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MicroReview

Non-canonical roles of tRNAs and tRNA mimics in bacterial cell biology

Assaf Katz,[1]* Sara Elgamal,[2] Andrei Rajkovic[2] and Michael Ibba[2]

[1]*Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago 8380453, Chile.*

[2]*Department of Microbiology and The Center for RNA Biology, Ohio State University, Columbus, Ohio 43210, USA.*

Summary

Transfer RNAs (tRNAs) are the macromolecules that transfer activated amino acids from aminoacyl-tRNA synthetases to the ribosome, where they are used for the mRNA guided synthesis of proteins. Transfer RNAs are ancient molecules, perhaps even predating the existence of the translation machinery. Albeit old, these molecules are tremendously conserved, a characteristic that is well illustrated by the fact that some bacterial tRNAs are efficient and specific substrates of eukaryotic aminoacyl-tRNA synthetases and ribosomes. Considering their ancient origin and high structural conservation, it is not surprising that tRNAs have been hijacked during evolution for functions outside of translation. These roles beyond translation include synthetic, regulatory and information functions within the cell. Here we provide an overview of the non-canonical roles of tRNAs and their mimics in bacteria, and discuss some of the common themes that arise when comparing these different functions.

Introduction

All currently known genetic information is coded in nucleic acids (RNA or DNA). In order to be functional, much of this genetic information must first be decoded into proteins that are composed of amino acids that do not have any specific affinity to the message coded in nucleic acids (Crick, 1958; Crick, 1970; Francklyn and Minajigi, 2010). In order to translate this genetic information cells need adaptors, molecules that can translate nucleic acid into amino acid sequences (Crick, 1958; Hoagland *et al.*, 1958). This adaptor function is performed by aminoacyl-transfer RNAs (aa-tRNA), which have a nucleic acid portion composed of a highly structured transfer RNA (tRNA) of about 80 nucleotides. The second part of an aa-tRNA molecule is composed of a single amino acid bound through an ester bond to the 2′ or 3′ OH of the 3′-end of the tRNA. The secondary structure of the tRNA is composed of an acceptor arm that carries the amino acid, the anticodon arm where there is an anticodon triplet which recognizes codons on mRNA during translation, and the deoxyuridine, TΨC and variable arms (D, T, and V arms, respectively) (Fig. 1) (Ladner *et al.*, 1975; Giegé and Frugier, 2000; Marck and Grosjean, 2002). Transfer RNAs are functionally grouped into families of isoacceptors, which may differ in sequence, but all of which carry a unique amino acid for protein synthesis. This amino acid is added by aminoacyl-tRNA synthetases (aaRS), a group of enzymes that specifically recognize and activate a particular amino acid and transfer it to the correct tRNA. This reaction is central to the process of translation as it ensures that the correct amino acid is transferred to the nascent peptide (Cavarelli and Moras, 1993; Schimmel *et al.*, 1993; Ibba *et al.*, 2005). In order to select the correct tRNAs, aaRS recognize specific nucleotides called 'identity elements'. These nucleotides are scattered throughout the tRNA structure, but are usually concentrated in the anticodon loop and the acceptor stem (Beuning and Musier-Forsyth, 1999). Ribosomes also contact tRNAs throughout their surface and require them to be flexible molecules. Nevertheless, with the exception of discrimination between initiator and elongator tRNAs, interactions with anticodon nucleotides have a predominant role in selection of the correct tRNA. Additionally, other regions of the tRNA have a role in

Accepted 9 May, 2016. *For correspondence. E-mail: akatz@med.uchile.cl; Tel. +56-2 2978 9584; Fax +56-2 2735 5580.

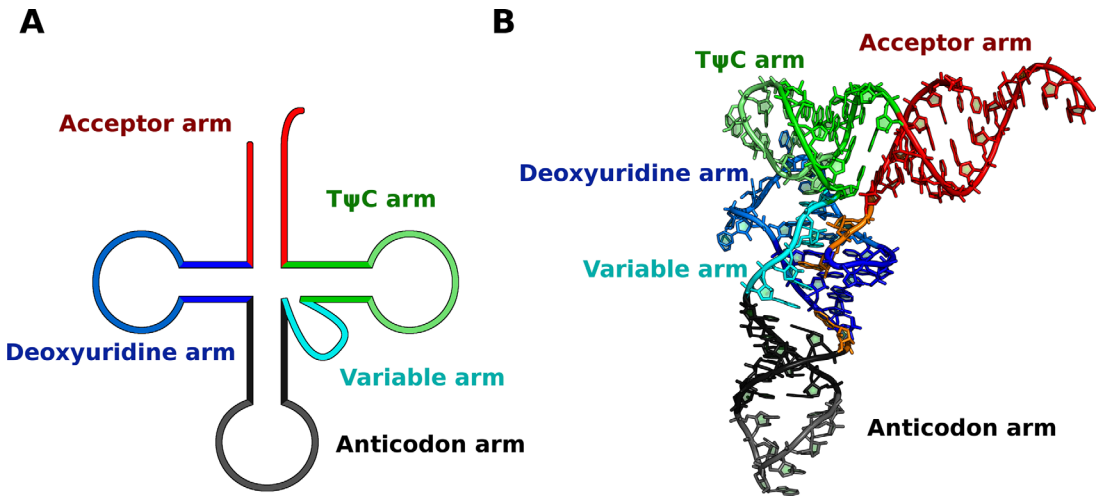

Fig. 1. Structure of tRNA. Schematic representation of (A**)** secondary and (B**)** tertiary structure of tRNA. Tertiary structure is based on the backbone of yeast $tRNA^{Asp}$ as in pdb model 1VTQ (Moras *et al.*, 1980). The main parts of tRNA are highlighted in different colours, using darker colours for stems and lighter colours for loops. Used colours are red: acceptor stem, blue: deoxyuridine arm, gray: anticodon arm, cyan: variable arm, green: TΨC arm and orange: segments linking tRNA arms.

compensating for the different affinity of the diverse anticodons and amino acids that are used in translation either directly interacting with the ribosome or allowing for optimal flexibility (Dale and Uhlenbeck, 2005; Khade and Joseph, 2010; Shepotinovskaya and Uhlenbeck, 2013).

Phylogenetic studies and the observation that tRNA structure and the genetic code are essentially maintained in all known organisms, suggest that tRNAs (or their direct and structurally similar predecessor) originated at the time of the last universal common ancestor or earlier (Schimmel *et al.*, 1993; Di Giulio, 1995; Di Giulio, 2006; Sun and Caetano-Anollés, 2008; Moura *et al.*, 2010). Precursors of tRNA might even predate the existence of proteins and could have had a catalytic or metabolic role outside of translation in the "RNA world" (Rodin *et al.*, 2011; de Vladar, 2012; Morgens, 2013). Regardless of their exact origin, the high degree of similarity between extant tRNAs probably derives from their role in a very complex machinery where changes in one component could necessitate modification of other parts of the genetic code and/or translation apparatus to maintain function. An example of this can be observed in the mitochondria of several metazoans where many tRNAs have lost either their T or D arms. In the cases that have been studied in more detail, it was observed that EF-Tu, rRNA, ribosomal proteins and aaRS coevolved to allow the recognition of these non-canonical tRNAs (Watanabe *et al.*, 2014). Changes in the 'sense' of codons (where they are reassigned to encode a different amino acid) also require major modifications as the appearance of this new sense for a codon would otherwise force the introduction of amino acids in an erroneous context. For instance, comparative genomic studies have suggested that Leu CUG codon reassignment to Ser in several Candida and Debaryomyces fungi led to a drastic reduction in the usage of these codons (Moura *et al.*, 2010). Replicating this codon reassignment in the model organism Saccharomyces cerevisiae resulted in decreased fitness in several growth media and decreased thermal stability for at least one protein. Nevertheless, these experiments also showed a great growth advantage in some culture conditions (e.g. a 500% growth advantage in the presence of copper sulphate). This change in niche preference and a probable low level of misincorporation in the original conditions may have allowed the fixation of these changes to the genetic code (Mateus *et al.*, 2013). Other codon reassignments may have required the prior elimination of the original codon from the genetic code, for instance due to abnormally high or low CG usage in the genome (Moura *et al.*, 2010). Although such tRNA-dependent modifications to the translation apparatus are rare, they probably had an important role in the original establishment and/or expansion of the genetic code by facilitating the coding of new amino acids (Moura *et al.*, 2010; Rodin *et al.*, 2011; de Vladar, 2012). Beyond its integral role in defining and translating the genetic code, tRNA has also evolved a surprisingly wide range of alternative functions (Fig. 2). The aim of this review is to analyze the pathways that have evolved to utilize tRNAs, sometimes by hijacking them to function beyond the translation machinery. Emphasis will be placed on bacterial systems, and examples from other organisms will be mentioned when relevant.

