whereas the evolutionary divergence of the tRNAs has been clarified, recent studies have also shown that the role of tRNA is not limited to translating the genetic code (22). Therefore, we review the recently proposed alternative tRNA functions, which provide an important new perspective on tRNA, as a member of the new class of regulatory RNAs.

Nematode tRNAs that decode an alternative genetic code

tRNA can be classified into two groups based on structural differences in their variable regions. Class I tRNAs have a short variable region of four to five nucleotides, whereas class II tRNAs (e.g., $tRNA^{Leu}$, $tRNA^{Ser}$, and bacterial $tRNA^{Tyr}$) have a long variable arm (V-arm) structure containing 10 or more nucleotides (23). The long V-arm acts as a crucial recognition site for aaRSs during class II tRNA aminoacylation (24–27). Our research group identified a novel type of tRNA, which is based on the class II tRNA structural characteristics but with a class I anticodon, in nematode worms (20). For example, although the common $tRNA^{Gly}$ is classified into class I and has a short variable loop, nematode $tRNA^{Gly}$, with a CCC anticodon, has a long V-arm, which is the class II tRNA structure (Figure 1A). These tRNAs are found only in nematode worms, such as *Caenorhabditis elegans* and *Caenorhabditis brenneri*; thus, they have been

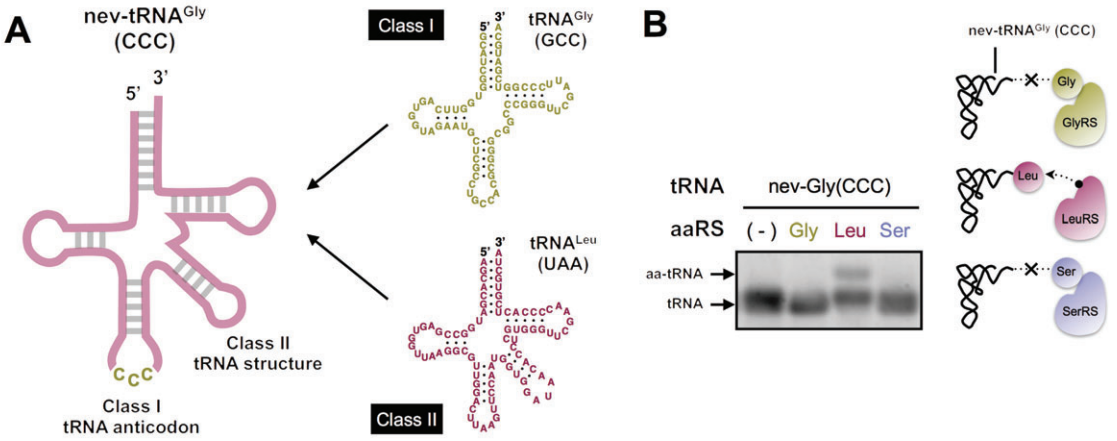

Figure 1 Nematode-specific tRNAs (nev-tRNAs) that decode an alternative genetic code.
(A) nev-tRNAs have both class I and class II tRNA characteristics. (B) nev-tRNA can be solely charged with leucine. *In vitro* transcribed tRNAs were aminoacylated using recombinant *C. elegans* aminoacyl-tRNA synthetases (aaRSs). The aminoacylated tRNAs (aa-tRNAs) were separated on an acid-urea polyacrylamide gel (83).

designated 'nematode-specific V-arm-containing tRNAs' (nev-tRNAs). The numbers of nev-tRNAs and their anticodon variations have increased during the evolution of the nematode taxon. Notably, nev-tRNAs with the CCC or UAU anticodon are two major types of nev-tRNAs and are widely conserved among the nematodes. This observation suggests that nev-tRNAs have evolved specifically in the nematodes. In addition to their unusual structural characteristics, nev-tRNAs exhibit unique aminoacylation and decoding properties, directly involving the genetic code. *In vitro* aminoacylation assays suggest that the nev-tRNA with the CCC anticodon is specifically aminoacylated with leucine and not glycine (Gly) or serine (Figure 1B). The nev-tRNA with the UAU anticodon is also only aminoacylated with leucine. This has been shown to be primarily attributable to the V-arm domains of the nev-tRNAs, which are very similar to that of $tRNA^{Leu}$ and are known to be a major determinant of leucyl-aminoacyl tRNA synthetase (LeuRS) recognition. Moreover, cell-free protein expression assays have suggested that nev-tRNAs can be incorporated into eukaryotic ribosomes and used in translation *in vitro*. These findings indicate that nev-tRNAs decode an alternative genetic code for leucine, at least *in vitro*.

