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Noncanonical Roles of tRNAs: tRNA Fragments and Beyond

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Abstract

As one of the most abundant and conserved RNA species, transfer RNAs (tRNAs) are well known for their role in reading the codons on messenger RNAs and translating them into proteins. In this review, we discuss the noncanonical functions of tRNAs. These include tRNAs as precursors to novel small RNA molecules derived from tRNAs, also called tRNA-derived fragments, that are abundant across species and have diverse functions in different biological processes, including regulating protein translation, Argonaute-dependent gene silencing, and more. Furthermore, the role of tRNAs in biosynthesis and other regulatory pathways, including nutrient sensing, splicing, transcription, retroelement regulation, immune response, and apoptosis, is reviewed. Genome organization and sequence variation of tRNA genes are also discussed in light of their noncanonical functions. Lastly, we discuss the recent applications of tRNAs in genome editing and microbiome sequencing.

1. INTRODUCTION

Transfer RNA (tRNA) is an important adaptor molecule that converts information from messenger RNA (mRNA) to peptide chains (proteins). tRNA performs this function (i.e., reads the genetic code) by using its unique cloverleaf structure, where the anticodon pairs with the triplet codon on mRNA and the amino acid attached to the 3′ end of tRNA forms a peptide bond with another aminoacyl-tRNA (**Figure 1*a***). In addition to its canonical role in protein translation, accumulating evidence suggests that tRNA also performs other, noncanonical functions (**Figure 1*b***).

tRNAs have several unique features. First, tRNA (transcribed by RNA polymerase III) makes up 15% of all RNA inside the cell and therefore is very abundant (130). Second, mature tRNAs are short [~70–90 nucleotides (nt)] and fold into a conserved L-shape structure that has an important canonical function in protein translation. Third, tRNAs are heavily modified (see 7): Every tRNA molecule is estimated to have 13 modifications on average, and a large number of modifications have been reported on tRNA (87, 88, 93). These modifications are important for the decoding activity and structural stability of tRNA. The ample abundance of tRNA and its unique tertiary structure led to a previous assumption that tRNA is relatively stable and is not under much biological regulation. We now know that tRNA is under dynamic regulation intrinsically or in response to stress or during pathogenesis via regulation of tRNA transcription, nuclear-cytoplasm transport, posttranscriptional modifications, quality check pathways, nontemplated cytidine-cytidine-adenosine sequence (CCA) addition/removal, and aminoacylation. The new insights about tRNA regulation with respect to its canonical function in protein translation are not discussed here, but we refer the reader to several excellent reviews (60, 87, 88, 93, 106). We do not cover some of the unique tRNA processes in bacteria or archaea; instead, for most of this review, we focus on eukaryotic systems and particularly mammalian systems.

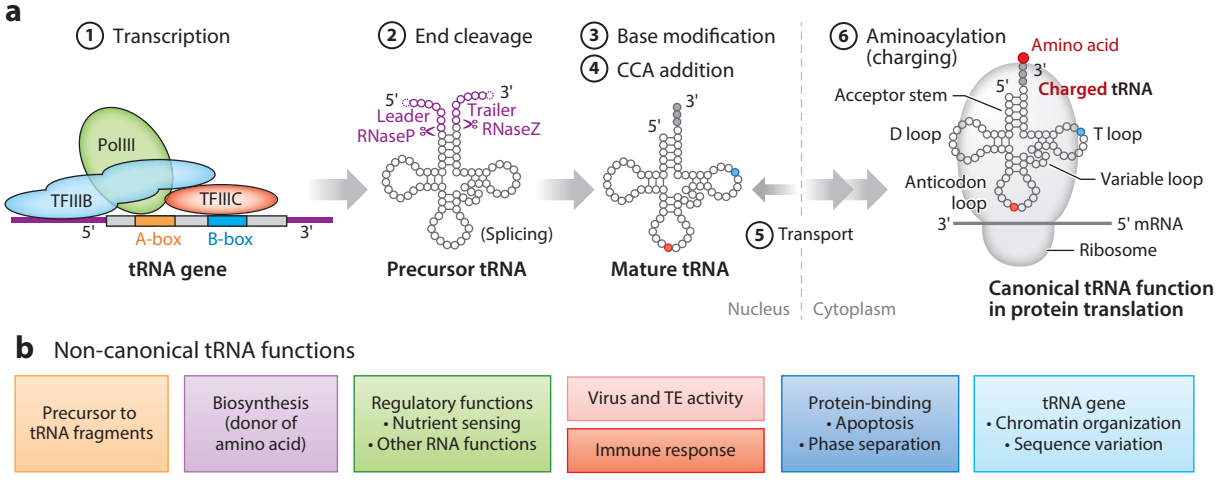

Figure 1

Overview of tRNA processes and functions. (*a*) A simplified scheme of tRNA processes in mammalian cells. Precursor tRNA is first transcribed by PolIII, then processed into mature tRNA, and finally aminoacylated for its canonical function in protein translation. (*b*) Summary of noncanonical tRNA functions. Abbreviations: CCA, cytidine-cytidine-adenosine sequence; mRNA, messenger RNA; PolIII, RNA polymerase III; TE, transposable element; TF, transcription factor; tRNA, transfer RNA.

2. tRNA-DERIVED FRAGMENTS: A NEW GROUP OF SMALL NONCODING RNAs

One unexpected observation from the Human Genome Project is the variation among different tRNA gene sequences for the same anticodon, known as isodecoders (89). Functional analysis reveals divergent tRNA decoding activity among isodecoders, suggesting that some tRNA isodecoders might have other roles outside of translation since they are not optimal for decoding (33). A relatively recent discovery for the noncanonical roles of tRNAs was that they could give rise to smaller RNA fragments called tRNA-derived small RNA fragments (tRFs) that have their own functions. Indeed, a recent analysis suggests that the expression of different tRNA genes affects tRNA fragment levels more than it affects mature tRNA levels (126). These tRNA fragments have emerged as a new group of small noncoding RNAs that are implicated in various biological contexts. Since we have previously reviewed the biogenesis and functions of tRFs (64), in this review, we focus on the progress made over the last few years in this topic. We focus on the nuclear-encoded tRNAs that function in the cytoplasm, but mitochondrial tRNAs have also been shown to produce tRNA fragments. However, since mitochondrial tRNA fragment functions are less clear, we do not discuss them here.

2.1. General Features of tRNA Fragments

Characterization of tRFs has been greatly facilitated by next-generation sequencing (NGS). Profiling tRF identity and abundance in various species and tissues is necessary to establish the foundation for future discoveries. To this end, several tRF databases, constructed from mining NGS data sets, are available to researchers (65, 94, 148). These tRF databases include several species, which reveal that tRFs are present in all domains of life (63) and can be differentiated from random degradation products through their higher abundance, defined lengths, and site-specific termini (148). In general, tRFs can be categorized into six major groups, depending on their start and end positions (**Figure 2*a***): tRF-5, tRF-3, 5′ tRNA half, 3′ tRNA half, tRF-1, and internal tRF. tRF-5s begin at the extreme 5′ end of the mature tRNA and extend at a minimum into the D-loop and at maximum into the anticodon stem. This variation in size allows further classification of tRF-5s: tRF-5a extends to the D-loop, tRF-5b consists of a 3′ end that goes beyond the D-loop and into the D-arm, and tRF-5c represents the longest tRF-5 class that goes into the anticodon stem and ranges from 27 to 30 nt. tRFs derived from the 3′ cytidine-cytidine-adenosine sequence (CCA)-end of a mature tRNA are known as tRF-3s. These tRFs have two major size classifications: tRF-3a, about 18 nt in length, and tRF-3b, about 22 nt long. 5′ and 3′ tRNA halves (also called tRNA-derived stress-induced RNAs) represent the small RNAs generated by cleavage at the anticodon loop of mature tRNAs, usually ranging from 31 to 40 nt. tRF-1s come from the 3′ trailer of the precursor tRNAs. The 5′ end of tRF-1s is cleaved by RNase Z during the tRNA maturation process, and the 3′ end corresponds to the poly-U termination signal by RNA polymerase III; therefore, tRF-1s are also called 3′ U tRFs and have various lengths. Internal tRFs (previously named tRF-2s) represent another group of tRFs from mature tRNAs that arise from the anticodon loop. Other minor groups of tRFs are also present that have not been clearly defined.

