RNA BIOLOGY
2021, VOL. 18, NO. S1, 4–18
https://doi.org/10.1080/15476286.2021.1931756

REVIEW

OPEN ACCESS

Variations in transfer and ribosomal RNA epitranscriptomic status can adapt eukaryote translation to changing physiological and environmental conditions

Arnaud Dannfald[a,b], Jean-Jacques Favory[a,b], and Jean-Marc Deragon[a,b,c]

[a]CNRS LGDP-UMR5096, Pepignan, France; [b]Université de Perpignan via Domitia, Perpignan, France; [c]Institut Universitaire de France, Paris, France

ABSTRACT

The timely reprogramming of gene expression in response to internal and external cues is essential to eukaryote development and acclimation to changing environments. Chemically modifying molecular receptors and transducers of these signals is one way to efficiently induce proper physiological responses. Post-translation modifications, regulating protein biological activities, are central to many well-known signal-responding pathways. Recently, messenger RNA (mRNA) chemical (i.e. epitranscriptomic) modifications were also shown to play a key role in these processes. In contrast, transfer RNA (tRNA) and ribosomal RNA (rRNA) chemical modifications, although critical for optimal function of the translation apparatus, and much more diverse and quantitatively important compared to mRNA modifications, were until recently considered as mainly static chemical decorations. We present here recent observations that are challenging this view and supporting the hypothesis that tRNA and rRNA modifications dynamically respond to various cell and environmental conditions and contribute to adapt translation to these conditions.

ARTICLE HISTORY
Received 9 March 2021
Revised 7 May 2021
Accepted 13 May 2021

KEYWORDS
Epitranscriptome; tRNA; rRNA; ribosome; translation; stress; environment; acclimation; adaptation; post-transcriptional modifications

Introduction

Co- or post-transcriptional modification of RNA is an evolutionarily conserved process that drastically increases the biological potential of these crucial molecules. In all life kingdoms, at least 143 distinct chemical modifications can occur on RNA molecules, 111 on transfer RNAs (tRNAs), 33 on ribosomal RNAs (rRNAs) and 17 on messenger RNAs (mRNAs) [1]. Considering only eukaryotes, these numbers are 68, 21 and 17 for respectively tRNAs, rRNAs and mRNAs. In eukaryotes, mRNA epitranscriptomic marks have a dramatic impact on mRNA splicing, transport, stability, storage and translation and can regulate genes involved in development and stress responses [2,3]. The chemical modification of mRNAs is a dynamic process, with enzymes responsible for setting up (writers), removing (erasers) and reading (readers) marks in response to various developmental and stress conditions [2,3].

Compared to mRNA, tRNA modifications are much more diverse, ranging from simple modifications (i.e. methylation of the nucleobase or the sugar moiety), to the addition of complex compounds (i.e. isopentenylation of adenosine or the formation of cyclopentendiol derivates or imidazopurines from guanosine). tRNA biogenesis and functions, including tRNA maturation, stability, structure, aminoacylation, interaction with ribosomes and mRNA decoding properties, can be modulated by these chemical modifications [4]. Not all tRNA ribonucleotides are equal in terms of modifications as some positions in the T-loop (54 and 55), the D-loop (16 and 20) and the anticodon-loop (32, 34 and 37) are more frequently targeted. The most complex modifications are mainly found in the anticodon-loop region while modifications in the tRNA core are usually simpler. On average, 17% of tRNA ribonucleotides are modified (representing 13 modifications on each tRNA) [5].

From 2% to around 3% of all ribonucleotides of the four eukaryote cytosolic rRNAs (28S (25S in yeast, 26S in nematodes), 18S, 5.8S and 5S), representing over 100 nucleotides in *Saccharomyces cerevisiae* (yeast) and 200 in human, are modified co- or post-transcriptionally [6]. Most of these modifications are 2′-*O*-methylation of ribose (to generate 2′-*O*-methyladenosine (Am), 2′-*O*-methyluridine (Um), 2′-*O*-methylguanosine (Gm) or 2′-*O*-methylcytidine (Cm)) or uridine conversion to pseudouridine (Ψ), and are largely contributing to the biogenesis and stabilization of ribosomes [7]. Almost all rRNA modifications are found on either 18S and 28S rRNAs while 5.8S and 5S rRNAs are not modified or presenting a small number of modified positions. For example, in yeast, 5.8S rRNA is not modified and a single position is converted to Ψ in 5S rRNA [6]. In contrast with yeast, human 5S rRNA is not modified but four modifications (Um, Gm and two Ψ) are present on 5.8S rRNA [8]. The majority of 2′-*O*-methylations and pseudouridylations are guided by C/D or H/ACA snoRNPs [9]. Other known rRNA modifications include 5-methylcytidine (m^5C), 6-methyladenosine (m^6A), N^6,N^6-dimethyladenosine ($m^{6,6}A$), 7-methylguanosine (m^7G), 1-methyladenosine (m^1A), N^4-acetylcytidine (ac^4C),