Each nematode worm also expresses the usual class I $tRNA^{Gly}$ with the UCC anticodon and $tRNA^{Ile}$ with the UAU anticodon to decode the GGG and AUA codons as Gly and isoleucine (Ile), respectively (20). Therefore, nev-tRNAs might compete with these 'normal' tRNAs, so that both Leu and Gly/Ile are incorporated at GGG/AUA positions, creating genetic code 'ambiguity'. Similar codon ambiguity has been observed in several species of the genera *Candida* and *Debaryomyces* (28), the ciliate *Euplotes crassus* (29), and *Bacillus subtilis* (4), and is thought to be an important intermediate stage in codon reassignment [explained by the 'ambiguous intermediate' hypothesis (30)]. For instance, in *Candida zeylanoides*, deviant tRNAs with the CAG anticodon have a dual identity, and are charged with not only Leu but also Ser, and decode the Leu CUG codon as either Leu or Ser, resulting in the mistranslation of CUG (31). The positive outcomes of codon ambiguity are still unclear; however, the artificial reconstruction of the CUG codon ambiguity in *Saccharomyces cerevisiae* showed that codon misreading confers putative advantages, such as proteome novelty, phenotypic diversity, and adaptation to new environmental conditions (32, 33). Even so, the negative impact of the misreading phenotype is minimized by a gradual reduction in the use of the ambiguous codon (34). Interestingly, major nev-tRNAs, such as those with the CCC, CCU, and GGG anticodons, tend to selectively correspond to the rare codons and are weakly expressed in the cell (20). Similarly, because isoleucine and leucine are chiral amino acids with highly similar chemical properties, the penetration of the nev-tRNA against the Ile AUA codon would have the least effect on the proteome. On the basis of these observations, the 'ambiguous intermediate' hypothesis also applies to the genetic code ambiguity in nematodes, in which the tRNAs might still be in an intermediate phase of genetic code reassignment.

As well as the nev-tRNAs, a great variety of non-canonical types of tRNAs have been found in the Archaea and primitive eukaryotes with recent comprehensive computational approaches (21). All these tRNA genes can be categorized as 'disrupted' because their precursors are disrupted by intervening sequences, such as introns,

which are removed during tRNA maturation. For example, intron-containing tRNAs that contain a maximum of three introns located at various nucleotide positions have been found predominantly in crenarchaeal species (35). *Trans*-spliced tRNAs, the 5′ and 3′ halves of which are encoded by two or three separated genes, have only been found in a few archaeal species (36, 37). Permuted tRNAs, in which the 5′ and 3′ halves are encoded with a permuted orientation, have been found in unicellular red/green algae (38, 39) and the crenarchaeon *Thermofilum pendens* (40). These alternative tRNA conformations differ essentially from the genetic code reassignment described in the Introduction; however, the evolutionary divergence of the tRNAs strongly suggests that they have a great potential to change very markedly with the diversification of living organisms.

Expansion of tRNA functions

In the 1960s–1970s, the role of tRNA was considered to be restricted to the delivery of specific amino acids to the ribosome during protein synthesis. However, since the 1980s, a growing number of studies have demonstrated alternative tRNA functions (Figure 2) (22). For example, uncharged tRNAs have been shown to function as (i) primers for DNA synthesis (41); (ii) sensors of amino acid concentrations (42, 43); and (iii) regulators of gene transcription termination (44). During the replication of human immunodeficiency virus 1 (HIV-1), uncharged $tRNA^{Lys}$ is used to prime the reverse transcription of the viral RNA genome to double-stranded DNA, which is then integrated into the host genome (41). In certain eukaryotic cells under amino acid starvation, uncharged tRNAs interact with the protein Gcn2p, which induces eIF2 phosphorylation and reduces the overall level of translation (42). This phosphorylation causes the activation of the transcriptional regulator Gcn4p and consequently increases amino acid production (42). In Gram-negative bacteria, such as *Escherichia coli*, uncharged tRNAs block protein synthesis by penetrating the A site of the ribosome and induce the production of the global transcriptional regulator ppGpp in response to amino acid starvation, as in eukaryotes (43). In Gram-positive bacteria, uncharged