2.2. Detection of tRNA Fragments: Current Approaches, Challenges, and Limitations

In order to detect tRFs, classic small RNA detection methods, such as Northern blotting and small-RNA sequencing, have been used, and tRNA detection approaches have also been

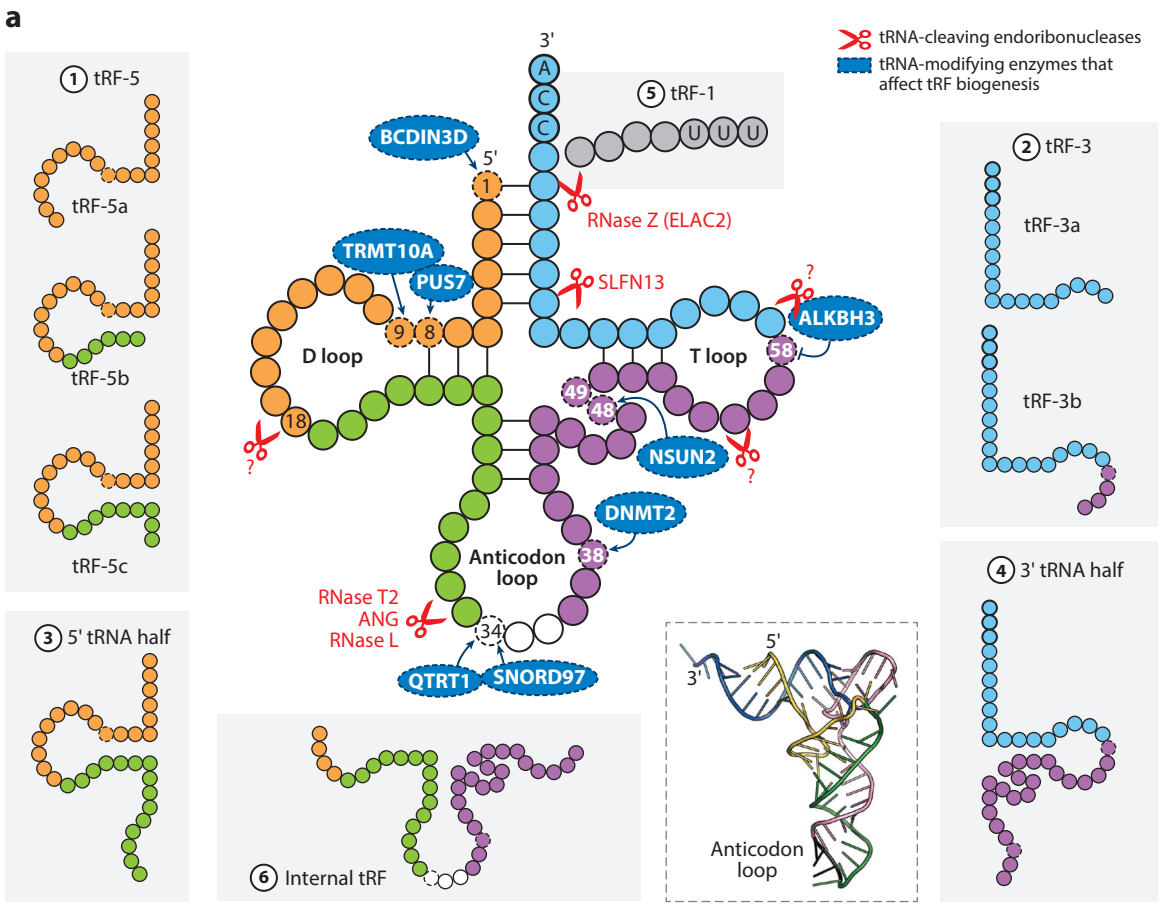

Figure 2

tRNA-derived fragments: types, biogenesis, and functions. (*a*) Six major types of tRFs represented in each box. tRNA-cleaving endoribonucleases are shown as red scissors at indicated locations, whereas tRNA-modifying enzymes that have been shown to affect tRF biogenesis are highlighted in blue at corresponding base positions in dashed circles. tRNA sequence is color-coded according to the $tRNA^{Phe}$ crystal structure (PDB ID 4TRNA) displayed in the inset. Scissors with question marks indicate known tRNA cleavage sites that could result from unknown endoribonucleases or exoribonucleases. (*b*) Summary of tRF functions and mechanisms of action. Abbreviations: PDB, Protein Data Bank; RBP, RNA-binding protein; TE, transposable element; tRF, tRNA-derived small RNA fragments; tRNA, transfer RNA.

repurposed for tRF detection. It is worth noting that tRF and tRNA detection method development is still a growing area due to certain inherent challenges: First, tRFs often have very similar sequences, which require highly specific detection methods, ideally with base resolution. For example, human tRF-3021a and tRF-3020a from $tRNA^{Ala}$ differ only by a single nucleotide. This issue can be overcome by high-throughput sequencing or a more specific TaqMan-based quantitative polymerase chain reaction approach. Second, certain tRFs contain terminal modifications that are not compatible with adaptor ligation, which typically requires 3′ OH and 5′ monophosphate on the RNA molecules. This issue is relevant for tRNA halves in particular, since tRNA halves often have 3′ 2′-3′-cyclic phosphate (cP) or 5′ OH as a result of RNase cleavage (113). To circumvent this issue, investigators have developed two methods to enrich cP-containing RNAs for sequencing. The first approach, cP-RNA-seq, depletes RNAs with a 3′ OH or 3′ phosphate for cloning by phosphatase plus periodate treatment, which has been used to identify tRNA halves that are sex-hormone-dependent (45). One limitation of this approach is that RNAs with 3′ modifications (e.g., 2′-*O*-methyl ribose) may not be efficiently removed by periodate and would remain detectable in cP-RNA-seq. The second approach utilizes RtcB, an RNA ligase that can convert 3′ cP to 3′ monophosphate, thus making it amenable for adaptor ligation. This method has been used to identify tRNA halves produced by RNase L (21). Third, though not systematically evaluated yet, tRFs are highly likely to inherit internal modifications that are common on tRNA (18, 39, 146). Some of these modifications have been known to strongly impede reverse transcription (RT) during cloning, which could lead to inaccurate quantification of tRFs. For small-RNA sequencing, this effect could lead to either underestimation (RT after adaptor ligation) or overestimation (RT before adaptor ligation) of tRFs, according to the cloning protocol. Because of these limitations in tRF detection, it is advisable to corroborate the results by complementary approaches.