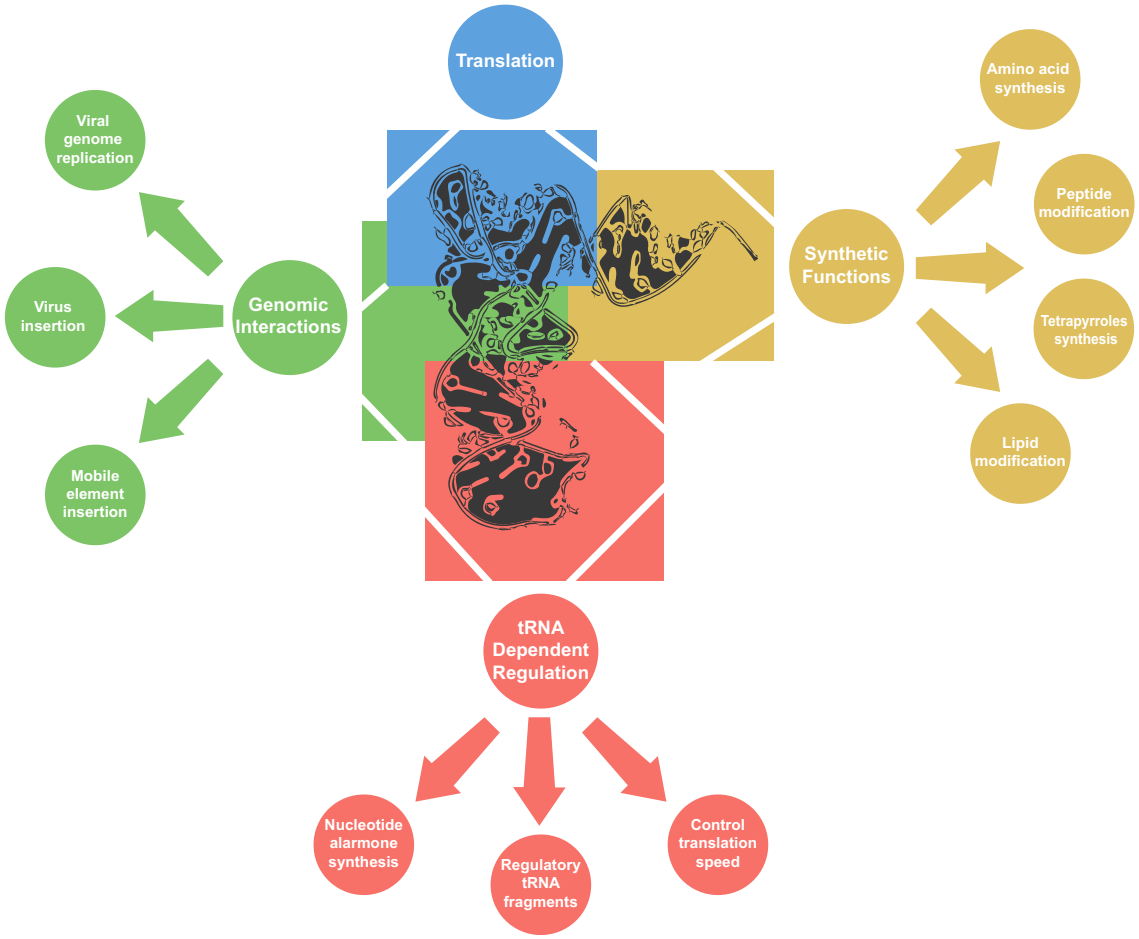

Fig. 2. Biological roles of tRNA. Classification of the diverse roles that tRNAs play in cell biology. Functions of tRNAs were classified in four main groups, translation (blue), synthetic functions beyond translation (yellow), tRNA dependent regulation (red) and genomic interactions (green). Positioning of each group in the figure is not related to the parts of tRNA involved in specific functions.

aa-tRNA based synthesis beyond translation

Modification of macromolecules. Many different aa-tRNAs are used outside of translation to provide activated amino acids for a wide variety of purposes. These include the synthesis of several metabolites as well as the modification of macromolecules (RajBhandary and Söll, 2008; Banerjee *et al.*, 2010; Francklyn and Minajigi, 2010; Dare and Ibba, 2012; Katz and Orellana, 2012a; Belin *et al.*, 2012; Raina and Ibba, 2014). Some of the earliest described examples of such roles outside of translation are related to modification of cellular envelopes (RajBhandary and Söll, 2008; Francklyn and Minajigi, 2010). For example, the addition of amino acids to phosphatidylglycerol through ester bonds to the glycerol moiety and synthesis of certain peptide cross bridges for peptidoglycan both require aa-tRNA substrates (RajBhandary and Söll, 2008). Modification of lipids is used to change the membrane charge distribution, thereby protecting the cell against some antibiotics or bactericidal peptides produced by competing species (Dare and Ibba, 2012; Raina and Ibba, 2014; Goldfine, 2014). Lipid aminoacylation also participates in resistance to osmotic or pH stresses (Dare and Ibba, 2012; Goldfine, 2014) and might even have a role in bacterial replication by modulating the ability of DnaA to exchange ADP with ATP (Ichihashi *et al.*, 2003). In addition to lipid modification, aa-tRNAs can also be used as substrates for the addition of amino acids to peptides in bacteria. This includes the synthesis of the pentapeptide bridge from peptidoglycan peptide by MurM or Fem proteins, modification of the amino terminus in proteins starting with basic amino acids by leucyl/phenylalanyl transferases (L/F-transferases; in eukaryotes Arg-tRNA-protein transferase modifies proteins with Arg) and the synthesis or modification of some small peptides using enzymes like AlbC, PacB or VlmA (Berger-Bächi and Tschierske, 1998; Garg *et al.*, 2008; Zhang *et al.*, 2011; Dougan *et al.*, 2012; Dare and Ibba, 2012; Belin *et al.*, 2012; Shepherd and Ibba, 2013b; Raina and Ibba, 2014). These amino acid additions to peptides have

diverse functions. The peptidoglycan bridge changes the structural properties of the cell wall, enhancing antibiotic resistance and binding of some extracellular proteins (Berger-Bächi and Tschierske, 1998; Guan *et al*., 2004; Scheffers and Pinho, 2005; Dare and Ibba, 2012; Shepherd and Ibba, 2013b). By contrast, modification of the amino terminus of cytoplasmic proteins modulates the half-life of specific proteins by targeting them for recognition by ClpS and subsequent degradation by ClpAP protease in bacteria or by UBR proteins and subsequent degradation by the 26S proteasome in eukaryotes (Dougan *et al*., 2012; Raina and Ibba, 2014). Finally, small peptides synthesized using the activated amino acid on aa-tRNA as a precursor correspond to secondary metabolites with diverse physiological functions ranging from antibiotics and inter-cellular signalling molecules to metal chelators (Garg *et al*., 2008; Zhang *et al*., 2011; Belin *et al*., 2012).

In all cases that have so far been studied, the specificity for aa-tRNA in the modification of macromolecules depends primarily on recognition of the amino acid with a minor but relevant role for the tRNA, particularly the acceptor stem. For instance, FemX from *Weissella viridescens* uses Ala-tRNA^{Ala} as substrate, excluding from its active site any amino acid that is bigger than Ala. The only aa-tRNA carrying a smaller amino acid, Gly-tRNA^{Gly}, is excluded by recognition of tRNA 'anti-determinants', that is, the exclusion of any tRNA that presents a specific sequence (in this case base pair C2-G71) that is absent from the natural substrate of the enzyme (Fonvielle *et al*., 2009). Base pairs that are outside of the acceptor stem of tRNA, or even 3 to 4 base pairs away from the amino acid, have a negligible effect on the aa-tRNA specificity of FemX (Villet *et al*., 2007; Fonvielle *et al*., 2009; 2013). Studies on aminoacyl-phosphatidylglycerol synthases (MprF or aa-PGS) that catalyze lipid modification (Gould *et al*., 1968; Roy and Ibba, 2008; Hebecker *et al*., 2011; Dare and Ibba, 2012), L/F-transferases that modify the amino terminus of proteins tagging them for degradation (Suto *et al*., 2006; Watanabe *et al*., 2007; Wagner *et al*., 2011; Fung *et al*., 2014), and AlbC that catalyzes the synthesis of a cyclic dipeptide (Bonnefond *et al*., 2011; Sauguet *et al*., 2011; Moutiez *et al*., 2014) all showed similar dependence on acceptor stem sequences. From this perspective, AlbC is particularly interesting as the enzyme uses two aa-tRNAs that bind sequentially to different regions of the enzyme. The enzyme is more specific for the recognition of the first aa-tRNA (where it uses only one amino acid) than for the second aa-tRNA (where it uses one amino acid preferentially, but accepts others). In the case of AlbC, recognition of the second aa-tRNA depends strongly on the identity of the first base pair of the tRNA acceptor stem (G1-C72 in AlbC substrates), while the identity of these nucleotides is not relevant for the recognition of the first aa-tRNA substrate (Moutiez *et al*., 2014).

Synthesis of small metabolites. In addition to their roles in the synthesis or modification of macromolecules, tRNAs participate in the production of several much smaller metabolites. Beside the synthesis of di-peptides mentioned above, tRNAs have central roles in the synthesis of tetrapyrroles (such as heme or chlorophyll) and several amino acids. Many organisms lack the complete set of aaRSs required to aminoacylate every tRNA isoacceptor with the corresponding amino acid. In these organisms some tRNAs are aminoacylated in an indirect pathway where the cognate tRNA is first 'mis-acylated' with a precursor of the correct amino acid that is subsequently modified while on the tRNA. These indirect pathways have been observed for the synthesis of glutamine on tRNA^{Gln} (in all known archaea, as well as in most bacteria and eukaryal organelles), asparagine on tRNA^{Asn} (in several bacteria and archaea), formylmethionine on tRNA^{fMet} (in bacteria and eukaryotic organelles), cysteine on tRNA^{Cys} (in some archaea) and selenocysteine on tRNA^{Sec} (in some bacteria, archaea and eukarya) (Ibba *et al*., 2000; O'Donoghue *et al*., 2005; Sheppard *et al*., 2008; Yuan *et al*., 2008; Katz and Orellana, 2012a). In several organisms the main function of these pathways is to provide aa-tRNA for the synthesis of proteins. Furthermore, some organisms lack alternative pathways to synthesize these amino acids and for them, tRNA dependent synthesis is the sole means to produce these metabolites (Sauerwald *et al*., 2005; O'Donoghue *et al*., 2005; Katz and Orellana, 2012a; Mladenova *et al*., 2014). The indirect aminoacylations of 'amide' (Gln and Asn) tRNAs were among the first of these pathways to be described. In these examples, tRNA^{Gln} or tRNA^{Asn} are first aminoacylated with the 'acid' amino acid (Glu or Asp) by a non-discriminating aaRS (ND-GluRS or ND-AspRS). In some organisms, these non-discriminating enzymes aminoacylate the tRNAs for both acid (tRNA^{Glu} or tRNA^{Asp}) and amide (tRNA^{Gln} or tRNA^{Asn}) amino acids, but in other organisms there are specialized enzymes for each kind of tRNA. In a second step, a tRNA dependent amidotransferase (AdT) catalyzes the formation of the amide (Gln or Asn) on the tRNA (Katz and Orellana, 2012a). The other indirect pathways follow similar steps, first adding a precursor (phosphoserine (Sep) on tRNA^{Cys}, Met on tRNA^{fMet} or Ser on tRNA^{Sec}) that is converted into the final product in one or two additional reactions (catalyzed by Sep-tRNA:Cys-tRNA synthase (SepCysS) for Cys-tRNA^{Cys}, methionyl-tRNA formyltransferase (FMT) for fMet-tRNA^{fMet} and selenocysteine synthase (SelA) in bacteria or phosphoseryl-

tRNA kinase (PSTK) plus Sep-tRNA:Sec-tRNA synthase (SepSecS) in archaea and eukarya for Sec-tRNA^{Sec}). In all these pathways, the production of a "mis-acylated" tRNA as an intermediary could potentially allow the mis-incorporation of amino acids during translation. This is prevented mainly by two processes: (i) the ability of translation elongation factors and, possibly the ribosomal A site, to partially discriminate tRNAs, binding preferentially the correctly acylated ones (LaRiviere *et al*., 2001; Dale and Uhlenbeck, 2005; Yuan *et al*., 2008; Katz and Orellana, 2012a) and (ii) the formation of complexes between the non-discriminatory aaRS and the modifying enzyme from the second step of the pathway that sequester the mis-acylated tRNAs (Bailly *et al*., 2007; Zhang *et al*., 2008; Rampias *et al*., 2010; Katz and Orellana, 2012a). Additionally, in some cases channeling is not required as kinetic competition between the amino acid modifying enzyme and elongation factor prevents accumulation of mis-acylated intermediates (Bhaskaran and Perona, 2011).