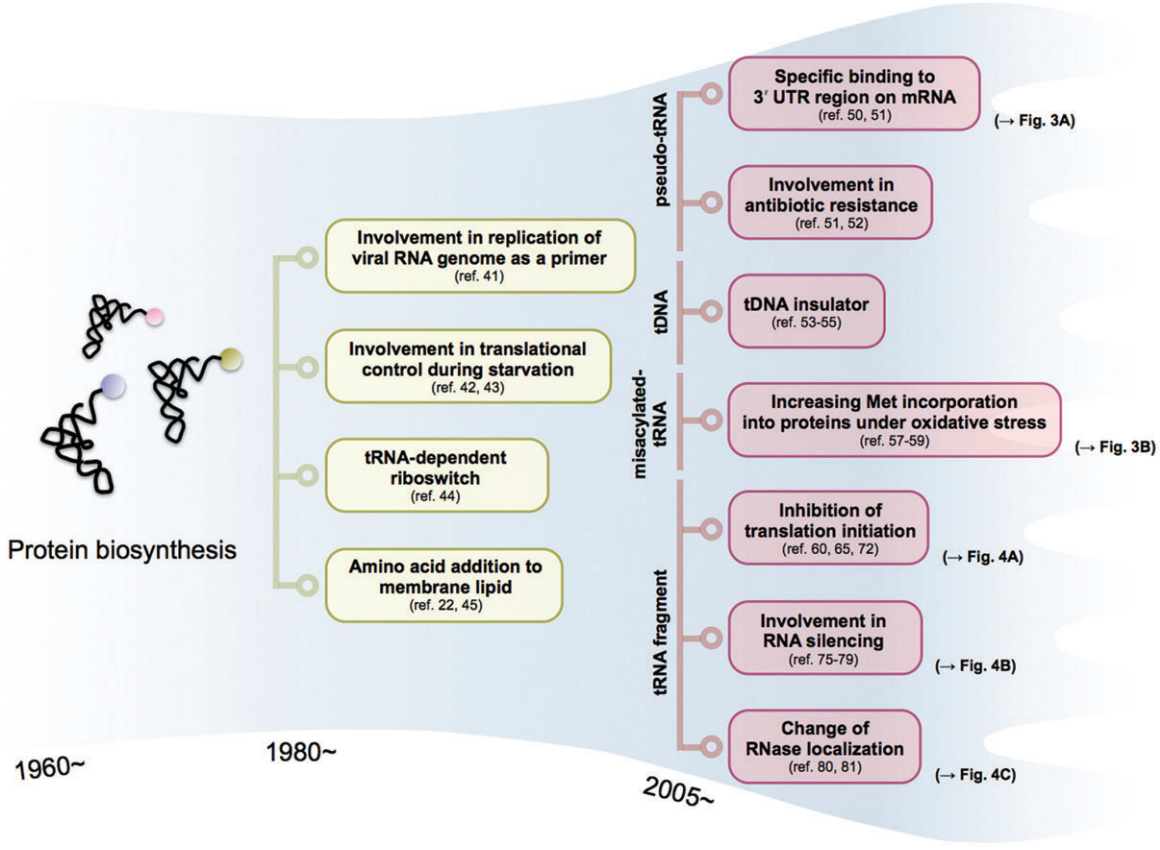

Figure 2 Expansion of known tRNA functions during the past 50 years.

tRNAs act as riboswitches in the T-box transcription termination system (44). When a tRNA is poorly charged, an uncharged tRNA binds to the 5′ untranslated region (UTR) on the mRNA and prevents the formation of a stable terminator helix, causing read-through of the termination site and the transcription of the downstream genes, such as those encoding aaRS and the proteins involved in amino acid biosynthesis and transport (44). It has also been shown that the ribosome is not the only destination to which tRNAs deliver amino acids. tRNAs are used in other amino acid addition pathways, ranging from lipid modification to antibiotic biosynthesis (45).