2.3. tRF Biogenesis: Factors and Crosstalk with tRNA Modifications

Meta-analysis has revealed the presence of tRFs in organisms from bacteria to humans (63). Profiling of tRFs revealed specific cleavage sites on tRNAs. Some factors have been implicated in the biogenesis of tRFs and are summarized in **Supplemental Table 1**.

Supplemental Material >

2.3.1. tRF generation as part of the tRNA maturation process. Some tRFs are generated as part of the tRNA maturation process, such as tRF-1s that are cleaved from the 3′ trailer of precursor tRNAs by RNase Z (68). Theoretically, leader tRFs could also be generated from the 5′ leader of pre-tRNA by RNase P cleavage but have not been described so far, most likely due to their short length. Sequencing of pre-tRNAs reveals that most tRNAs have a leader sequence that is less than 15-nt long, which is too short for small-RNA detection (36). Additionally, intron sequences spliced from certain tRNAs are known to generate stable noncoding RNA, tRNA intronic circular RNA (tricRNA) (74). The tricRNAs were detected in *Drosophila* and *Caenorhabditis elegans*, but their functions are as yet unclear. Lastly, when exon ligation for tRNA splicing is impaired in *CLP1*-deficient mice, pre-tRNA-derived tRFs accumulate as splicing intermediates (41, 53).

2.3.2. Regulation of tRNA halves: more than stress responsive. tRFs are ubiquitously expressed. In particular, cleavage in the anticodon loop to produce tRNA half-molecules is conserved across organisms in response to various stress conditions, including oxidative stress, nutrient deprivation, hyperosmotic stress, and virus infection (120). These tRNA half-molecules also exist in nonstressed conditions and are especially abundant in the hematopoietic system, including serum; fetal liver and placenta (19, 118); body fluids such as sperm, cerebrospinal fluid, and urine;

and extracellular vesicles (EVs) (35, 49, 116, 138). They can also be regulated by physiological cues, including diet (12, 103, 111), sex hormones (45), cell differentiation (62), and development (118). 5′ tRNA half-molecules are prone to form dimers (5′ halves from $tRNA^{Gly}$ and $tRNA^{Glu}$) or tetramers (5′ halves from $tRNA^{Ala}$ and $tRNA^{Cys}$), which could explain their higher stability and abundance (77, 127).

Ribotoxins in bacteria and fungi cleave tRNA at the anticodon loop as a defense mechanism to stall protein translation and growth in response to stress, such as phage infection (87). This action could even be suicidal and lead to cell death, but it is believed to be protective for the microbe community. In yeast and plants, the RNase T2 family is responsible for tRNA half-generation, while *Arabidopsis* RNase T2 has an additional role to produce shorter tRF-5s (82, 123). Human RNase T2 also has tRNA cleavage activity in vitro (82, 123), but knocking down RNase T2 failed to decrease arsenite-induced tRNA halves (141). What, if anything, triggers mammalian RNase T2 to generate tRNA halves is still unclear.

In mammalian cells, tRNA half-biogenesis studies have focused on angiogenin (ANG or RNase 5), a member of the vertebrate-specific RNase A family. The function of ANG in angiogenesis, hematopoiesis, neurogenesis, oncogenesis, and inflammation has been reviewed (76, 112). Cellular ANG is inactivated by binding with the RNase inhibitor RNH1, which is sensitive to oxidative stress. During oxidative stress, ANG is activated and mediates cleavage in the anticodon loop of various tRNAs; ANG cleavage does not decrease tRNA concentration, but instead produces tRNA halves that repress global protein translation (141). ANG is likely not the only RNase to produce tRNA halves since tRNA halves are still present in the ANG knockout cells (119).

In addition to ANG, several members from the interferon-stimulated gene (ISG) family have also been shown to cleave tRNAs, likely as part of the immune response. For example, double-stranded RNA (dsRNA)-responsive RNase L generates tRNA halves from $tRNA^{His}$, $tRNA^{Pro}$, and $tRNA^{Gln}$, which correlate with protein translational arrest without diminishing the tRNA levels (21). Schlafen 11 (SLFN11) binds and cleaves $tRNA^{Ser}$ and $tRNA^{Thr}$, which have a codon-usage-dependent effect on protein translation during human immunodeficiency virus (HIV) infection and DNA damage response (69, 70). Similarly, SLFN13 (RNase S13) cleaves tRNA and restricts HIV replication (142). While SLFN13 recognizes the length of the acceptor stem and cleaves between the acceptor stem and T stem, the SLFN11 cleavage site is unclear.

2.3.3. Biogenesis of shorter tRFs: The jury is still out. The question of how the shorter tRFs are generated remains open: They could be generated independently from tRNA halves or further processed from tRNA halves. Shorter tRF-5s in the size range of 26–30 nt that could interact with PIWI-like proteins are also called td-piRNAs; these tRF-5s are most likely to originate from the 5′ tRNA halves (44, 119). On the other hand, we recently showed that short tRF-3s in human cancer cell lines are generated independently from tRNA halves and not by ANG (119).

We have previously discussed the role of Dicer and Drosha, the RNase III family members that generate mature microRNAs, in tRF biogenesis (64). Our conclusion remains that Dicer and Drosha are not responsible for tRF biogenesis on a global scale. This has been further validated in *Arabidopsis* (82) and new data sets in human cell lines with knockouts (66), in addition to the previous analyses in *Schizosaccharomyces pombe*, *Drosophila*, mouse, and human. In particular, tRF-3-mediated gene repression was not impaired and was even better in *Dicer* knockout cells (66). This is not to overlook Dicer-dependent biogenesis of certain individual tRFs. For example, pre-$tRNA^{Ile}$ adopts alternative folding with a pre-microRNA-like hairpin structure and is cleaved by Dicer to produce miR-1983, which is regulated by Lupus autoantigen (La) protein binding and

viral RNAs binding to La (42). The mechanism of biogenesis for the bulk of short tRFs is still unclear at this point.