While the enzymes used for aa-tRNA-dependent modification of macromolecules recognize their substrates mainly through direct recognition of the amino acid and the nearby nucleotides of the acceptor stem on the tRNA, enzymes involved in the modification of amino acids on tRNAs may recognize a bigger surface of the tRNA including the D and/or T arms. For example, bacterial AdT (GatCAB) (Katz and Orellana, 2012a), eukaryotic and archaeal PSTK (Sherrer *et al*., 2008; Yuan *et al*., 2008) and FMT from bacteria and mitochondria of mammals (Lee *et al*., 1991; Varshney *et al*., 1991; Guillon *et al*., 1992; Li *et al*., 1996; Newton *et al*., 1999; Takeuchi *et al*., 2001) have all been shown to recognize their substrates through interactions with the acceptor and D arms. In contrast, FMT from yeast mitochondria (Vial *et al*., 2003) and SepCys (Fukunaga and Yokoyama, 2007; Helgadóttir *et al*., 2012) have been suggested to recognize tRNA through its acceptor stem and SepSecS through its acceptor and T stems (Palioura *et al*., 2009).

Unlike the amino acid synthesis reactions described above, the first step of tRNA-dependent tetrapyrrole synthesis in archaea, chloroplast and most bacteria involves removal of the amino acid attached to Glu-tRNA^{Glu}. In this reaction Glu is detached from tRNA^{Glu} and transferred to the enzyme glutamyl-tRNA reductase (GluTR) where it is reduced to form glutamate 1-semialdehyde (GSA). Subsequently, GSA is channeled to the second enzyme of the pathway GSA aminotransferase where it is isomerized to δ-aminolevulinic acid, which is the universal precursor for tetrapyrrole synthesis. GluTR can use Gln-tRNA^{Glu} as a substrate indicating that the enzyme is not very stringent in amino acid recognition. Additionally, experiments performed with several mutants indicated that GluTR accepts several variations in the tRNA sequence. In contrast to all the other enzymes discussed previously, GluTR does not tightly recognize the amino acid or any specific sequence features. Instead, the enzyme seems to recognize the global folding of tRNA^{Glu} mainly due to the nucleotide in position 47, which is absent in tRNA^{Gln} (O'Brian and Thöny-Meyer, 2002; Randau *et al*., 2004; Heinemann *et al*., 2008; Katz and Orellana, 2012a).

In some organisms that use these alternative pathways, specialized tRNAs have been found. For instance, in *Staphylococcus epidermidis* and apparently *Staphylococcus aureus* there are specialized tRNA^{Gly} isoacceptors that are used for peptidoglycan synthesis, but not for mRNA translation (Bumsted *et al*., 1968; Giannouli *et al*., 2009). Conversely, in some strains of *Acidithiobacillus ferrooxidans* a specialized tRNA^{Glu} used in translation, but unable to participate in tetrapyrrole synthesis, has been found (Levicán *et al*., 2005). In both cases such specialized tRNAs might ensure aa-tRNA availability for both pathways or alternatively prevent sudden changes in tRNA aminoacylation levels when usage by one of the alternative pathways changes. Nevertheless, neither of these hypotheses has been tested. When a tRNA is released from the constraints imposed by its participation in translation, its sequence can deviate from the canonical. As an example, all tRNA^{Gly} from *S. aureus* that apparently are specialized in peptidoglycan synthesis have an altered sequence that prevents binding to EF-Tu, including one that has deviated in structure (mainly at its D and T arms) to the degree that tRNA prediction software categorizes the sequence as belonging to a pseudogene (Giannouli *et al*., 2009). Another potential example of this is tRNA^{Other} in *Bacillus cereus*, which presents several deviations from canonical tRNA structure including a G2:A71 bulge and the fact that it presents a Trp anticodon whereas it is aminoacylated with Lys (Ataide *et al*., 2005). Although originally shown to be a substrate for aminoacylation *in vitro* (Ataide *et al*., 2005), this tRNA was later shown to be absent from ribosome fractions *in vivo*. Instead of participating in translation, it was shown to be part of a larger regulatory RNA, where it was proposed to represent an important structural element (Rogers *et al*., 2012). The role of the smaller tRNA like fragment has yet to be determined.

Hijacking of tRNA for non-synthetic processes

The information in tRNAs can be used for several 'non-synthetic' roles which do not require donating an activated amino acid. Some of these roles, such as the usage of tRNAs as primers for viral genome replication

(Mak and Kleiman, 1997) or the use of their genes as insertion sites in the genome for invading viruses (Hacker and Kaper, 2000; Williams, 2002) depend on the mere existence of these molecules, while others such as the use for regulatory functions take advantage of tRNA's ability to act as a messenger for the metabolic status of the cell.

tRNA in regulatory processes. The ratio of aa-tRNA to deacylated tRNA depends on the availability of amino acids for aminoacylation and the speed of translation. Thus, the aminoacylation status of tRNA is a good indicator of the balance between the pathways that use amino acids (including translation and central metabolism) and amino acid synthesis or acquisition from the environment (Henkin and Yanofsky, 2002; Elf and Ehrenberg, 2005). In this context, it is not surprising that tRNAs have been hijacked out of their biosynthetic roles for use in regulatory processes. These regulatory systems usually require very small amounts of molecules and sometimes use only RNA. Thus, it has been proposed that such tRNA-based regulatory systems are cheap to produce and easy to evolve, and that for these reasons they might have already been in use in a probable ancestral RNA world (Henkin and Yanofsky, 2002). Furthermore, it has recently been proposed that the high specificity of aaRSs additionally allows tRNA-based regulatory systems to be highly sensitive and substrate-specific (Bullwinkle and Ibba, 2016).

Some of the best-described regulatory mechanisms involving tRNA are the stringent response and transcription attenuation. Both take advantage of different "side effects" of changing the aa-tRNA/tRNA ratio. In the stringent response, a sudden decrease in amino acid availability allows accumulation of deacylated tRNAs that bind the ribosome where they stimulate the transfer of PPi from ATP to either GDP or GTP by RelA/SpoT enzymes. These reactions form ppGpp and pppGpp, alarmones with pleiotropic effects on bacterial physiology including changes in metabolism of amino acids and nucleotides and regulation of synthesis of stable RNAs (rRNAs and tRNAs) (Liu *et al.*, 2015). Under transcription attenuation, instead the state of tRNA aminoacylation can be directly sensed by riboswitches that control the formation of Rho independent transcription terminators and thus, modulate expression of genes coded by the transcript downstream of the terminator site. The riboswitch may sense tRNA aminoacylation levels by two different strategies. In one strategy, represented by the classic example of the *trp* operon in *E. coli*, a small open reading frame at the 5′ leader region of the operon contains several Trp codons. Changes in the abundance of the specific aa-tRNA^{Trp} will change the speed of translation of this leader peptide modulating the time of residence of ribosomes in this area of the mRNA and controlling the formation of the terminator structure. In a second strategy, most frequently found in Gram-positive bacteria, the mRNA may present a riboswitch structure termed the T-box. Binding of the non-aminoacylated tRNA to the T-box prevents the formation of the transcription terminator, allowing transcription of the genes located downstream (Henkin and Yanofsky, 2002).

Similar to the effect of aa-tRNA abundance on the speed of translation of the leader peptide for transcription attenuation, changes in the availability of a specific aa-tRNA can also modify the speed of translation of genes that are rich in codons translated by this aa-tRNA. These genes are usually less sensitive than leader peptides and require large changes in amino acid availability that can potentially have strong effects on the abundance of aa-tRNA. Additionally, genes involved in signal transduction pathways can be extremely sensitive to small changes in protein levels (Elf and Ehrenberg, 2005; Sørensen *et al.*, 2005; Subramaniam *et al.*, 2013b; Subramaniam *et al.*, 2014). As an example of this extreme sensitivity, *Bacillus subtilis* can sense decreases in environmental serine abundance through changes in the speed of translation of the *sinR* biofilm repressor and use this signal to trigger the formation of biofilms. Changes in codon usage of *sinR* produce only minor changes in the protein's cellular concentration. Nevertheless, as SinR has a cooperative behaviour and is part of a negative feedback mechanism, this small change in protein concentration induces a strong change in colony morphology (Subramaniam *et al.*, 2013a).

The modification of several tRNA nucleotides is also known to be involved in regulatory pathways. Usually these modified nucleotides are located in the anticodon loop, although there are some exceptions. One of the first to be identified is located at position 37 (next to the 3′ end of the anticodon) of tRNAs reading codons with a 5′ U (tRNA^{Tyr}, tRNA^{Trp}, tRNA^{Phe}, tRNA^{Leu}, tRNA^{Ser}, tRNA^{Cys}) from *E. coli*. Cells cultured in a medium lacking iron were found to have lost a methylthio modification (presenting an i6A nucleotide instead of the normal hypermodified ms^{2}i^{6}A nucleotide), an alteration that induced the synthesis of enterobactin (a high affinity siderophore) and its aromatic amino acid precursors (Wettstein and Stent, 1968; Rosenberg and Gefter, 1969; Buck and Ames, 1984). Since then, several other modifications have been found to be involved in modulation of aerobic/respiratory metabolism (Buck and Ames, 1984; Persson *et al.*, 1998; Björk *et al.*, 1999; Nakayashiki *et al.*, 2013), expression of virulence factors (Björk *et al.*, 1999; Durand *et al.*, 2000; Shippy and Fadl, 2014), the response to changes in amino acid

availability (Laxman *et al*., 2013) or other stressful conditions (Golovina *et al*., 2009; Murata *et al*., 2011; Chan *et al*., 2012; Caballero *et al*., 2012; Dedon and Begley, 2014; Gu *et al*., 2014) in both bacteria and eukaryotes including mammals (Wei and Tomizawa, 2011; Gu *et al*., 2014; Endres *et al*., 2015). Additionally, high-throughput experiments have found changes of tRNA nucleotide modifications or the enzymes involved in the modification pathways related to stress conditions or diseases (Wang and He, 2014) suggesting that modification of tRNA might play a central role in regulation of gene expression.