Since 2005, pseudo-tRNAs, tDNAs, and tRNA-derived fragments (as described in the next section) have, like mature common tRNAs, also been attributed more extensive roles. Recent genome sequencing projects have identified a large number of tRNA isodecoders, which share the same anticodon but differ in their body sequences. However, many of these annotated tRNA genes were automatically predicted using a computational approach, such as tRNAscan-SE (46), ARAGORN (47), and SPLITS (48, 49), and parts of these tRNA molecules lack canonical features, such as the conserved secondary structure, and are classified as pseudo-tRNAs. Therefore, their functions were largely unknown. Rudinger-Thirion et al. (50) have shown that one human $tRNA^{Asp}$ is poorly aminoacylated but is used in a regulatory role beyond translation. This tRNA isodecoder binds directly to a partial Alu sequence in the 3′ UTR of the aspartyl-tRNA synthetase (AspRS) mRNA, and modulates the stability of the mRNA (Figure 3A) (50). Similarly, in *Bacillus cereus* and several other *Bacillus* species, even though a predicted pseudo-$tRNA^{Trp}$ is poorly aminoacylated and does not associate with polysomes *in vitro*, it plays a role in the regulation of tryptophanyl-tRNA synthetase (TrpRS) gene expression in the stationary phase (51). The deletion of this tRNA isodecoder led to significant changes in the cell wall morphology and antibiotic resistance, and was accompanied by changes in the expression of numerous genes involved in the cellular responses to oxidative stress (52). Furthermore, expanded tRNA roles have been reported not only at the level of RNA but also at the DNA level (53). In certain eukaryotic cells, tRNA genes (tDNAs) are repetitive sequences dispersed throughout the whole genome and function as chromatin insulators, helping separate active chromatin domains from silenced ones (54, 55). tDNAs can also block enhancers from activating promoters transcribed by RNA polymerase II (54, 55). This finding indicates that tRNA (tDNA) can potentially mediate the spatial and functional organization of the genome and drive genome change and evolution (53).

These biological functions are accomplished by uncharged tRNAs (or pseudo-tRNAs and tDNAs); however, recent studies have demonstrated that aminoacylated tRNAs also have extended roles. As explained in the Introduction, the most crucial role of tRNAs is to transfer the

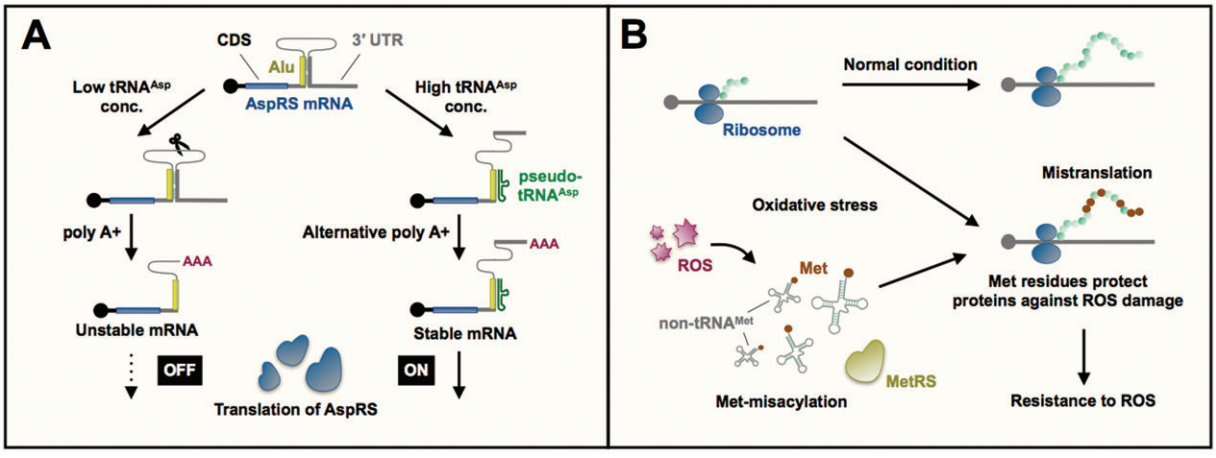

Figure 3 Recently proposed novel tRNA functions beyond the translation of the genetic code.
(A) Regulation of target-specific gene expression by pseudo-tRNA. Human pseudo-$tRNA^{Asp}$ binds to an embedded Alu RNA element in the 3′ UTR of AspRS mRNA. The tRNA-Alu interaction modulates the accessibility of the alternative polyadenylation sites and regulates the stability of the mRNA, leading to the activation of AspRS expression. (B) Modification of translational fidelity triggered by oxidative stress. ROS induce Met-misacylation to produce specific non-methionyl-tRNA families in the mammalian cell. These Met-misacylated tRNAs are used in translation to increase the incorporation of Met into proteins because Met residues are known to protect proteins against ROS-mediated damage.