CONTACT Jean-Marc Deragon jean-marc.deragon@univ-perp.fr Université de Perpignan via Domitia, LGDP-UMR5096 58 Av. Paul Alduy, Perpignan 66860, France

This article has been republished with minor changes. These changes do not impact the academic content of the article.
Supplemental data for this article can be accessed here.

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

and 3-methyluridine (m^3U) [1]. Some of these modifications (such as 25S/28S rRNA NOP2/NSUN1-dependent m^5C [10] or the RRP8-dependent m^1A [11] modifications) are clearly important for normal ribosome biogenesis, others (like human m^6A or yeast m^3U modifications) have no clear impact on this process [12,13]. In general, little is known on the impact of these modifications on ribosome function, although many of them are evolutionarily conserved in eukaryotes and localized within or near catalytic centres, suggesting that they should play important roles in translation [6].

A first line of evidence supporting the hypothesis that tRNA and rRNA modifications are not static comes from the observation that these chemical decorations vary in human diseases including cancer [14–22]. For example, two recent studies revealed a global reduction of rRNA 2′-*O*-methylation at some rRNA sites (named variable sites) in two types of human tumours compared to their corresponding level in normal tissue [21,22]. These new cancer-related hypomethylated patterns were found to be tumour-specific and associated with tumour aggressiveness [21,22]. In contrast, the global level of tRNA modifications is generally higher in cancer cells compared to their corresponding level in normal tissue [23]. Based on these variations, both rRNA and tRNA modifications are now proposed as useful prognostic markers for cancer [14,21,22,24,25]. More generally, eukaryote tRNA modification levels have been shown to vary during cell cycle and in response to environmental stresses [5,26–31]. Although the kinetic of these variations has not been investigated *in vivo*, the fact that they can be observed from 15 min to 1 h after yeast cell exposure to stressing agents suggests that they result of either increased enzyme activity and/or variations in tRNA copy numbers [27,28,30,32,33]. For example, yeasts grown at high temperature (37°C) or exposed for 15 min to hyperosmotic conditions (0.4 M NaCl) show changes in their global tRNA modification levels, with induction of new chemical marks and elimination of others [28]. A global analysis of tRNA marks revealed that a combination of 14 modified ribonucleotides has strong predictive power to distinguish exposure of yeast to oxidative or alkylating agents, in a manner similar to transcriptional, proteomic and metabolomic profiling [30], although the exact profiles of stress-induced tRNA variations can be different among different yeast strains [34]. In plants, tRNA modification levels were also shown to vary in different stress situations. For example, in *Arabidopsis thaliana* (Arabidopsis) and *Oryza sativa* (rice), several tRNA marks increase in response to 1–5 days of exposure to drought, salt or cold temperature conditions [31]. Also, while tRNAs are stable molecules, with estimated half-lives from 9 h to days, stress-induced tRNA modifications can affect their stability in one way or the other [32,35].

The dynamic, and not static, nature of tRNA and rRNA modifications suggests that it represents a sensing system linking cellular and environmental stimuli to translation and metabolism. For this system to work, variations in tRNA and rRNA chemical status must be exploited to adapt ribosome mRNA decoding potential to particular cellular conditions. One way to do this is to target tRNA subsets, especially in the anticodon loop [26,29,30,36,37], in order to better translate physiologically relevant mRNAs preferentially decoded by these tRNAs. Another way to modulate translation is to synthesize new 'specialized' ribosomes with variable levels of key rRNA modifications [6,38]. These specialized ribosomes could then preferentially bind and translate mRNA subsets involved in responding to specific cellular and/or environmental conditions [39,40]. We review here evidence supporting the idea that variations in tRNA and rRNA epitranscriptomic marks are not only passive consequences of different cellular conditions but can fine-tune translation to adapt cellular activities and the physiology of organisms to environmental changes. The principal tRNA- and rRNA-modified positions discussed in the following sections are presented in Figure 1, and a short summary of major information concerning these positions and their impact on translation is presented in Supplementary Table S1. In addition, Figure 2 gives an outline of the translation adjustment through tRNA and rRNA epitranscriptomic landscape modifications leading to adaptation to new environmental conditions.