In addition to nucleotide modifications, a series of bacterial toxins can cleave initiator or elongation tRNAs, thus inhibiting translation (Ogawa *et al*., 1999; Kaufmann, 2000; Winther and Gerdes, 2011; Ruhe *et al*., 2013; Cruz *et al*., 2015). This usually forms part of stress defense mechanisms, as in the case of PrrC, that inhibit protein synthesis under phage infection (Kaufmann, 2000) or some toxins from the VapC family that induce a dormancy state under other stressful conditions (Winther and Gerdes, 2011; Cruz *et al*., 2015). Nevertheless, other toxins have a very different ecological role and are used to attack nearby bacteria. This is the case of colicins D and E5 (Ogawa *et al*., 1999; Tomita *et al*., 2000) as well as some toxins from contact dependent growth inhibition systems such as WapA in *Bacillus subtilis* or some CDI toxins from *Burkholderia pseudomallei* (Koskiniemi *et al*., 2013; Ruhe *et al*., 2013). The reduction of functional tRNAs could have other consequences, as it has been shown that it can trigger the formation of dinucleotide second messengers (Kramer *et al*., 1988; Katz and Orellana, 2012b) or an increased usage of elongator tRNAs at initiation (Winther and Gerdes, 2011; Samhita *et al*., 2013; Shetty *et al*., 2015). Nevertheless, a physiological role for these phenomena in the context of tRNA fragmentation has not been studied yet. In eukaryotes, tRNA fragments are also produced. In addition to the effects derived from a decrease in tRNA concentration, in these organisms the fragments *per se* have a physiological role, binding polysomes or proteins involved in the siRNA and miRNA pathways and consequently affecting gene expression by mechanisms that are still not well understood (Raina and Ibba, 2014; Keam and Hutvagner, 2015).

tRNA mimics. The structural and functional adaptability of tRNA have allowed not only their use for alternative roles beyond protein synthesis, but also the appearance of processes that take advantage of their existence albeit not using them directly. For instance, several virus and mobile genetic elements use genes coding for tRNAs as an insertion site in genomes. These mobile elements have to include segments that mimic part of the tRNA gene sequence in order to enable recombination and to allow for reconstitution of the tRNA gene in cases where it is essential (Hacker and Kaper, 2000; Williams, 2002). Along the same lines, several molecules mimic the structure of tRNAs in order to control translation or use its machinery for other purposes. An impressive example of this are the tRNA-like structures (TLS) in the RNA genomes of several viruses (Dreher, 2009). A prototype of these mimics is the TLS located at the 3`end of the turnip yellow mosaic virus (TYMV) (Dreher, 2009; Colussi *et al*., 2014). When folded, the TLS adopts the classic L-shaped tRNA structure, although with a different topology based on idiosyncratic intramolecular interactions. Although the elements analogous to the D-loop, T-loop and V-loop are positioned similar to tRNA, they interact in a different manner. This configuration allows the TLS to present two faces. One face of the TLS is the 'tRNA-like face', which closely mimics tRNA and achieves tRNA-like valylation efficiencies and eIF1A binding. The opposing side presents tRNA-deviating features, an upstream pseudoknot domain and the genomic RNA interacting to enable additional functionality (Dreher and Goodwin, 1998; Colussi *et al*., 2014). In contrast to tRNA, the unique topology and intramolecular interactions of the viral RNA also allows the TLS to unfold, possibly to allow the replication of the RNA genome (Colussi *et al*., 2014).

Beside mimicry of foreign molecules (mobile elements and viruses) there are also several molecules within a cell that mimic its own tRNAs. One remarkable example is established by the participation of two molecules: tmRNA (small transfer-messenger RNA also known as SsrA and 10Sa RNA) and a tmRNA-specific binding protein called SmpB (small protein B). Together they mimic the upper (tmRNA) and lower (SmpB) halves of a tRNA, respectively. tmRNA owes its name to its action as both a transfer and a messenger RNA. The tmRNA-SmpB complex targets and rescues stalled ribosomes in a process known as trans-translation (Himeno *et al*., 2014; Keiler, 2015). This conserved translation surveillance pathway prevents accumulation of non-functional proteins from truncated mRNA (Karzai *et al*., 1999; Chadani *et al*., 2010). The tmRNA-SmpB complex mimics tRNA throughout the process. First, it is aminoacylated with alanine by alanyl-tRNA synthetase. Ala-tmRNA then enters the A-site of the stalled ribosome on a truncated mRNA to receive the nascent polypeptide from peptidyl-tRNA in the P-site. Then peptidyl-Ala-tmRNA translocates to the P-site, which exchanges the template switching the stalled ribosome from the translation of the defective mRNA to the translation of the mRNA domain of tmRNA. Translation of the tmRNA reading frame tags the defective protein to be degraded by

cellular proteases (Richards *et al*., 2008; Giudice *et al*., 2014).

In principle, any macromolecule could mimic tRNAs and in fact, even a tRNA has been found to mimic another tRNA. In this interesting case of structural mimicry between the anticodon arm of $tRNA^{Asp}$ and the acceptor stem of $tRNA^{Glu}$, a paralog of glutamyl-tRNA synthetases, glutamyl-Q-$tRNA^{Asp}$ synthetase (Glu-Q-RS, previously YadB), activates glutamate and transfers it to the queuosine at the wobble position of $tRNA^{Asp}$ (Blaise *et al*., 2004; Campanacci *et al*., 2004; Dubois *et al*., 2004; Salazar *et al*., 2004). This is considered an hypermodification as queuosine corresponds already to a modified guanine and Glu-Q-RS was the first aaRS paralog known to catalyze this kind of reaction. Several aspects of Glu-Q-RS are unusual. First, in contrast to GluRS the enzyme can activate glutamate in the absence of tRNA (Campanacci *et al*., 2004; Salazar *et al*., 2004) and transfer it to $tRNA^{Asp}$ instead of $tRNA^{Glu}$ (Dubois *et al*., 2004; Salazar *et al*., 2004). Second, it does not glutamylate $tRNA^{Asp}$ at its 3′ terminal adenosine, but instead it transfers the amino acid to a modified queuosine nucleoside, which is the first anticodon nucleoside in $tRNA^{Asp}$ (Blaise *et al*., 2004; Salazar *et al*., 2004). Finally, the orientation in which a tRNA interacts with Glu-Q-RS is different from that with GluRS since the tRNA needs to come close to Glu-Q-RS's active site with its anticodon loop instead of its acceptor helix (Blaise *et al*., 2004). The role of this hypermodification is poorly understood. It is speculated that it might reverse the effect of a single queuosine on GAU codon binding by $tRNA^{Asp}$ Q34 during translation (Blaise *et al*., 2005). The high K_M for glutamate of Glu-Q-RS suggests that this glutamylation might only occur when the glutamate concentration rises in the cell (Salazar *et al*., 2004). If this is true, the hypermodification may be a sensor, allowing Glu to affect the speed of GAU decoding during translation (Blaise *et al*., 2005) and, consistent with this, Glu-Q-RS seems to have a role in regulating the response to osmotic stress in *Shigella flexneri* (Caballero *et al*., 2012).

Similar to the example of SmpB in the mimicry of tRNA, there are several other proteins that mimic tRNA. Also similar to SmpB, all these proteins bind to the ribosome and have a function in translation. Presumably, primordial ribosomes prior to the last common ancestor catalyzed peptide-bond formation without the addition of translation factors, however these specialized factors greatly enhance the rate of translation. For instance, elongation factor G (EF-G) is a GTPase that binds the ribosome and upon GTP hydrolysis, accelerates translocation fourfold to fivefold (Shoji *et al*., 2009). EF-G is organized into five domains, of which domains I and II resemble in EF-Tu structure, while III-V resemble that of A-site tRNA. By virtue of structurally mimicking the tRNA-EF-Tu complex, domains III-V occupy the A-site tRNA to occlude tRNA from entering (Gao *et al*., 2009; Zhou *et al*., 2013). EF-G is not the only protein to have evolved A-site tRNA mimicry, in addition ribosome release (RRF) and recycling factors, (RF1/2, RF3) mimic tRNA and bind to similar regions. Despite these factors all binding at or near the A-site, each have functions that differ considerably. RF1/2 and RF3 bind ribosomes that have reached a stop codon to form an anticodon-codon interaction and hydrolyze the tRNA-peptidyl linkage with a conserved GQQ motif that mimics the CCA end of a tRNA (Zhou *et al*., 2012). RRF traverses between the A- and P-site to disassemble the ribosome into separate subunits, mediated by EF-G catalyzed translocation (Weixlbaumer *et al*., 2007). Therefore access to the A-site of the ribosome provides translation factors the opportunity to control each aspect of protein synthesis, however the cavity of the ribosome's entrance imposes a structural barrier that requires such proteins to mimic tRNA.

Protein-tRNA mimics are not all confined to interact with the entrance of the ribosome. Elongation factor P (EF-P) is an acidic protein comprised of three ß-barrel domains, arranged in a format that notably resembles tRNA, though the domains corresponding to the anticodon arm are somewhat smaller (Hanawa-Suetsugu *et al*., 2004). According to biochemical studies and a co-crystal structure with the ribosome, EF-P is positioned between the P/E site, making various contacts with the peptidyl-tRNA to entropically provide the ribosome the necessary velocity when translating consecutive proline residues, as proline forms the slowest peptide bond (Blaha *et al*., 2009; Doerfel *et al*., 2013; 2015). The considerable structural similarity between EF-P and tRNA gives EF-P a distinct opportunity to interact with enzymes that tRNA may also interact with. In particular, EF-P is post-translationally modified in some organisms by a mechanism reminiscent of tRNA-aminoacylation (Navarre *et al*., 2010; Roy *et al*., 2011). The addition of (R)-ß-lysine onto the conserved lysine residue at the acceptor stem tip of EF-P augments EF-P's function by increasing the observed K_M for paused ribosomes (Doerfel *et al*., 2013). Interestingly, EF-P is glycosylated in some beta- and gamma-proteobacteria with a rhamnose molecule at an analogous arginine residue, calling into question how this evolutionary partnership arose while still accommodating important contacts with the ribosome (Rajkovic *et al*., 2015; Lassak *et al*., 2015).