2.3.4. tRNA fragment biogenesis is regulated by tRNA modifications. Modifications are important regulators of tRNA function and are dynamically regulated by environmental stimuli. tRF biogenesis is tightly connected with tRNA modifications (**Figure 2*a***). DNMT2 and NSUN2 are methyltransferases that catalyze cytosine-C5 methylation, a modification that is important for tRNA decoding activity and tRNA stability (129). DNMT2 installs m^5C_{38} on $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Val}$ and limits 5′ tRNA half production upon heat stress in *Drosophila* (25, 104); similar tRNA-protective effects of DNMT2 were observed in mammalian hematopoietic cells (128) and sperm (146). Likewise, *NSUN2* deficiency in neurons decreases $m^5C_{48/49}$ on tRNAs and increases 5′ tRNA half levels due to the higher binding affinity of hypomodified tRNAs with ANG (6). NSUN2 and the tRNA/tRF balance could be regulated by stress to control global protein translation (34). Pseudouridylation is another prevalent modification on tRNAs. Loss of stem cell–specific pseudouridine synthase PUS7 downregulates levels of 18-nt 5′ terminal oligoguanine (TOG) tRF-5s [known as mini TOGs (mTOGs)] from $tRNA^{Ala}$, $tRNA^{Cys}$, and $tRNA^{Val}$ (39). Furthermore, these mTOG-tRF-5s harbor PUS7-dependent ψ8 that are necessary to rescue the translational defects in *PUS7* knockout cells (39). Decreased m^1G_9 by *TRMT10A* deficiency in pancreatic islet β-cells leads to the generation of $tRF\text{-}5^{Gln}$ (17). Queuosine (Q) modification of guanosine on the anticodon loop wobble base 34 is known to regulate tRNA decoding. Human queuosine is catalyzed by the QTRT1/QTRT2 enzyme utilizing queuine from diet or gut microbes. Depleting queuosine in human cells rendered more tRNA halves from $tRNA^{His}$ and $tRNA^{Asn}$ (135). A lower abundance of 5′ tRNA halves was observed in knockout cells of ALKBH3, a tRNA demethylase of m^1A and m^3C (13). Methyltransferase BCDIN3D was shown to methylate 5′ monophosphate of $tRNA^{His}$ and protect $tRNA^{His}$ from fragmentation into tRF-3 (78, 98). Recently, 2′-*O*-methylation of C34 on $tRNA^{eMet}$ catalyzed by box C/D SNORD97 guide RNP was shown to prevent stress-induced cleavage in the anticodon loop (134). Overall, these examples highlight how tRNA modifications could affect tRF biogenesis, often without changing the parental tRNA levels. So far, most of the tRNA modifications have not been shown to be reversible, with the only exception being m^1A (73). While some in vitro studies indicate that ribotoxins might show higher activity in the presence of tRNA modifications (87), most cases in mammalian cells, as discussed above, suggest that tRNA hypomodification increases tRNA fragmentation. It is not yet clear whether certain patterns (e.g., sequence motifs and/or modification states) are actually being recognized by tRNA-cleaving enzymes to facilitate tRF and tRNA half-biogenesis or if the modification-dependent protein binding or tRNA folding regulates nuclease accessibility to tRNAs.

2.4. Functions of tRNA Fragments and Mechanisms Thereof

It has become clear that tRFs could have diverse functions and mechanisms of action, and not necessarily with a unifying rule. Based on current knowledge, we summarize the mechanisms of action of tRFs in **Figure 2*b***. Below, we describe tRF functions in more detail.

2.4.1. tRFs regulate protein translation. It has been demonstrated that tRFs have both positive and negative effects on the regulation of translation. $tRNA^{Ala}$ and $tRNA^{Cys}$ 5′ halves inhibit global protein translation and promote stress granule (SG) formation by interacting with YBX1 (YB-1) (47). YBX1-tRNA-half-binding is required for bringing target mRNAs into SGs but is dispensable for the translational repression (75). Interestingly, these two tRNA halves contain a TOG

(terminal oligoguanine) motif and could form a G-quadruplex structure; such unique conformation is important for translational repression and SG function (48, 77). The shorter tRF-5s have also been shown to repress protein translation. For example, 19-nt tRF-5^{Gln} was shown to inhibit protein translation in a sequence-independent manner, and it was found to interact with a multisynthetase complex that contains multiple AARS (aminoacyl tRNA synthetase) proteins (115). In another report, 18-nt tRF-5s from tRNA^{Ala}, tRNA^{Cys}, and tRNA^{Val} repress global protein translation by interaction with translation initiation factor PABPC1, and this effect is dependent on the PUS7-dependent ψ8 modification (39).

A small number of reports implicate tRFs in promoting protein translation. For example, 22-nt tRF-3011b from tRNA^{Leu} promotes ribosome biogenesis by base-pairing with ribosomal protein mRNAs to enhance their translation (58). In *Trypanosoma brucei*, a 3′ half from tRNA^{Thr} stimulates global protein translation by interacting with ribosomes (30).

2.4.2. tRFs silence genes by base-pairing with target mRNAs. Shorter tRFs that are similar in size to microRNAs have been found that are associated with Argonaute (AGO) proteins and suggested to have microRNA-like functions (63). Indeed, tRFs have been detected in RISC (RNA-induced silencing complex) proteins like AGO and GW182/TNRC6 along with complementary target mRNAs (63, 66). 22-nt tRF-3027b from tRNA^{Gly} was shown to repress expression of *RPA1* via its association with AGO proteins in normal B cells (80). We have recently shown that three tRF-3s (tRF-3001, -3003, and -3009), when overexpressed by their parental tRNAs, could repress target genes on a global level similar to microRNAs (66). Recently, rhizobial tRF-3s were shown to hijack host plant AGO1 to repress host genes involved in nodule formation and symbiosis (99). Thus, although the tRF-3s are produced independently of Dicer or Drosha, they could function as part of AGO-containing RISC proteins to repress target mRNAs.

tRFs have also been shown to interact with PIWI proteins and perform gene-silencing activity. For example, tRF-5c from tRNA^{Glu} can be regulated by IL-4 and interacts with PIWIL4; this interaction downregulates *CD1A* transcription by recruiting histone-modifying enzymes to the *CD1A* promoter region in human monocytes, which seems to be dependent on the tRF complementarity (143). This is in addition to the identification of global tRF-5c, which is enriched by PIWIL4 in breast cancer cell lines (56). In another report, tRF-1s were shown to interact with PIWIL2 in addition to their binding with AGO1/2 (92). It is worth noting that so far most papers have predicted tRF targets based on miR-target algorithms, which might not be the most suitable algorithm for this purpose.

2.4.3. tRFs sequester RNA-binding proteins. tRFs can interact with RNA-binding proteins (RBPs) and sequester them from other RNAs. Internal tRFs (i-tRFs) from tRNA^{Gly}, tRNA^{Asp}, tRNA^{Tyr}, and tRNA^{Glu} interact with YBX1 and sequester it from the 3′ untranslated region (UTR) of other transcripts; these other transcripts are normally stabilized by YBX1 and thus are destabilized by i-tRFs (37). In another case, long tRF-5^{Gln} interacts with IGF2BP1 and sequesters IGF2BP2 from *c-Myc* RNA, which leads to lowered stability of *c-Myc* RNA (62). Similarly, stress-induced 5′ tRNA halves interact with *Drosophila* Dicer-2; this interaction inhibits Dicer-2's cleavage activity on its regular dsRNA substrate and decreases the production of siRNAs (25). tRF-3s in *Tetrahymena* can interact with nuclear PIWI protein Twi12; this interaction is important for Twi12 localization and regulates Xrn2 RNase activity on other cellular RNAs (18).

Lupus autoantigen (La, or SSB) is another RBP, and it is known to stabilize RNA Pol III transcripts, including pre-tRNAs and some viral RNAs. Recently, La was found to interact with tRF-1s, which sequester La from viral RNAs that are conferring resistance to RNA viruses, which use La protein as a chaperone to facilitate viral gene expression (16). The opposite scenario has also been

described when viral RNAs are overexpressed and sequester La proteins so that the lowered La binding causes the pre-tRNA to enter microRNA biogenesis pathways (42). Overall, RBP binding by tRFs can change the balance of many different cellular and viral RNAs.