Stress-modulated tRNA modifications and impact on translation

A key tRNA function is to decode mRNAs in the context of ribosome-directed translation. The 20 amino acids are encoded by 61 sense codons so that, for each amino acid, several 'isoacceptor' tRNAs (i.e. charged with the same amino acid but having distinct anticodons) are required. For example, the plant Arabidopsis has 597 tRNA genes, producing 198 unique sequences and 46 isoacceptors [41]. tRNA position 34 is named 'wobble' as it allows non-Watson and Crick parings with codon's third positions. In yeast, stress-responsive mRNAs named MoTTs for Modified Tunable Transcripts are biased for codons that necessitate the presence of corresponding tRNAs decorated with the proper chemical modification at the wobble position to be efficiently decoded [26,29,30,36,37]. Stress-induced variations of chemical modifications at the wobble position can therefore directly impact MoTTs (as well as other mRNAs) translation elongation speed [42], potentially influence co-translational mRNA decay [43], and affect the capacity of yeasts to survive stress. Apart from position 34, other stress-responsive modifications in the anticodon loop, such as the ones in positions 32 and 37, can also directly impact translation. Finally, modifications outside the anticodon loop can also affect cell translational capacities during stress. The next sections will focus on the description of these stress-responsive tRNA modifications involved in modulating the translational potential of cells.

Stress-responsive modifications of tRNA 'wobble' position 34

Uridine in position 34 of eukaryote tRNAs almost invariably carries a modification. In many instances, U_{34} is transformed to 5-methoxycarbonylmethyluridine (mcm^5U) or 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) by

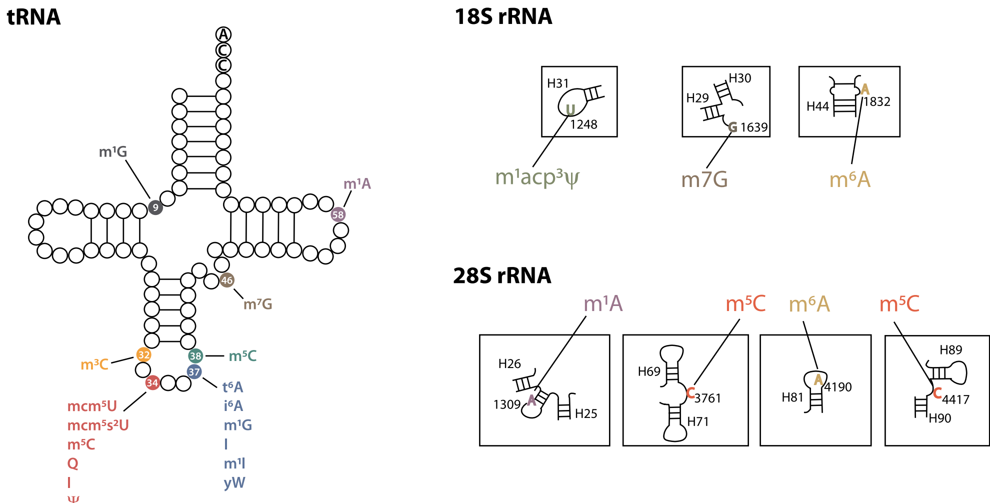

Figure 1. Schematic representation of tRNA and rRNA molecules with the position of major modifications discussed in the main text: 5-methylcytidine (m^5C), 3-methylcytidine (m^3C), 1-methyladenosine (m^1A), 6-methyladenosine (m^6A), 1-methylguanosine (m^1G), 7-methylguanosine (m^7G), queuosine (Q), wybutosine (yW), 5-methoxycarbonylmethyluridine (mcm^5U), 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U), Inosine (I), Pseudouridine (Ψ), 1-methylinosine (m1I), N6-threonylcarbamoyladenosine (t^6A), N6-isopentenyladenosine (i^6A) and 1-methyl-3-amino-carboxyl-propyl pseudouridine (m^1acp^3 Ψ). Positions of each modified tRNA ribonucleotides are indicated by their corresponding number. For 18S and 28S rRNAs, only close-ups of the different regions are presented. The numbering of the different 18S and 28S positions and helices (H) are taken from human sequences. A short summary of key information concerning these modifications is presented in Table S1.