Recent crystal and cyroEM structures revealed the energy-dependent translational throttle A (Etta) protein enters the E-site of the ribosome to regulate translation elongation in a manner dependent on the ratio of ATP/ADP (Boël *et al*., 2014). EttA is composed of four

domains that bind ADP or ATP and a long C-terminal α-helix that interacts with the E and P-site of the ribosome. The C-terminal extension mimics tRNA to control protein synthesis directly by interacting with the peptidyl-transfer center when initiator tRNA is bound. For example, when ADP levels are high, such as in stationary phase for bacteria, EttA-ADP restricts synthesis of the first peptide bond, while in the presence of ATP, EttA marginally enhances the synthesis of the first peptide bond. This provides the first example of a protein tRNA mimic as having a regulatory function within the context of translation. Furthermore, it will be interesting to see whether EttA and EF-P accelerate peptide bond formation in a similar way.

Mimics from pathogens and parasites (such as TLS) have a strong selective pressure to maintain similitude to their host models. The hosts instead, have a strong selective pressure to change the structure of the model (in this case tRNA) or of the molecules that interact with it (in this case the ribosome or an aaRS), such that they stop recognizing the mimic and become more resistant to the pathogen/parasite (Elde *et al*., 2009; Elde and Malik, 2009). Nevertheless, most tRNA mimics are from within the same organism. Thus, there is strong selective pressure over both the mimic and the model for maintenance of mimicry (Katz *et al*., 2014).

Final remarks

Aa-tRNAs are central in translation of the genetic information, connecting cellular memory stored in nucleic acids with structural and catalytic functions performed mainly by proteins. Thus, these molecules are believed to have performed a major role in the evolution from a probable 'RNA world' where RNA had both memory and catalytic functions to a world where these functions are performed mainly by specialized molecules: catalysis by proteins and memory by DNA. In this scenario, it is probable that beside tRNA, there are only a few other information molecules in living organisms that are as old and as well conserved in the three domains of life plus viruses. Although there are some differences between organisms, the fact that bacterial and eukaryal tRNAs can easily be interchanged for both aminoacylation and mRNA translation (Shafritz and Anderson, 1970; Berthelot *et al*., 1973; Weisblum, 1999) is evidence for how well these molecules have retained their structure. Such a long period of time has created multiple chances for nature to evolve new functions for these old molecules that carry an activated amino acid with very high specificity. As discussed in this review, several of these newer functions correspond to pathways unrelated to translation *per se*. These functions include several synthetic and regulatory pathways, some of which might be very ancient themselves. For instance, indirect pathways for synthesis of aminoacyl-tRNA are thought to be very old, preceding the corresponding direct pathways (Woese *et al*., 2000; Ibba *et al*., 2000; Sheppard *et al*., 2008). The tRNA dependent synthesis of tetrapyrroles has also been proposed to be significantly older than the corresponding tRNA independent pathway (Schulze *et al*., 2006) maybe even predating LUCA and having arisen in the RNA world (Benner *et al*., 1989). Surprisingly, although many of these alternative pathways are old and usually use the same tRNAs as in translation, there are very few cases where a 'cross-talk' between translation, alternative synthetic pathways and regulatory processes has been proposed. Potential cross-talk has been suggested for pathways that use large amounts of aa-tRNA, such as synthesis of peptidoglycan in gram-positive bacteria (Shepherd and Ibba, 2013a,b) or tetrapyrroles in organisms with high demand of them for either photosynthesis (Jahn, 1992) or oxygen consumption (Levicán *et al*., 2005; 2007; Katz and Orellana, 2012a; Farah *et al*., 2014). Nevertheless, for the most part we are unaware of the effect that changes in tRNA have on most of the pathways using tRNA outside of translation. For instance, the effects of tRNA fragmentation or modification on the regulation of protein stability or tRNA dependent synthesis of amino acids are completely unknown. We expect that the mass-spec and high-throughput sequencing tools that have been developed in the last years, as well as the vast amount of genomic sequences that have been made available, will enhance research about the roles of tRNAs in regulating translation and the alternative pathways where these molecules have a central role.

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References

Ataide, S.F., Jester, B.C., Devine, K.M., and Ibba, M. (2005) Stationary-phase expression and aminoacylation of a transfer-RNA-like small RNA. *EMBO Rep* **6**: 742–747.

Bailly, M., Blaise, M., Lorber, B., Becker, H.D., and Kern, D. (2007) The transamidosome: a dynamic ribonucleoprotein particle dedicated to prokaryotic tRNA-dependent asparagine biosynthesis. *Mol Cell* **28**: 228–239.

Banerjee, R., Chen, S., Dare, K., Gilreath, M., Praetorius-Ibba, M., Raina, M., *et al*. (2010) tRNAs: cellular barcodes for amino acids. *FEBS Lett* **584**: 387–395.

Belin, P., Moutiez, M., Lautru, S., Seguin, J., Pernodet, J.-L., and Gondry, M. (2012) The nonribosomal synthesis of diketopiperazines in tRNA-dependent cyclodipeptide synthase pathways. *Nat Prod Rep* **29**: 961–979.

Benner, S.A., Ellington, A.D., and Tauer, A. (1989) Modern metabolism as a palimpsest of the RNA world. *Proc Natl Acad Sci USA* **86**: 7054–7058.

Berger-Bächi, B., and Tschierske, M. (1998) Role of fem factors in methicillin resistance. *Drug Resist Updat Rev Comment Antimicrob Anticancer Chemother* **1**: 325–335.

Berthelot, F., Bogdanovsky, D., Schapira, G., and Gros, F. (1973) Interchangeability of factors and tRNA's in bacterial and eukaryotic translation initiation systems. *Mol Cell Biochem* **1**: 63–72.

Beuning, P.J., and Musier-Forsyth, K. (1999) Transfer RNA recognition by aminoacyl-tRNA synthetases. *Biopolymers* **52**: 1–28.

Bhaskaran, H., and Perona, J.J. (2011) Two-step aminoacylation of tRNA without channeling in Archaea. *J Mol Biol* **411**: 854–869.

Björk, G.R., Durand, J.M.B., Hagervall, T.G., Leipuviene, R., Lundgren, H.K., Nilsson, K., *et al*. (1999) Transfer RNA modification: influence on translational frameshifting and metabolism. *FEBS Lett* **452**: 47–51.

Blaha, G., Stanley, R.E., and Steitz, T.A. (2009) Formation of the first peptide bond: the structure of EF-P bound to the 70S ribosome. *Science* **325**: 966–970.

Blaise, M., Becker, H.D., Keith, G., Cambillau, C., Lapointe, J., Giegé, R., and Kern, D. (2004) A minimalist glutamyl-tRNA synthetase dedicated to aminoacylation of the $tRNA^{Asp}$ QUC anticodon. *Nucleic Acids Res* **32**: 2768–2775.

Blaise, M., Becker, H.D., Lapointe, J., Cambillau, C., Giegé, R., and Kern, D. (2005) Glu-Q-tRNA(Asp) synthetase coded by the yadB gene, a new paralog of aminoacyl-tRNA synthetase that glutamylates tRNA(Asp) anticodon. *Biochimie* **87**: 847–861.

Boël, G., Smith, P.C., Ning, W., Englander, M.T., Chen, B., Hashem, Y., *et al*. (2014) The ABC-F protein EttA gates ribosome entry into the translation elongation cycle. *Nat Struct Mol Biol* **21**: 143–151.

Bonnefond, L., Arai, T., Sakaguchi, Y., Suzuki, T., Ishitani, R., and Nureki, O. (2011) Structural basis for nonribosomal peptide synthesis by an aminoacyl-tRNA synthetase paralog. *Proc Natl Acad Sci USA* **108**: 3912–3917.

Buck, M., and Ames, B.N. (1984) A modified nucleotide in tRNA as a possible regulator of aerobiosis: synthesis of cis-2-methyl-thioribosylzeatin in the tRNA of Salmonella. *Cell* **36**: 523–531.

Bullwinkle, T.J., and Ibba, M. (2016) Translation quality control is critical for bacterial responses to amino acid stress. *Proc Natl Acad Sci USA* **113**: 2252–2257.

Bumsted, R.M., Dahl, J.L., Söll, D., and Strominger, J.L. (1968) Biosynthesis of the peptidoglycan of bacterial cell walls. X. Further study of the glycyl transfer ribonucleic acids active in peptidoglycan synthesis in *Staphylococcus aureus*. *J Biol Chem* **243**: 779–782.

Caballero, V.C., Toledo, V.P., Maturana, C., Fisher, C.R., Payne, S.M., and Salazar, J.C. (2012) Expression of Shigella flexneri gluQ-rs gene is linked to dksA and controlled by a transcriptional terminator. *BMC Microbiol* **12**: 226.

Campanacci, V., Dubois, D.Y., Becker, H.D., Kern, D., Spinelli, S., Valencia, C., *et al*. (2004) The *Escherichia coli* YadB gene product reveals a novel aminoacyl-tRNA synthetase like activity. *J Mol Biol* **337**: 273–283.

Cavarelli, J., and Moras, D. (1993) Recognition of tRNAs by aminoacyl-tRNA synthetases. *FASEB J Off Publ Fed Am Soc Exp Biol* **7**: 79–86.

Chadani, Y., Ono, K., Ozawa, S.-I., Takahashi, Y., Takai, K., Nanamiya, H., *et al*. (2010) Ribosome rescue by *Escherichia coli* ArfA (YhdL) in the absence of trans-translation system. *Mol Microbiol* **78**: 796–808.

Chan, C.T.Y., Pang, Y.L.J., Deng, W., Babu, I.R., Dyavaiah, M., Begley, T.J., and Dedon, P.C. (2012) Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* **3**: 937.

Colussi, T.M., Costantino, D.A., Hammond, J.A., Ruehle, G.M., Nix, J.C., and Kieft, J.S. (2014) The structural basis of transfer RNA mimicry and conformational plasticity by a viral RNA. *Nature* **511**: 366–369.

Crick, F. (1958) On Protein Synthesis. *Symp Soc Exp Biol* **12**: 138–163.

Crick, F. (1970) Central dogma of molecular biology. *Nature* **227**: 561–563.

Cruz, J.W., Sharp, J.D., Hoffer, E.D., Maehigashi, T., Vvedenskaya, I.O., Konkimalla, A., *et al*. (2015) Growth-regulating Mycobacterium tuberculosis VapC-mt4 toxin is an isoacceptor-specific tRNase. *Nat Commun* **6**: 7480.