2.4.4. tRFs regulate transposable element and noncoding RNA activity. tRFs have been implicated to defend the host genome against transposable element (TE) activity. Abundant tRF-3s in mouse stem cells inhibit long terminal repeat (LTR) retrotransposon activity via two distinct mechanisms: The 18-nt tRF-3s block RT and suppress replication of a large number of endogenous retroviruses (ERVs), possibly by competing with tRNAs that work as RT primers; meanwhile the 22-nt tRF-3s silence proviral *MusD* ERV posttranscriptionally by targeting its primer-binding site (PBS) (108). Long tRF-5s from mouse sperm and mouse embryonic stem cells (ESCs) could globally repress MERVL-associated genes (111). In their search for the underlying mechanism for MERVL regulation, the Rando group (9) found that tRF-5c^{Gly} interacts with hnRNPF/H and positively regulates the production of noncoding RNAs in Cajal bodies, including *U7* small nuclear RNA molecules that are important for histone RNA processing. The lowered expression of histone leads to transcriptional repression of MERVL elements, an effect that appears to be specific in mouse and not observed in human ESCs. This also suggests a role for tRFs in noncoding RNA regulation.

3. AMINOACYLATED tRNA IN BIOSYNTHESIS PATHWAYS

Aminoacylated tRNAs (AA-tRNAs) can act as amino acid donors to other molecules (reviewed in more detail in 28, 55, and 85), similar to their primary role in amino acid transfer during protein synthesis. For instance, tRNA plays vital roles in regulating the N-terminal amino acid on peptides to destabilize proteins. This phenomenon (i.e., N-terminal amino acid regulating protein stability) exists in both prokaryotes and eukaryotes, and the specific N-end rule differs according to the organism. The N-terminal amino acid can be regulated by changing the level of specific AA-tRNA, such as formylated methionine tRNA as recently shown in yeast (59), or by transferring the amino acid moiety from AA-tRNA to the peptide chain at its N terminus. For example, Arg-tRNA^{Arg} is used to transfer arginine to target peptides in mammalian cells by arginyl-tRNA transferase (ATE), and Leu-tRNA^{Leu} and Phe-tRNA^{Phe} are used similarly in prokaryotes (121). Another example of using AA-tRNA (where AA is Gly, Ser, Thr, or Ala) in peptide conjugation is building the pentapeptide interpeptide bridge (crosslink) in peptidoglycan polymer biogenesis (catalyzed by Muramyl ligase and FemXAB peptidyl transferase), which is important for maintaining bacterial cell wall functions. Furthermore, cyclic dipeptides (2,5-diketopiperazines) are generated by AA-tRNA-dependent cyclodipeptide synthase (where AA is Phe, Pro, Tyr, Leu, His, or Gly). Intriguingly, these small compounds are important for bacterial quorum sensing and mammalian immune regulation and have been proposed to participate in gut-brain communication and neurodegenerative disease (4). AA-tRNA can also transfer amino acids to lipids. Amino acid (Lys, Ala, Arg) transfer to phosphatidylglycerol (PG) on bacterial cell membranes by aminoacyl-PG synthase is triggered by stress (including certain antibiotics) and neutralizes the negative charge of PG, which increases antibiotics resistance and bacterial pathogenicity (27). Other lipid substrates include cardiolipin (Lys) and diacylglycerol (Ala), but they are not well studied. Interestingly, lipid aminoacylation on bacterial membrane was also found to regulate DNA replication, gene expression, and membrane proteome composition, although these effects may be indirect. In other cases, AA-tRNA transfers amino acid to promote antibiotic synthesis, such as Val-tRNA and Ser-tRNA for valanimycin synthesis. Lastly, Glu-tRNA is important for the multistep biosynthesis of tetrapyrroles (e.g., heme/chlorophyll) in bacteria, algae, and plants,

compounds important for photosynthesis and metabolism. So far, several hundreds of AA-tRNA-utilizing enzymes have been identified (mostly in bacteria) (85). Interestingly, in some cases, separate tRNA species were used for biosynthesis functions that do not enter into protein synthesis pathways.

4. REGULATORY FUNCTIONS OF tRNAs

4.1. Uncharged tRNAs in Nutrient Sensing

Though tRNA aminoacylation is generally believed to be a very efficient process, it has become clear that aminoacylation fraction or fidelity is an important aspect of tRNA functional regulation. Under certain cellular or environmental stress conditions, uncharged tRNAs accumulate and alter downstream signaling pathways to adjust for changes in energy needs. In prokaryotes, uncharged tRNAs stalled in the ribosome A site trigger RelA [ribosome-associated (p)ppGpp synthetase I] to synthesize alarmone signaling molecules 5′-diphosphate-3′-diphosphate-guanosine (ppGpp) from ATP and guanosine-5′-triphosphate (GTP). Alarmone signals for the bacterial stringent response and alters replication, transcription, and translation to regulate metabolism and growth (100). Also in bacteria, uncharged tRNAs could be sensed by the T-box leader, a riboswitch RNA structure that base-pairs with charged or uncharged tRNAs through different conformations to regulate gene expression (43)—a detailed interaction map between $tRNA^{Gly}$ and T-box has recently been revealed by structural studies (reviewed in 136). In eukaryotes, the tRNA charging status is monitored by GCN2 (general control nondepressible 2) kinase, whose substrate eIF2α is central in the integrated stress response pathway. GCN2 can be activated by uncharged tRNA through its HisRS-like domain or alternatively by ribosome stalling without changes in tRNA via the ribosome P-stalk (reviewed in 79).

4.2. Uncharged tRNAs Regulating Other RNA or DNA Processes

Specific uncharged tRNAs could also have regulatory functions. Human $tRNA^{iMet}$ has been shown to regulate pre-mRNA splicing; this regulation is dependent on base-pairing between $tRNA^{iMet}$ and the start codon on the mRNAs, but not dependent on the aminoacylation on the $tRNA^{iMet}$ (52). Moreover, uncharged $tRNA^{Asp}$ adopts a noncanonical fold that interacts with the *Alu* element in the 3′ UTR to affect the choice of polyadenylation site (101). The T-box riboswitch that regulates transcription by tRNA interaction, as discussed above, also fits into this category. Lastly, *Escherichia coli* tRNA can serve as a primer in the DNA replication of T7 phage, which is regulated by tRNA binding with phage protein gp5.5 and *E. coli* protein H-NS (149). A more global role of tRNAs in RNA or DNA regulatory pathways is yet to be explored.

4.3. tRNAs in Retrovirus and Retrotransposon Regulation

Retroelements are DNA sequences that can be amplified and inserted into genomes (i.e., copy and paste) via an RNA intermediate. They are important for exogenous virus activity. They also commonly exist in the host genome as TEs, regulating genome stability and gene expression and therefore development and ultimately evolution (31). tRNAs have been known to prime RT in retroelements (reviewed in 50 and 109). These retroelements include LTR retroviruses, such as HIV ($tRNA^{Lys}$), human T cell leukemia virus type 1 ($tRNA^{Pro}$), Rous sarcoma virus ($tRNA^{Trp}$), Moloney murine leukemia virus ($tRNA^{Pro}$ and $tRNA^{Gln}$), and mouse mammary tumor virus ($tRNA^{Lys}$), as well as LTR retrotransposons (including Ty1/Copia, Ty3/Gypsy, and ERVs)

that employ $tRNA^{Phe}$, $tRNA^{Lys}$, and $tRNA^{Leu}$. To initiate RT for cDNA synthesis, the 18-nt 3′ end of specific mature tRNA from the host binds to the complementary sequence (also known as the PBS) downstream from the 5′ LTR and serves as a primer for RT, with additional interactions with other tRNA regions. It was recently discovered that tRNA fragments could regulate TE activities (as discussed in Section 2.4.4), further expanding the functional linkage between tRNA and TE regulation.