correct amino acids to the ribosome in accordance with the genetic code. Translational fidelity is essential for protein and cell integrity, which is achieved by accurate tRNA aminoacylation. The error rate of aminoacylation has been shown to be 1 per 10,000–1,000,000 couplings when purified aaRSs were used (56). However, in certain eukaryotic cells, Met is misacylated to specific non-methionyl-tRNA families and these Met-misacylated tRNAs are used in translation in response to oxidative stress (57–59). The Met-misacylation function is thought to protect cells against oxidative stress by increasing Met incorporation into proteins because Met residues protect proteins from reactive oxygen species (ROS)-mediated damage (Figure 3B) (57). These unexpected functional repertoires of pseudo-tRNAs, tDNAs, and aminoacylated tRNAs imply the existence of further regulatory roles for tRNA harbored in the genome.

A new class of regulatory small RNAs derived from tRNA

Recently, it has been demonstrated that tRNA fragments are often generated by endonucleolytic cleavage of tRNAs under specific conditions. However, there are significant differences between the mechanisms involved in this phenomenon in prokaryotes and eukaryotes (22, 60). In prokaryotes, tRNA cleavage mainly occurs to rapidly reduce the levels of tRNA, thereby reducing protein translation, as a cellular defense mechanism against competing organisms (61). For instance, in *E. coli*, the plasmid-encoded nuclease PrrC cleaves tRNAs in their anticodon loops and completely depletes full-length tRNAs in response to bacteriophage infection (62). In eukaryotes, although similar tRNA cleavage within the anticodon loop has been reported (22, 60), in most cases, full-length tRNA levels do not decline markedly and tRNA fragment levels are consistently lower than those of full-length tRNAs (63–65). This suggests that eukaryotic tRNA cleavage has functions other than to reduce tRNA levels. Recent studies have reported regulatory roles for these tRNA fragments, as reviewed here.

In eukaryotic cells, tRNA cleavage is a conserved part of the responses to a variety of stresses (22, 60). For example, in *S. cerevisiae*, *Arabidopsis*, and humans, tRNA cleavage occurs during oxidative stress (64). In yeast, Rny1, a member of the RNase T7 family, is activated and moves into the cytoplasm from the vacuole in response to oxidative stress, where it cleaves tRNAs into fragments (66). tRNAs are also cleaved in mammalian cells and the potential impact of this cleavage there is more fully understood. Although mammalian cells express an orthologue of Rny1, called RNASET2, stress-induced tRNA cleavage depends on a member of the RNase A family, angiogenin, rather than on RNASET2 (65, 67–69). Angiogenin is normally localized in the nucleus and is regulated by the ribonuclease inhibitor RNH1 (70, 71). In response to oxidative stress, angiogenin is dissociated from RNH1 and enters the cytoplasm, where it cleaves the anticodon loops of specific tRNAs (65). A subset of 5′ tRNA fragments, which contain four to five consecutive guanine residues at their 5′ ends, interacts with the translational silencer YB-1 (72). This complex inhibits the initiation of translation by recruiting the general translation initiation factors eIF4E/G/A from capped mRNAs or eIF4G/A from uncapped mRNAs (Figure 4A) (72). In addition to oxidative stress, amino-acid-starvation-induced, age-associated, or tissue-specific tRNA fragmentation has also been observed in many eukaryotic cells (63, 73, 74); however, its biological roles are still unclear.