Dale, T., and Uhlenbeck, O.C. (2005) Amino acid specificity in translation. *Trends Biochem Sci* **30**: 659–665.

Dare, K., and Ibba, M. (2012) Roles of tRNA in cell wall biosynthesis. *Wiley Interdiscip Rev RNA* **3**: 247–264.

Dedon, P.C., and Begley, T.J. (2014) A system of RNA modifications and biased codon use controls cellular stress response at the level of translation. *Chem Res Toxicol* **27**: 330–337.

Di Giulio, M. (1995) The phylogeny of tRNAs seems to confirm the predictions of the coevolution theory of the origin of the genetic code. *Orig Life Evol Biosph* **25**: 549–564.

Di Giulio, M. (2006) The non-monophyletic origin of the tRNA molecule and the origin of genes only after the evolutionary stage of the last universal common ancestor (LUCA). *J Theor Biol* **240**: 343–352.

Doerfel, L.K., Wohlgemuth, I., Kothe, C., Peske, F., Urlaub, H., and Rodnina, M.V. (2013) EF-P is essential for rapid synthesis of proteins containing consecutive proline residues. *Science* **339**: 85–88.

Doerfel, L.K., Wohlgemuth, I., Kubyshkin, V., Starosta, A.L., Wilson, D.N., Budisa, N., and Rodnina, M.V. (2015) Entropic Contribution of Elongation Factor P to Proline Positioning at the Catalytic Center of the Ribosome. *J Am Chem Soc* **137**: 12997–13006.

Dougan, D.A., Micevski, D., and Truscott, K.N. (2012) The N-end rule pathway: from recognition by N-recognins, to destruction by AAA+proteases. *Biochim Biophys Acta* **1823**: 83–91.

Dreher, T.W. (2009) Role of tRNA-like structures in controlling plant virus replication. *Virus Res* **139**: 217–229.

Dreher, T.W., and Goodwin, J.B. (1998) Transfer RNA mimicry among tymoviral genomic RNAs ranges from highly efficient to vestigial. *Nucleic Acids Res* **26**: 4356–4364.

Dubois, D.Y., Blaise, M., Becker, H.D., Campanacci, V., Keith, G., Giegé, R., *et al*. (2004) An aminoacyl-tRNA synthetase-like protein encoded by the *Escherichia coli* yadB gene glutamylates specifically $tRNA^{Asp}$. *Proc Natl Acad Sci USA* **101**: 7530–7535.

Durand, J.M., Dagberg, B., Uhlin, B.E., and Björk, G.R. (2000) Transfer RNA modification, temperature and DNA superhelicity have a common target in the regulatory network of the virulence of *Shigella flexneri*: the expression of the virF gene. *Mol Microbiol* **35**: 924–935.

Elde, N.C., Child, S.J., Geballe, A.P., and Malik, H.S. (2009) Protein kinase R reveals an evolutionary model for defeating viral mimicry. *Nature* **457**: 485–489.

Elde, N.C., and Malik, H.S. (2009) The evolutionary conundrum of pathogen mimicry. *Nat Rev Microbiol* **7**: 787–797.

Elf, J., and Ehrenberg, M. (2005) Near-critical behavior of aminoacyl-tRNA pools in *E. coli* at rate-limiting supply of amino acids. *Biophys J* **88**: 132–146.

Endres, L., Dedon, P.C., and Begley, T.J. (2015) Codon-biased translation can be regulated by wobble-base tRNA modification systems during cellular stress responses. *RNA Biol* **12**: 603–614.

Farah, C., Levicán, G., Ibba, M., and Orellana, O. (2014) Effect of hydrogen peroxide on the biosynthesis of heme and proteins: potential implications for the partitioning of Glu-tRNA(Glu) between these pathways. *Int J Mol Sci* **15**: 23011–23023.

Fonvielle, M., Chemama, M., Villet, R., Lecerf, M., Bouhss, A., Valéry, J.-M., *et al*. (2009) Aminoacyl-tRNA recognition by the FemXWv transferase for bacterial cell wall synthesis. *Nucleic Acids Res* **37**: 1589–1601.

Fonvielle, M., de La Sierra-Gallay, I., Sagheer, A.H. El-, Lecerf, M., Patin, D., Mellal, D., *et al*. (2013) The structure of FemX(Wv) in complex with a peptidyl-RNA conjugate: mechanism of aminoacyl transfer from Ala-tRNA(Ala) to peptidoglycan precursors. *Angew Chem Int Ed Engl* **52**: 7278–7281.

Francklyn, C.S., and Minajigi, A. (2010) tRNA as an active chemical scaffold for diverse chemical transformations. *FEBS Lett* **584**: 366–375.

Fukunaga, R., and Yokoyama, S. (2007) Structural insights into the second step of RNA-dependent cysteine biosynthesis in archaea: crystal structure of Sep-tRNA:Cys-tRNA synthase from Archaeoglobus fulgidus. *J Mol Biol* **370**: 128–141.

Fung, A.W.S., Leung, C.C.Y., and Fahlman, R.P. (2014) The determination of $tRNA^{Leu}$ recognition nucleotides for *Escherichia coli* L/F transferase. *RNA N Y N* **20**: 1210–1222.

Gao, Y.-G., Selmer, M., Dunham, C.M., Weixlbaumer, A., Kelley, A.C., and Ramakrishnan, V. (2009) The structure of the ribosome with elongation factor G trapped in the posttranslocational state. *Science* **326**: 694–699.

Garg, R.P., Qian, X.L., Alemany, L.B., Moran, S., and Parry, R.J. (2008) Investigations of valanimycin biosynthesis: elucidation of the role of seryl-tRNA. *Proc Natl Acad Sci USA* **105**: 6543–6547.

Giannouli, S., Kyritsis, A., Malissovas, N., Becker, H.D., and Stathopoulos, C. (2009) On the role of an unusual tRNAGly isoacceptor in *Staphylococcus aureus*. *Biochimie* **91**: 344–351.

Giegé, R., and Frugier, M. (2000) Transfer RNA Structure and Identity. In *Madame Curie Bioscience Database [Internet]*. Landes Bioscience, Austin (TX), USA. [WWW document]. URL http://www.ncbi.nlm.nih.gov/books/NBK6236/.

Giudice, E., Macé, K., and Gillet, R. (2014) Trans-translation exposed: understanding the structures and functions of tmRNA-SmpB. *Front Microbiol* **5**: 113.

Goldfine, H. (2014) Charge counter charge: bacterial response to antimicrobial cationic peptides and more. *Virulence* **5**: 451–453.

Golovina, A.Y., Sergiev, P.V., Golovin, A.V., Serebryakova, M.V., Demina, I., Govorun, V.M., and Dontsova, O.A. (2009) The yfiC gene of *E. coli* encodes an adenine-N6 methyltransferase that specifically modifies A37 of tRNA1Val(cmo5UAC). *RNA N Y N* **15**: 1134–1141.

Gould, R.M., Thornton, M.P., Liepkalns, V., and Lennarz, W.J. (1968) Participation of aminoacyl transfer ribonucleic acid in aminoacyl phosphatidylglycerol synthesis. II. Specificity of alanyl phosphatidylglycerol synthetase. *J Biol Chem* **243**: 3096–3104.

Gu, C., Begley, T.J., and Dedon, P.C. (2014) tRNA modifications regulate translation during cellular stress. *FEBS Lett* **588**: 4287–4296.

Guan, R., Roychowdhury, A., Ember, B., Kumar, S., Boons, G.-J., and Mariuzza, R.A. (2004) Structural basis for peptidoglycan binding by peptidoglycan recognition proteins. *Proc Natl Acad Sci USA* **101**: 17168–17173.

Guillon, J.M., Meinnel, T., Mechulam, Y., Lazennec, C., Blanquet, S., and Fayat, G. (1992) Nucleotides of tRNA governing the specificity of *Escherichia coli* methionyl-tRNA(fMet) formyltransferase. *J Mol Biol* **224**: 359–367.

Hacker, J., and Kaper, J.B. (2000) Pathogenicity islands and the evolution of microbes. *Annu Rev Microbiol* **54**: 641–679.

Hanawa-Suetsugu, K., Sekine, S., Sakai, H., Hori-Takemoto, C., Terada, T., Unzai, S., *et al*. (2004) Crystal structure of elongation factor P from Thermus thermophilus HB8. *Proc Natl Acad Sci USA* **101**: 9595–9600.

Hebecker, S., Arendt, W., Heinemann, I.U., Tiefenau, J.H.J., Nimtz, M., Rohde, M., *et al*. (2011) Alanyl-phosphatidylglycerol synthase: mechanism of substrate recognition during tRNA-dependent lipid modification in *Pseudomonas aeruginosa*. *Mol Microbiol* **80**: 935–950.

Heinemann, I.U., Jahn, M., and Jahn, D. (2008) The biochemistry of heme biosynthesis. *Arch Biochem Biophys* **474**: 238–251.

Helgadóttir, S., Sinapah, S., Söll, D., and Ling, J. (2012) Mutational analysis of Sep-tRNA:Cys-tRNA synthase reveals critical residues for tRNA-dependent cysteine formation. *FEBS Lett* **586**: 60–63.

Henkin, T.M., and Yanofsky, C. (2002) Regulation by transcription attenuation in bacteria: how RNA provides instructions for transcription termination/antitermination decisions. *BioEssays News Rev Mol Cell Dev Biol* **24**: 700–707.

Himeno, H., Kurita, D., and Muto, A. (2014) tmRNA-mediated trans-translation as the major ribosome rescue system in a bacterial cell. *Front Genet* **5**: 66.

Hoagland, M.B., Stephenson, M.L., Scott, J.F., Hecht, L.I., and Zamecnik, P.C. (1958) A Soluble Ribonucleic Acid Intermediate in Protein Synthesis. *J Biol Chem* **231**: 241–257.

Ibba, M., Becker, H.D., Stathopoulos, C., Tumbula, D.L., and Söll, D. (2000) The adaptor hypothesis revisited. *Trends Biochem Sci* **25**: 311–316.

Ibba, M., Francklyn, C., and Cusack, S. (eds) (2005) *The aminoacyl-tRNA synthetases*. Landes Bioscience: Eurekah.com, Georgetown, TX., USA.

Ichihashi, N., Kurokawa, K., Matsuo, M., Kaito, C., and Sekimizu, K. (2003) Inhibitory effects of basic or neutral phospholipid on acidic phospholipid-mediated dissociation of adenine nucleotide bound to DnaA protein, the initiator of chromosomal DNA replication. *J Biol Chem* **278**: 28778–28786.