4.4. tRNAs Regulating Immune Sensing of Foreign Nucleic Acids

Bacterial RNA is a strong activator of the mammalian innate immune response by Toll-like receptors (107), which need to distinguish foreign RNA from self-RNA. One strategy is by RNA modification that is uniquely or differentially expressed in bacteria or eukaryotes (26). For example, 2′-*O*-methylation, an abundant modification in mammalian cells, on guanosine 18 (G_m18) in bacterial tRNA was shown to suppress immune activation (32, 51), suggesting that bacteria might use tRNA modifications to attenuate host defense. Other than G_m18, 2′-*O*-methylthymidine (m^5Um) of position 54 has also been shown to suppress TLR7 activation (57). The relative immunostimulatory contribution from bacterial tRNAs compared to other RNA types is unclear on the organism level, though a recent attempt to determine its contribution shows that the loss of G_m18 in live *E. coli* does not alter cytokine production by peripheral blood mononuclear cells (29), suggesting that tRNA might be sufficient but not necessary to trigger an immune response on the organism level. Whether the host defense response is regulated by bacterial tRNA and how the host immune response regulates bacterial tRNA and tRNA fragment homeostasis will be interesting future topics, given the recent example of rhizobial tRNA fragments regulating symbiosis (99).

4.5. tRNA-Protein Interaction: Apoptosis, Phase Separation, and More?

Both canonical and noncanonical tRNA functions rely on tRNA-binding proteins. Elongation factor Tu binds with correctly aminoacylated tRNA with subnanomolar K_d (67) and GCN2 binds to uncharged tRNA with a similarly strong affinity (20). However, considering the high abundance of tRNAs (around 100-μM cellular concentration for total tRNAs), even weaker-affinity protein binding might constitute a significant bound proportion for the specific protein. For example, tRNA inhibits cytochrome c (cyt c)-mediated apoptosis by specific interaction with cyt c and promotion of cyt c reduction; as a result, degradation of tRNAs enhances apoptosis (83). This binding ($K_d \sim 1$ μM) happens to both mitochondria and cytoplasmic tRNAs, regardless of its aminoacylation status, but prefers the reduced form of cyt c (72). This could be a mechanism for the cells to prevent apoptosis when tRNAs are abundantly expressed, e.g., growth conditions. Several ISG proteins have been shown to bind tRNA. For example, with regard to the interferon-stimulated Schlafen family members, mentioned in Section 2.3.2, their nuclease activity against tRNAs attenuates protein translation upon virus infection (69, 70, 142). RNase L has a similar function (21). As another tRNA-binding ISG protein, IFIT5 (interferon-induced protein with tetratricopeptide repeats 5, also known as ISG58) specifically binds to a broad range of RNAs, including pre-tRNAs and poly(U)-tailed tRFs, such binding was hypothesized to decrease tRNA concentration or increase tRNA degradation by DIS3L2 (54). A common function for the ISG protein:tRNA interaction is protein translation, but more functions could be discovered in the future. Intriguingly, a structure-based algorithm predicts 37 potential tRNA-binding proteins (90), suggesting that there might be more to be characterized in the future.

The relatively weak RNA protein binding has attracted more interest, especially in light of liquid-liquid phase separation (LLPS) and membraneless organelles, now appreciated as a

common mechanism for increasing the local concentration of specific molecules in functional compartments (10, 71). Examples of LLPS include SGs, Cajal bodies, RISC complexes, and more. So far, there has only been one study about tRNAs in phase separation regarding their binding to τ protein, the main component of neurofibrillary tangles in Alzheimer's disease. Such binding is relatively weak (submicromolar K_d) and may not be highly specific, since other RNAs could also phase separate with τ (144). In the future, it will be interesting to identify novel tRNA or tRF binding proteins and test whether tRNA or tRF protein interactions could regulate phase separation formation or dynamics.

5. NONCANONICAL FUNCTIONS OF tRNA GENES

5.1. tRNA Genes and Chromatin Organization

tRNA genes (tDNAs) are highly transcribed by RNA polymerase III. tDNA transcription not only produces tRNA molecules but can also affect other gene expression. Early work shows that active tRNA gene transcription in *Saccharomyces cerevisiae* can repress a gene divergently transcribed upstream from a tRNA gene by RNA polymerase II (known as position effect or tRNA gene-mediated silencing) (8). Interestingly, *S. cerevisiae* LTR retrotransposon elements Ty1 and Ty3 are frequently integrated upstream of tRNA genes, and so Ty1/Ty3 activity could also be regulated by tRNA transcription. Alternatively, a tRNA gene can block the spreading of chromatin silencing (i.e., barrier insulator activity) in yeast (22, 86). An updated view suggests that gene regulation by tRNA genes might be explained by their ability to regulate the higher-order organization of chromatin (131). Fluorescent imaging shows that tRNA genes in *S. cerevisiae* are clustered in the nucleolus, which depends on RNA polymerase III–complex formation (124). Furthermore, the tRNA position effect is perturbed when tRNA genes fail to cluster to the nucleolus and a single tRNA gene is insufficient to act as an insulator (86). It was proposed that tDNA insulation is due to the combined action of cohesins and chromatin remodeling/modifying proteins, including SMC (structural maintenance of chromosomes) proteins that regulate chromatin loops (23, 86) or sister chromatid cohesion (24). While most work was done in yeast (due to the difficulty in editing individual tRNA genes in human cells), chromatin insulator activity has also been described for human tRNA genes to block repression mediated by PcG (Polycomb group) proteins and enhancer-mediated activation (96). Chromosome conformation capture analysis by enhanced 4C or Hi-C identifies long-range interactions among tDNA molecules (96, 132), which are dependent on CTCF (132). All these data suggest that tDNAs and their associated transcription are tightly linked to chromatin higher-order structure. A recent report of tDNA deletion in *S. cerevisiae* and its effect on local nucleosome positioning and gene silencing but not global chromosome mobility or chromatin folding (40) suggests that tDNA probably plays a local role in the chromatin structure and is not as important for maintaining higher-order chromatin structure.

5.2. Sequence Variation in tRNA Genes

Not much was known about tRNA sequences on a genome-wide scale before the Human Genome Project, which shows a surprisingly large number of tRNA genes compared to the number of amino acids or codons that need decoding. Since then, although genomics research has grown substantially to understand different parts of the genome, tRNA genes have not been a major focus. Unexpectedly, sequence variations in tDNAs that are different from the reference genome have been described in the 1,000 Genomes Project (89) and a more recent tDNA-targeted sequencing approach (5). Such variation in tDNAs could affect both canonical and noncanonical functions of tRNAs. It was recently suggested that tRNA genes might experience 7–10-fold higher mutation rates compared to other parts in the genome, due to transcription-associated mutagenesis (125).

Whether tDNA variation also happens in other organisms, particularly viruses, and contributes to their adaptation could be an interesting future topic of research, given that tRNA genes have been identified with unclear functions in phage genomes, including the newly discovered giant phages (1). Altogether, the sequence variation in tDNAs could hint at some undiscovered functions of tRNAs in natural selection and evolution.

6. tRNA DYSREGULATION IN DIFFERENT DISORDERS

tRNAs have been implicated in different diseases, attributed to both their canonical and noncanonical functions. Although tRNA dysregulation has been linked to proteome dysregulation (60), this does not exclude the dysregulation of possible noncanonical functions of the tRNAs. In this section, we summarize recent reports where mechanistic details of tRNA dysregulation were reported in human disease, focusing on tRFs.