Another possible role of these tRNA fragments is as regulatory RNAs for gene silencing pathways, such as siRNAs or miRNAs (75). In human embryonic kidney 293 cells, two types of tRNA-derived small RNAs (sRNAs) have been identified (Figure 4B) (76). One type corresponds to the 3′ ends of mature tRNAs and their presence is Dicer dependent (76). Interestingly, a dual *Renilla*-firefly luciferase assay using a reporter gene carrying a target for one of these tRNA fragments indicated that the tRNA fragment could modestly downregulate the target gene in *trans* (76). The other type of sRNA corresponds to the 3′ ends of pre-tRNAs (3′ trailer regions), which are generated by a common pre-tRNA 3′-processing enzyme, RNaseZ (76). In contrast to the first type of sRNA, canonical gene silencing was not observed; however, the addition of an antisense oligonucleotide to the tRNA fragment triggered an increase in gene silencing (76). Both types of tRNA fragments were found to be complexed with Argonautes (Agos) 1–4, but their *in vivo* activities and target mRNAs have not yet been identified (76). Similarly, sRNAs derived from the 5′ or 3′ ends of mature tRNAs and the 3′ trailer regions of pre-tRNAs have been found in human prostatic carcinoma cells (77). The expression of one of these RNAs, corresponding to the 3′ trailer of pre-tRNA^{Ser} (UGA), correlated with the cell proliferation rate and its knockdown slowed cell proliferation (Figure 4B) (77). In HeLa cells, Dicer-cleaved small RNAs corresponding to the 5′ ends of mature tRNAs were identified, which bound weakly to Ago 1 and Ago 2 (78). In HIV-1-infected cells, a host cell tRNA^{Lys}-derived sRNA, which is known to be the primer for viral genome replication (see the previous section), was bound to Ago 2 and silenced a