Jahn, D. (1992) Complex formation between glutamyl-tRNA synthetase and glutamyl-tRNA reductase during the tRNA-dependent synthesis of 5-aminolevulinic acid in Chlamydomonas reinhardtii. *FEBS Lett* **314**: 77–80.

Karzai, A.W., Susskind, M.M., and Sauer, R.T. (1999) SmpB, a unique RNA-binding protein essential for the peptide-tagging activity of SsrA (tmRNA). *EMBO J* **18**: 3793–3799.

Katz, A., and Orellana, O. (2012a) Glutamyl-tRNA in Bacteria. Multiple Identities for Multiple Functions. *Croat Chem Acta* **85**: 159–169.

Katz, A., and Orellana, O. (2012b) Protein Synthesis and the Stress Response. In *Cell-Free Protein Synthesis*. Biyani, M. (ed.). InTech, DOI: 10.5772/50311.

Katz, A., Solden, L., Zou, S.B., Navarre, W.W., and Ibba, M. (2014) Molecular evolution of protein-RNA mimicry as a mechanism for translational control. *Nucleic Acids Res* **42**: 3261–3271.

Kaufmann, G. (2000) Anticodon nucleases. *Trends Biochem Sci* **25**: 70–74.

Keam, S.P., and Hutvagner, G. (2015) tRNA-Derived Fragments (tRFs): emerging new roles for an ancient RNA in the regulation of gene expression. *Life* **5**: 1638–1651.

Keiler, K.C. (2015) Mechanisms of ribosome rescue in bacteria. *Nat Rev Microbiol* **13**: 285–297.

Khade, P., and Joseph, S. (2010) Functional interactions by transfer RNAs in the ribosome. *FEBS Lett* **584**: 420–426.

Koskiniemi, S., Lamoureux, J.G., Nikolakakis, K.C., Roodenbeke, C. t'Kint de , Kaplan, M.D., Low, D.A., and Hayes, C.S. (2013) Rhs proteins from diverse bacteria mediate intercellular competition. *Proc Natl Acad Sci USA* **110**: 7032–7037.

Kramer, G.F., Baker, J.C., and Ames, B.N. (1988) Near-UV stress in *Salmonella typhimurium*: 4-thiouridine in tRNA, ppGpp, and ApppGpp as components of an adaptive response. *J Bacteriol* **170**: 2344–2351.

Ladner, J.E., Jack, A., Robertus, J.D., Brown, R.S., Rhodes, D., Clark, B.F., and Klug, A. (1975) Structure of yeast phenylalanine transfer RNA at 2.5 A resolution. *Proc Natl Acad Sci USA* **72**: 4414–4418.

LaRiviere, F.J., Wolfson, A.D., and Uhlenbeck, O.C. (2001) Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. *Science* **294**: 165–168.

Lassak, J., Keilhauer, E.C., Fürst, M., Wuichet, K., Gödeke, J., Starosta, A.L., *et al*. (2015) Arginine-rhamnosylation as new strategy to activate translation elongation factor P. *Nat Chem Biol* **11**: 266–270.

Laxman, S., Sutter, B.M., Wu, X., Kumar, S., Guo, X., Trudgian, D.C., *et al*. (2013) Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. *Cell* **154**: 416–429.

Lee, C.P., Seong, B.L., and RajBhandary, U.L. (1991) Structural and sequence elements important for recognition of *Escherichia coli* formylmethionine tRNA by methionyl-tRNA transformylase are clustered in the acceptor stem. *J Biol Chem* **266**: 18012–18017.

Levicán, G., Katz, A., Armas, M. de , Núñez, H., and Orellana, O. (2007) Regulation of a glutamyl-tRNA synthetase by the heme status. *Proc Natl Acad Sci USA* **104**: 3135–3140.

Levicán, G., Katz, A., Valenzuela, P., Söll, D., and Orellana, O. (2005) A tRNA(Glu) that uncouples protein and tetrapyrrole biosynthesis. *FEBS Lett* **579**: 6383–6387.

Li, S., Kumar, N.V., Varshney, U., and RajBhandary, U.L. (1996) Important role of the amino acid attached to tRNA in formylation and in initiation of protein synthesis in *Escherichia coli*. *J Biol Chem* **271**: 1022–1028.

Liu, K., Bittner, A.N., and Wang, J.D. (2015) Diversity in (p)ppGpp metabolism and effectors. *Curr Opin Microbiol* **24**: 72–79.

Mak, J., and Kleiman, L. (1997) Primer tRNAs for reverse transcription. *J Virol* **71**: 8087–8095.

Marck, C., and Grosjean, H. (2002) tRNomics: analysis of tRNA genes from 50 genomes of Eukarya, Archaea, and Bacteria reveals anticodon-sparing strategies and domain-specific features. *RNA N Y N* **8**: 1189–1232.

Mateus, D.D., Paredes, J.A., Español, Y., Ribas de Pouplana, L., Moura, G.R., and Santos, M.A.S. (2013) Molecular reconstruction of a fungal genetic code alteration. *RNA Biol* **10**: 969–980.

Mladenova, S.R., Stein, K.R., Bartlett, L., and Sheppard, K. (2014) Relaxed tRNA specificity of the *Staphylococcus aureus* aspartyl-tRNA synthetase enables RNA-dependent asparagine biosynthesis. *FEBS Lett* **588**: 1808–1812.

Moras, D., Comarmond, M.B., Fischer, J., Weiss, R., Thierry, J.C., Ebel, J.P., and Giegé, R. (1980) Crystal structure of yeast tRNAAsp. *Nature* **288**: 669–674.

Morgens, D.W. (2013) The protein invasion: a broad review on the origin of the translational system. *J Mol Evol* **77**: 185–196.

Moura, G.R., Paredes, J.A., and Santos, M.A.S. (2010) Development of the genetic code: insights from a fungal codon reassignment. *FEBS Lett* **584**: 334–341.

Moutiez, M., Seguin, J., Fonvielle, M., Belin, P., Jacques, I.B., Favry, E., *et al*. (2014) Specificity determinants for the two tRNA substrates of the cyclodipeptide synthase AlbC from *Streptomyces noursei*. *Nucleic Acids Res* **42**: 7247–7258.

Murata, M., Fujimoto, H., Nishimura, K., Charoensuk, K., Nagamitsu, H., Raina, S., *et al*. (2011) Molecular strategy for survival at a critical high temperature in *Eschierichia coli*. *PloS One* **6**: e20063.

Nakayashiki, T., Saito, N., Takeuchi, R., Kadokura, H., Nakahigashi, K., Wanner, B.L., and Mori, H. (2013) The tRNA thiolation pathway modulates the intracellular redox state in *Escherichia coli*. *J Bacteriol* **195**: 2039–2049.

Navarre, W.W., Zou, S.B., Roy, H., Xie, J.L., Savchenko, A., Singer, A., *et al*. (2010) PoxA, yjeK, and elongation factor P coordinately modulate virulence and drug resistance in *Salmonella enterica*. *Mol Cell* **39**: 209–221.

Newton, D.T., Niemkiewicz, M., Lo, R.Y., and Mangroo, D. (1999) Recognition of the initiator tRNA by the *Pseudomonas aeruginosa* methionyl-tRNA formyltransferase: importance of the base-base mismatch at the end of the acceptor stem. *FEMS Microbiol Lett* **178**: 289–298.

O'Brian, M.R., and Thöny-Meyer, L. (2002) Biochemistry, regulation and genomics of haem biosynthesis in prokaryotes. *Adv Microb Physiol* **46**: 257–318.

O'Donoghue, P., Sethi, A., Woese, C.R., and Luthey-Schulten, Z.A. (2005) The evolutionary history of Cys-tRNA^{Cys} formation. *Proc Natl Acad Sci USA* **102**: 19003–19008.

Ogawa, T., Tomita, K., Ueda, T., Watanabe, K., Uozumi, T., and Masaki, H. (1999) A cytotoxic ribonuclease targeting specific transfer RNA anticodons. *Science* **283**: 2097–2100.

Palioura, S., Sherrer, R.L., Steitz, T.A., Söll, D., and Simonovic, M. (2009) The human SepSecS-tRNASec complex reveals the mechanism of selenocysteine formation. *Science* **325**: 321–325.

Persson, B.C., Ólafsson, Ó., Lundgren, H.K., Hederstedt, L., and Björk, G.R. (1998) The ms2io6A37 modification of tRNA in *Salmonella typhimurium* regulates growth on citric acid cycle intermediates. *J Bacteriol* **180**: 3144–3151.

Raina, M., and Ibba, M. (2014) tRNAs as regulators of biological processes. *Front Genet* **5**: 171.

RajBhandary, U.L., and Söll, D. (2008) Aminoacyl-tRNAs, the bacterial cell envelope, and antibiotics. *Proc Natl Acad Sci USA* **105**: 5285–5286.

Rajkovic, A., Erickson, S., Witzky, A., Branson, O.E., Seo, J., Gafken, P.R., *et al*. (2015) Cyclic Rhamnosylated elongation factor P establishes antibiotic resistance in *Pseudomonas aeruginosa*. *mBio* **6**: e00823.

Rampias, T., Sheppard, K., and Söll, D. (2010) The archaeal transamidosome for RNA-dependent glutamine biosynthesis. *Nucleic Acids Res* **38**: 5774–5783.

Randau, L., Schauer, S., Ambrogelly, A., Salazar, J.C., Moser, J., Sekine, S., *et al*. (2004) tRNA recognition by glutamyl-tRNA reductase. *J Biol Chem* **279**: 34931–34937.

Richards, J., Sundermeier, T., Svetlanov, A., and Karzai, A.W. (2008) Quality control of bacterial mRNA decoding and decay. *Biochim Biophys Acta* **1779**: 574–582.

Rodin, A.S., Szathmáry, E., and Rodin, S.N. (2011) On origin of genetic code and tRNA before translation. *Biol Direct* **6**: 14.

Rogers, T.E., Ataide, S.F., Dare, K., Katz, A., Seveau, S., Roy, H., and Ibba, M. (2012) A pseudo-tRNA modulates antibiotic resistance in *Bacillus cereus*. *PloS One* **7**: e41248.

Rosenberg, A.H., and Gefter, M.L. (1969) An iron-dependent modification of several transfer RNA species in *Escherichia coli*. *J Mol Biol* **46**: 581–584.