6.1. tRNA Dysregulation in Metabolic Disorders

Mutations in the tRNA methyltransferase *TRMT10A* cause young-onset diabetes and microcephaly. *TRMT10A* is expressed in all tissues but at significantly higher levels in pancreatic islets and brain compared to other tissues. *TRMT10A* deficiency leads to tRNA guanosine 9 hypomethylation, which results in $tRNA^{Gln}$ fragmentation and pancreatic β-cell death via oxidative stress (17). Though the enzyme generating tRNA fragments is not known, this unveils a novel mechanism of human β-cell death in diabetes due to the accumulation of tRF-5s.

Environmental factors such as diet can influence the metabolism of the offspring (11). Two recent studies demonstrated that sperm small RNAs have an impact on transmitting information to the offspring (12, 111). Sperm isolated from mice on a low-protein diet contained an excess of tRF-$5c^{GlyGCC}$ (tRF-5002c in tRFdb) that suppresses nearly 70 genes that are associated with the activity of endogenous retroelement MERVL (111). In the second study, mice on a high-fat diet produced offspring with impaired glucose tolerance. Sperm from F_0 mice on a high-fat diet had abundant 5′ tRNA halves (30–34 bases long). Injection of these 5′ tRNA halves into normal oocytes led to the downregulation of various early embryonic genes that have promoter regions with complementarity to the tRNA halves (12). Similar metabolic disorder across multiple generations was observed in response to a maternal high-fat diet, which is explained by tRF-5s from F_1 sperm (103). Interestingly, the intergenerational inheritance of metabolic disorder by the paternal diet is dependent on DNMT2 modification of tRNA halves (12, 146).

6.2. tRNA Dysregulation in Neurological Disorders

In addition to paternal cues that can shape offspring behaviors, maternal signals have been shown to affect progeny phenotypes, particularly those related to neurological disorders. A maternal high-fat diet increases hedonic behaviors, such as overconsumption of food and drug abuse, transgenerationally up to the third generation, in addition to the above-mentioned metabolic phenotypes; this phenotype is also transmitted by sperm tRFs via the paternal lineage (103). Moreover, maternal immune activation (MIA) during gestation increases autism-like behaviors in offspring, and recently placental/decidual tRFs (5′ tRNA halves and tRF-3s) were found to be acutely regulated by MIA (118). It is yet to be determined whether the placental tRF changes contribute to the autistic behaviors in the offspring.

ANG is one of the RNases that generate tRNA halves. Intriguingly, loss-of-function *ANG* mutations have been identified in neurodegenerative diseases, such as Parkinson's disease and amyotrophic lateral sclerosis (ALS) (112). Overall, wild-type ANG seems to play a neuroprotective role against different stress and neurotoxins (48, 102, 117) and might provide new treatments for

neurodegenerative disorders in the future. Since ANG regulates the production of tRNA halves in stress conditions (as discussed in Section 2.3.2), it is logical to speculate that certain tRNA halves could modulate the pathophysiological processes of these diseases. Indeed, the DNA mimics of the 5′ halves from $tRNA^{Ala}$ and $tRNA^{Cys}$ could form G-quadruplex structures that confer neuroprotective activity in cooperation with YBX1 by repressing translation and promoting SG formation (48). Interestingly, the neuroprotective activity of 5′ tRNA halves from $tRNA^{Ala}$ and $tRNA^{Cys}$ is inhibited by the pathological G-quadruplex forming the GGGGCC repeat of the *C9ORF72* gene, one of the leading causes of frontotemporal dementia and ALS (48). Meanwhile, interaction between ANG-generated 3′ tRNA halves and cyt c might protect primary cortical neurons from stress-induced apoptosis (102). Paradoxically, AND has also been held responsible for neurotoxicity: A genetic mutation in tRNA methyltransferase *NSUN2* is associated with a neurodevelopmental disorder that shows intellectual disability and Dubowtz-like syndrome. Hypomethylated tRNA in *NSUN2* deficiency leads to increased ANG-mediated tRNA halves, which increases the stress response, decreases cell size, and increases apoptosis in neurons of the cortex, hippocampus, and corpus striatum. The hypersensitivity of *NSUN2*-deficient mouse brains to oxidative stress was abrogated by ANG inhibition during embryogenesis, linking the tRNA half-generation by ANG to the neurotoxicity of *NSUN2* deficiency (6).

Neurodegeneration could come from defects in tRNA metabolic pathways and tRNA processing enzymes, such as CLP1, a component of the tRNA splicing machinery. *CLP1* mutation in patients with neurodegenerative disease displays progressive motor neuron loss (53, 105). In these patients, the neurons accumulate splicing intermediate tRFs, including 5′ leader-exon and 3′-exon from pre-$tRNA^{Tyr}$. Interestingly, these tRFs are induced by oxidative stress, and high levels of them caused neuronal cell death. Therefore, the accumulation of splicing intermediate tRFs could be a possible cause of the neurodegeneration in *CLP1*-deficient disease.

6.3. tRNA Dysregulation in Cancers

Global analyses of tRNAs and tRFs in different cancers are beginning to appear in the literature. These include databases that quantify tRFs (94, 148) and tRNAs (147) from The Cancer Genome Atlas (TCGA). In addition, tRF-1s have been profiled by custom microarrays with patient samples in chronic lymphocytic leukemia (CLL) and lung, colon, ovary, and breast cancer (2). Correlational analysis also reveals an unexplained relationship between tRFs and potential target pathways. In particular, tRF abundance was positively correlated with short genes with an increased density of ALU, MIR, and ERV repeats and with genes associated with cell proliferation and negatively correlated with long genes with a lower density of repeat elements. The mechanism underlying this relationship is unclear (122). Below we focus on recent reports about tRFs in different cancer types.

6.3.1. Breast cancer and prostate cancer. Changes in the expression of mature tRNAs have been described previously to regulate codon-mediated protein translation in breast cancer and are not detailed here (38, 91). Sex-hormone-dependent tRNA-derived RNAs (SHOT-RNAs) are abundantly expressed tRNA halves in ER^+ breast cancer or AR^+ prostate cancer, and the 5′ SHOT-RNAs from $tRNA^{Lys}$, $tRNA^{Asp}$, and $tRNA^{His}$ have a role in cell proliferation (45). The 5′ tRNA half from $tRNA^{Val}$ suppresses the Wnt/β-catenin signaling pathway by directly targeting the *FZD3* transcript and thus acts as a tumor suppressor in breast cancer cells (84). In another report, hypoxia in breast cancer cells induces internal tRFs derived from the anticodon loop of $tRNA^{Gly}$, $tRNA^{Asp}$, $tRNA^{Tyr}$, and $tRNA^{Glu}$; such induction is diminished in metastatic cells. These tumor-suppressive i-tRFs bind and displace YBX1, an RBP that otherwise binds to the 3′ UTR of various oncogenic transcripts and stabilizes them (37).

6.3.2. Colorectal cancer. The expression level of tRF-3001a from $tRNA^{Leu}$ (also known as miR-1280) is significantly decreased in human colorectal cancer tissues compared to adjacent normal tissues. tRF-3001a works as a tumor suppressor in colorectal cancer cells by interacting with the 3′ UTR of *JAG2* and suppressing its expression. The resulting repression of the Notch signaling pathway inhibits cancer stem-like cell functions in the cancer (46).