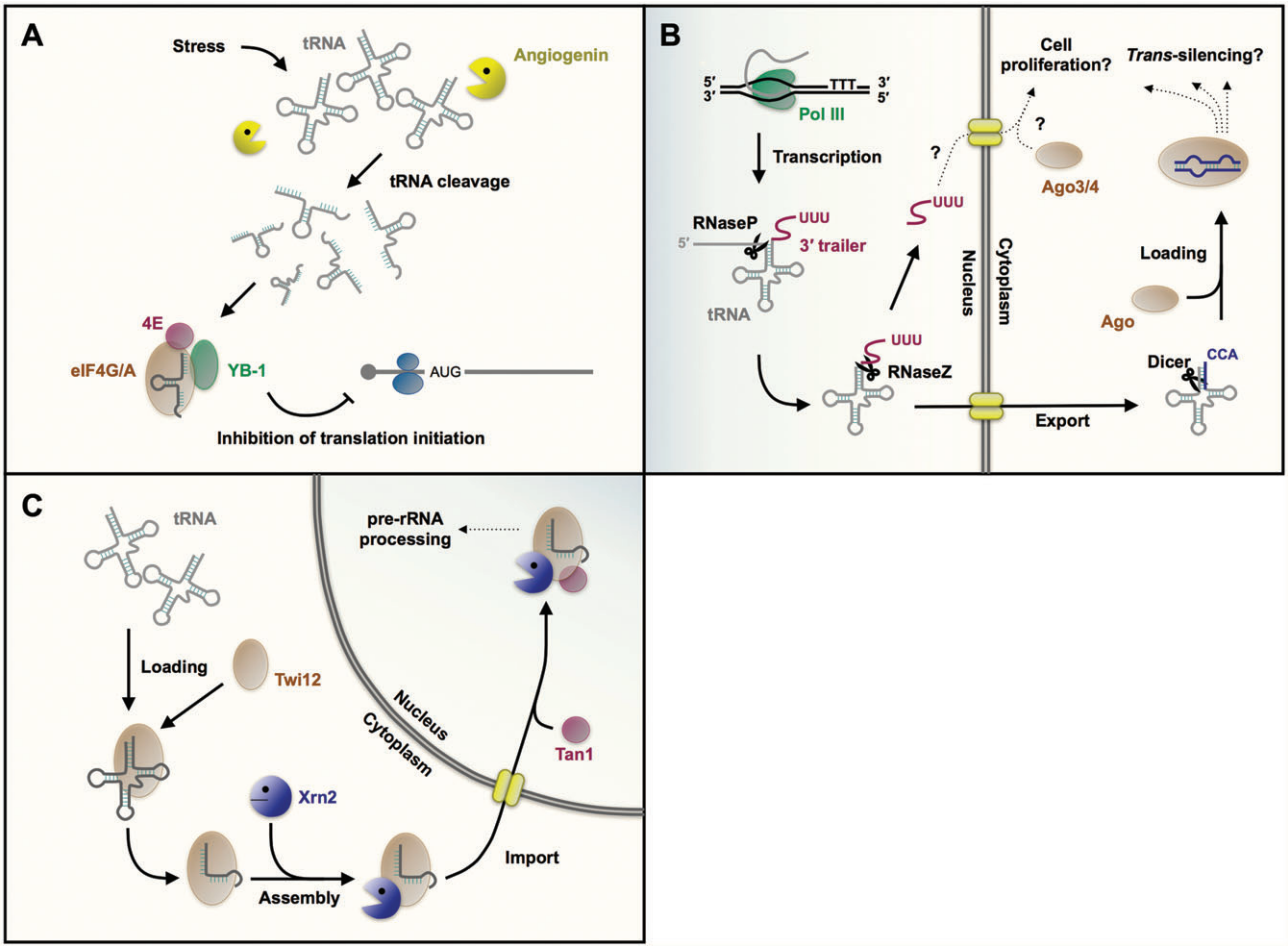

Figure 4 Three examples of tRNA-derived small RNA functions.
(A) Stress-induced tRNA-fragment-dependent inhibition of translation initiation. Stress conditions, such as oxidation and amino acid starvation, induce tRNA fragmentation by angiogenin (ribonuclease 5) in mammalian cells. The resulting tRNA fragments interact with YB-1 to inhibit the initiation of translation by recruiting the translation initiation factors (eIF4E/G/A) from mRNAs. (B) *Trans* RNA silencing by tRNA fragments. During typical tRNA maturation in humans, two types of tRNA-derived small RNAs are generated by either Dicer or RNase Z. Each Dicer-dependent tRNA fragment associates with Ago protein and modestly downregulates target genes in *trans*. The RNaseZ-generated tRNA 3′ trailer associates preferentially with Ago 3/4 and might be involved in certain biological processes (see the text for more information). (C) tRNA-fragment-dependent activation of the exonuclease Xrn2 for RNA processing in *Tetrahymena*. Twi12 (Ago/Piwi protein) is loaded with a mature tRNA and cleaves it in the cytoplasm. The complex of Twi12 and the tRNA 3′ fragment then forms a larger complex with Xrn2. This complex is imported into the nucleus and interacts with Tan1 (Twi-associated novel protein). The final complex is involved in cellular ribosomal RNA processing.

luciferase reporter engineered to be a target of the sRNA (79). A duplex of this RNA and the primer-binding site in the genomic HIV-1 during reverse transcription was a substrate for Dicer cleavage *in vitro*, suggesting its possible function in the host cellular RNA interference machinery that targets HIV-1 (79).

Current RNA cloning and high-throughput sequencing methods are sufficiently sensitive to capture even RNA fragments that are present in the cell in very few copies. This has allowed novel secreted tRNA-derived sRNAs to be identified. In the ciliate *Tetrahymena thermophila*, RNA fragments derived from the 3′ ends of mature tRNAs were detected by deep sequencing 18–22-nucleotide sRNAs co-purified with Twi12, which is a growth-essential *Tetrahymena* Piwi protein that forms a complex with the exonuclease Xrn2, which is involved in cellular ribosomal RNA processing (80, 81). The binding of tRNA fragments to Twi12 is required for the stabilization, localization, and activation of this complex (Figure 4C) (80, 81). Against this background, the presence of prokaryotic tRNA fragments and their possible functions have been revised, and further experiments have suggested that specific tRNA fragmentation occurs more frequently than previously thought.

For instance, Murakami et al. (82) collected hot spring water containing uncultured organisms directly from the underground environment and analyzed the sRNA sequences isolated from it. Their results demonstrated the presence of a large number of novel tRNA fragments and unique relations between tRNA anticodons and their cleavage sites (82). Taken together, these observations support the view that tRNA fragments have extensive biological functions.

Expert opinion

A comprehensive understanding of the genetic code requires a greater understanding of the tRNA molecule. The studies described in this review suggest that the evolution of tRNA correlates strongly with the evolution of the genetic code and with biodiversity in general. At the same time, this knowledge reveals a new series of tRNA functions, ranging from the regulation of translation to transcription and DNA replication. The existence of a large number of tRNA-derived fragments provides a new perspective on tRNAs as regulatory sRNAs. This finding highlights the need for additional analyses to identify the complete population of these RNAs, which have been misinterpreted as mere degradation products, and their detailed regulatory mechanisms.

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