Roy, H., and Ibba, M. (2008) RNA-dependent lipid remodeling by bacterial multiple peptide resistance factors. *Proc Natl Acad Sci USA* **105**: 4667–4672.

Roy, H., Zou, S.B., Bullwinkle, T.J., Wolfe, B.S., Gilreath, M.S., Forsyth, C.J., *et al*. (2011) The tRNA synthetase paralog PoxA modifies elongation factor-P with (R)-β-lysine. *Nat Chem Biol* **7**: 667–669.

Ruhe, Z.C., Low, D.A., and Hayes, C.S. (2013) Bacterial contact-dependent growth inhibition. *Trends Microbiol* **21**: 230–237.

Salazar, J.C., Ambrogelly, A., Crain, P.F., McCloskey, J.A., and Söll, D. (2004) A truncated aminoacyl-tRNA synthetase modifies RNA. *Proc Natl Acad Sci USA* **101**: 7536–7541.

Samhita, L., Virumäe, K., Remme, J., and Varshney, U. (2013) Initiation with elongator tRNAs. *J Bacteriol* **195**: 4202–4209.

Sauerwald, A., Zhu, W., Major, T.A., Roy, H., Palioura, S., Jahn, D., *et al*. (2005) RNA-dependent cysteine biosynthesis in archaea. *Science* **307**: 1969–1972.

Sauguet, L., Moutiez, M., Li, Y., Belin, P., Seguin, J., Du, M.-H. Le, *et al*. (2011) Cyclodipeptide synthases, a family of class-I aminoacyl-tRNA synthetase-like enzymes involved in non-ribosomal peptide synthesis. *Nucleic Acids Res* **39**: 4475–4489.

Scheffers, D.-J., and Pinho, M.G. (2005) Bacterial cell wall synthesis: new insights from localization studies. *Microbiol Mol Biol Rev* **69**: 585–607.

Schimmel, P., Giegé, R., Moras, D., and Yokoyama, S. (1993) An operational RNA code for amino acids and possible relationship to genetic code. *Proc Natl Acad Sci USA* **90**: 8763–8768.

Schulze, J.O., Schubert, W.-D., Moser, J., Jahn, D., and Heinz, D.W. (2006) Evolutionary relationship between initial enzymes of tetrapyrrole biosynthesis. *J Mol Biol* **358**: 1212–1220.

Shafritz, D.A., and Anderson, W.F. (1970) Isolation and partial characterization of reticulocyte factors M1 and M2. *J Biol Chem* **245**: 5553–5559.

Shepherd, J., and Ibba, M. (2013a) Lipid II-independent trans editing of mischarged tRNAs by the penicillin resistance factor MurM. *J Biol Chem* **288**: 25915–25923.

Shepherd, J., and Ibba, M. (2013b) Direction of aminoacylated transfer RNAs into antibiotic synthesis and peptidoglycan-mediated antibiotic resistance. *FEBS Lett* **587**: 2895–2904.

Shepotinovskaya, I., and Uhlenbeck, O.C. (2013) tRNA residues evolved to promote translational accuracy. *RNA N Y N* **19**: 510–516.

Sheppard, K., Yuan, J., Hohn, M.J., Jester, B., Devine, K.M., and Söll, D. (2008) From one amino acid to another: tRNA-dependent amino acid biosynthesis. *Nucleic Acids Res* **36**: 1813–1825.

Sherrer, R.L., Ho, J.M.L., and Söll, D. (2008) Divergence of selenocysteine tRNA recognition by archaeal and eukaryotic O-phosphoseryl-tRNASec kinase. *Nucleic Acids Res* **36**: 1871–1880.

Shetty, S., Bhattacharyya, S., and Varshney, U. (2015) Is the cellular initiation of translation an exclusive property of the initiator tRNAs? *RNA Biol* **12**: 675–680.

Shippy, D.C., and Fadl, A.A. (2014) tRNA Modification enzymes GidA and MnmE: potential role in virulence of bacterial pathogens. *Int J Mol Sci* **15**: 18267–18280.

Shoji, S., Walker, S.E., and Fredrick, K. (2009) Ribosomal translocation: one step closer to the molecular mechanism. *ACS Chem Biol* **4**: 93–107.

Sørensen, M.A., Elf, J., Bouakaz, E., Tenson, T., Sanyal, S., Björk, G.R., and Ehrenberg, M. (2005) Over expression of a tRNA(Leu) isoacceptor changes charging pattern of leucine tRNAs and reveals new codon reading. *J Mol Biol* **354**: 16–24.

Subramaniam, A.R., Deloughery, A., Bradshaw, N., Chen, Y., O'Shea, E., Losick, R., and Chai, Y. (2013a) A serine sensor for multicellularity in a bacterium. *eLife* **2**: e01501.

Subramaniam, A.R., Pan, T., and Cluzel, P. (2013b) Environmental perturbations lift the degeneracy of the genetic code to regulate protein levels in bacteria. *Proc Natl Acad Sci USA* **110**: 2419–2424.

Subramaniam, A.R., Zid, B.M., and O'Shea, E.K. (2014) An integrated approach reveals regulatory controls on bacterial translation elongation. *Cell* **159**: 1200–1211.

Sun, F.-J., and Caetano-Anollés, G. (2008) The origin and evolution of tRNA inferred from phylogenetic analysis of structure. *J Mol Evol* **66**: 21–35.

Suto, K., Shimizu, Y., Watanabe, K., Ueda, T., Fukai, S., Nureki, O., and Tomita, K. (2006) Crystal structures of leucyl/phenylalanyl-tRNA-protein transferase and its complex with an aminoacyl-tRNA analog. *EMBO J* **25**: 5942–5950.

Takeuchi, N., Vial, L., Panvert, M., Schmitt, E., Watanabe, K., Mechulam, Y., and Blanquet, S. (2001) Recognition of tRNAs by methionyl-tRNA transformylase from mammalian mitochondria. *J Biol Chem* **276**: 20064–20068.

Tomita, K., Ogawa, T., Uozumi, T., Watanabe, K., and Masaki, H. (2000) A cytotoxic ribonuclease which specifically cleaves four isoaccepting arginine tRNAs at their anticodon loops. *Proc Natl Acad Sci USA* **97**: 8278–8283.

Varshney, U., Lee, C.P., Seong, B.L., and RajBhandary, U.L. (1991) Mutants of initiator tRNA that function both as initiators and elongators. *J Biol Chem* **266**: 18018–18024.

Vial, L., Gomez, P., Panvert, M., Schmitt, E., Blanquet, S., and Mechulam, Y. (2003) Mitochondrial methionyl-tRNAfMet formyltransferase from Saccharomyces cerevisiae: gene disruption and tRNA substrate specificity. *Biochemistry (Mosc)* **42**: 932–939.

Villet, R., Fonvielle, M., Busca, P., Chemama, M., Maillard, A.P., Hugonnet, J.-E., *et al.* (2007) Idiosyncratic features in tRNAs participating in bacterial cell wall synthesis. *Nucleic Acids Res* **35**: 6870–6883.

Vladar, H.P. de (2012) Amino acid fermentation at the origin of the genetic code. *Biol Direct* **7**: 6.

Wagner, A.M., Fegley, M.W., Warner, J.B., Grindley, C.L.J., Marotta, N.P., and Petersson, E.J. (2011) N-terminal protein modification using simple aminoacyl transferase substrates. *J Am Chem Soc* **133**: 15139–15147.

Wang, X., and He, C. (2014) Dynamic RNA modifications in posttranscriptional regulation. *Mol Cell* **56**: 5–12.

Watanabe, K., Toh, Y., Suto, K., Shimizu, Y., Oka, N., Wada, T., and Tomita, K. (2007) Protein-based peptide-bond formation by aminoacyl-tRNA protein transferase. *Nature* **449**: 867–871.

Watanabe, Y.-I., Suematsu, T., and Ohtsuki, T. (2014) Losing the stem-loop structure from metazoan mitochondrial tRNAs and co-evolution of interacting factors. *Front Genet* **5**: 109.

Wei, F.-Y., and Tomizawa, K. (2011) Functional loss of Cdkal1, a novel tRNA modification enzyme, causes the development of type 2 diabetes. *Endocr J* **58**: 819–825.

Weisblum, B. (1999) Back to Camelot: defining the specific role of tRNA in protein synthesis. *Trends Biochem Sci* **24**: 247–250.

Weixlbaumer, A., Petry, S., Dunham, C.M., Selmer, M., Kelley, A.C., and Ramakrishnan, V. (2007) Crystal structure of the ribosome recycling factor bound to the ribosome. *Nat Struct Mol Biol* **14**: 733–737.

Wettstein, F.O., and Stent, G.S. (1968) Physiologically induced changes in the property of phenylalanine tRNA in *Escherichia coli*. *J Mol Biol* **38**: 25–40.

Williams, K.P. (2002) Integration sites for genetic elements in prokaryotic tRNA and tmRNA genes: sublocation preference of integrase subfamilies. *Nucleic Acids Res* **30**: 866–875.

Winther, K.S., and Gerdes, K. (2011) Enteric virulence associated protein VapC inhibits translation by cleavage of initiator tRNA. *Proc Natl Acad Sci USA* **108**: 7403–7407.

Woese, C.R., Olsen, G.J., Ibba, M., and Söll, D. (2000) Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. *Microbiol Mol Biol Rev MMBR* **64**: 202–236.

Yuan, J., Sheppard, K., and Söll, D. (2008) Amino acid modifications on tRNA. *Acta Biochim Biophys Sin* **40**: 539–553.

Zhang, C.-M., Liu, C., Slater, S., and Hou, Y.-M. (2008) Aminoacylation of tRNA with phosphoserine for synthesis of cysteinyl-tRNA(Cys). *Nat Struct Mol Biol* **15**: 507–514.

Zhang, W., Ntai, I., Kelleher, N.L., and Walsh, C.T. (2011) tRNA-dependent peptide bond formation by the transferase PacB in biosynthesis of the pacidamycin group of pentapeptidyl nucleoside antibiotics. *Proc Natl Acad Sci USA* **108**: 12249–12253.

Zhou, J., Korostelev, A., Lancaster, L., and Noller, H.F. (2012) Crystal structures of 70S ribosomes bound to release factors RF1, RF2 and RF3. *Curr Opin Struct Biol* **22**: 733–742.

Zhou, J., Lancaster, L., Donohue, J.P., and Noller, H.F. (2013) Crystal structures of EF-G-ribosome complexes trapped in intermediate states of translocation. *Science* **340**: 1236086.