6.3.3. B cell lymphoma. A 22-base tRF-3 from $tRNA^{GlyGCC}$, CU1276 (tRF-3027b in tRFdb), is abundantly expressed in normal B cells, and its expression is suppressed during the malignant transformation of B cells. Overexpression of tRF-3027b represses the expression of DNA-repair and replication gene *RPA1* via a microRNA-like mechanism, which regulates cell proliferation and DNA damage response (80).

6.3.4. Chronic lymphocytic leukemia and lung cancer. ts-4521 (tRF-1003 in tRFdb) from pre-$tRNA^{Ser}$ and ts-3676 (also renamed ts-53) from pre-$tRNA^{Thr}$ are downregulated in CLL and lung cancer patients; both tRFs bind with PIWIL2 (92). In another report from the same group, ts-3676 associates with AGO and targets the *TCL1* mRNA by targeting three consecutive 28-bp repeats in the 3′ UTR; overexpression of the *TCL1* oncogene, expected when the tRF is downregulated, has been known to promote the aggressive form of CLL (3). It is interesting to note that the authors also found that ts-3676 and ts-4521 have mutations in CLL patients (codeleted with *P53*) (3). PUS7 loss has been observed in myelodysplastic syndromes that have a high risk for acute leukemia; specific tRF-5s in *PUS7*-null cells are inhibited and lead to a failure to repress translation in hematopoietic stem cells, thus impairing differentiation (39).

6.4. tRFs or tRNAs in Extracellular Vesicles

In the last two years there have been more than a dozen papers published where researchers have studied the composition of EVs. EVs represent a newly described pathway of intercellular communication (133). tRFs have been reported to be differentially present in EVs that are isolated from patients with various diseases or in different conditions, providing evidence for them being packaged in exosomes. For example, tRFs were found in EVs secreted by T cells and might contribute to the repression of T cell activation (15). tRFs are highly expressed in plasma exosomes in liver cancer patients compared to healthy controls, suggesting the potential value of tRFs in plasma-based diagnoses of liver cancers (150).

Many reports showed the presence and relative enrichment of tRFs, especially 5′ tRNA halves in EVs (49, 116, 138). Since the EV field is relatively new, technical aspects such as EV isolation and sequencing strategy are being actively developed. Recently, TGIRT-seq was used to sequence EVs, which revealed abundant full-length tRNAs instead of tRFs in the EVs; the authors concluded that full-length tRNAs might be more abundant than previously measured (114). Interestingly, YBX1 plays an important role in sorting tRNAs and tRFs into exosomes. These experiments expand our understanding about how RNA is sorted into EVs for cellular export and the potential function of tRFs or tRNAs in intercellular communication.

7. tRNAS AS TOOLS IN BIOTECHNOLOGY APPLICATIONS

tRNA has emerged as a useful tool in biotechnology applications (**Figure 3**), including genetic code expansion using engineered tRNA and AARS pairs to install unnatural amino acid at specific codons (14) (**Figure 3*a***); this idea has also been employed to create a membraneless organelle

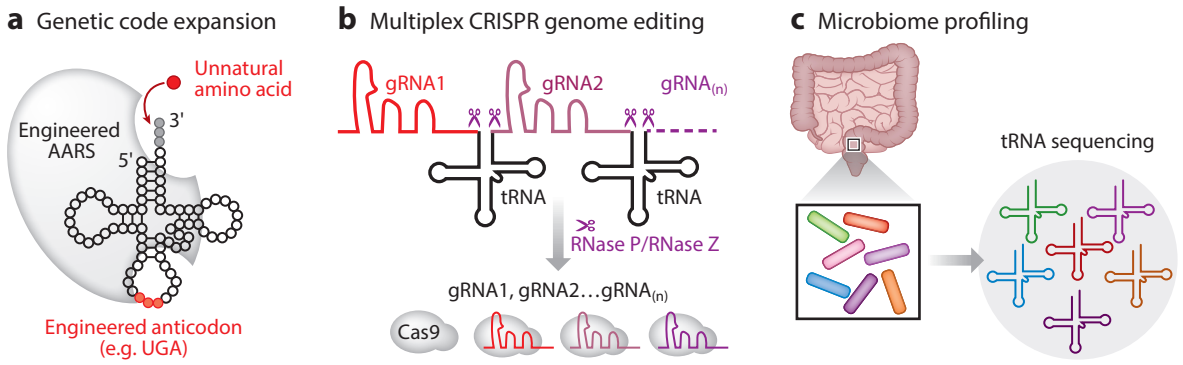

Figure 3

Applications using tRNAs. (*a*) An engineered tRNA-AARS pair can be used to incorporate unnatural amino acid in protein expression. (*b*) A tRNA scaffold can be used to express guide RNAs for CRISPR genome editing, utilizing endogenous tRNA processing machineries that cleave both ends of the tRNA molecules. Multiple guide RNAs can be expressed simultaneously by placing tRNA scaffolds in tandem. (*c*) tRNA sequencing can be used to profile microbiome composition. Abbreviations: AARS, aminoacyl tRNA synthetase; CRISPR, clustered regularly interspaced short palindromic repeat; gRNA, guide RNA; tRNA, transfer RNA.

(97). Since the incorporation of amino acids into polypeptides is considered a canonical role of tRNA, we do not go into details, and instead we highlight some recent developments below.

The clustered regularly interspaced short palindromic repeat (CRISPR) system has become a powerful tool for genome editing in the past decade. Central to this system is a guide RNA (gRNA) that directs CRISPR-associated nuclease protein to a specific genomic sequence. Compared to the traditional gRNA expression scaffold (usually ~350 bp), a single 70-bp tRNA sequence can be used to express and generate gRNA by harnessing the endogenous tRNA processing machinery that cleaves both ends of the tRNAs (81, 137). To inform the design of an engineered tRNA scaffold for better editing efficiency, a recent screening platform measured promoter strength and processing activity of different tRNA variants (61). Furthermore, tandemly arrayed tRNA-gRNA has enabled simultaneous gRNA expression and editing in various species (95, 139, 140, 145) (**Figure 3*b***).

Finally, tRNA sequencing has been used to profile the gut microbiome in mice, similar to traditional 16S ribosomal RNA sequencing (110) (**Figure 3*c***). Using a specific tRNA sequencing strategy (DM-tRNA-seq) that quantifies both tRNA expression and modification levels, the authors were able to infer bacteria taxonomy and even distinguish samples from different diets. Given the growing interest in studying the microbiome in human health, this approach might help shed light on whether bacterial tRNAs affect biosynthetic pathways and immune responses in the host.

8. CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we summarize the noncanonical functions of tRNAs outside their canonical role in protein translation. All of these tRNA functions could affect different aspects of biology: intracellular, intercellular, or even evolutional. Understanding these tRNA functions is also important for understanding functions or mechanisms of global regulators of tRNAs, such as RNA polymerase III, MYC, or MAF1, which have also been implicated in important biological pathways.

Currently tRNAs are missed in most RNA-seq data sets, including the Encyclopedia of DNA Elements (ENCODE) and TCGA. tRFs are represented to a certain extent in small RNA-seq data sets, but because these are usually optimized around 22-nt microRNAs, they are expected to be underrepresented, especially the longer tRFs and tRNA halves. We envision that the recent

technical advances in tRNA, tRF, and tRNA modification sequencing approaches, in conjunction with single-cell technologies, will bring new insights about their functions.

DISCLOSURE STATEMENT

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