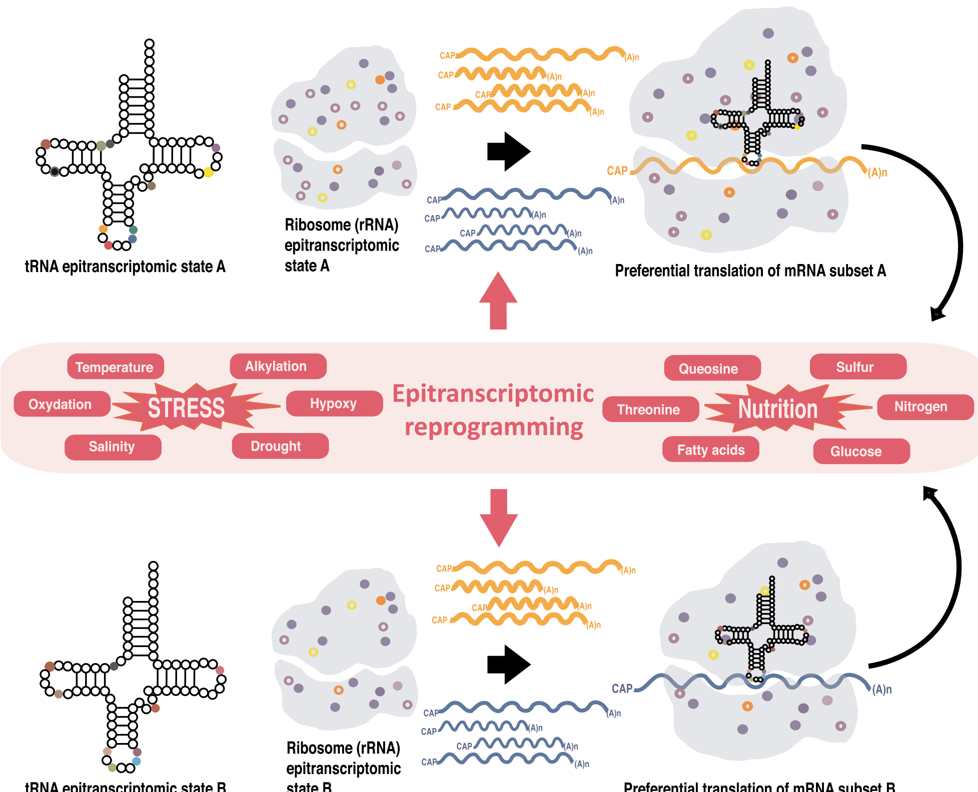

Figure 2. Reprogramming of tRNA and rRNA epitranscriptomic landscape to fit nutritional variation and stress condition. Nutrient availability and/or various stress conditions can reprogram the tRNA and/or rRNA epitranscriptomic landscape. This epitranscriptomic reprogramming than contributes to focus translation on different subsets of mRNAs facilitating acclimation and/or adaptation processes. Mechanisms by which various changes in tRNA and rRNA epitranscriptomic marks impact translation are summarized in Table S1.

a complex enzymatic process [1]. The elongator complex, composed of six subunits (ELP1–ELP6), is needed to transform U_{34} into 5-carboxymethyluridine (cm^5U) [44]. Next, the TRM9/TRM112 complex can methylate cm^5U_{34} to form mcm^5U_{34} [45]. Finally, thiolation of mcm^5U_{34}, to generate $mcm^5s^2U_{34}$, is a multistep process involving the ubiquitin-

related protein modifier 1 (URM1)-like proteins URM11 and URM12, and the CTU1/CTU2 complex [46]. tRNA arginine (UCU), glycine (UCC), glutamine (UUG), glutamic acid (UUC) and lysine (UUU) can be mcm^5U-modified while the last three tRNAs can be further thiolated [15,37]. The loss of ELP3, the catalytic subunit of the elongator complex [47] or TRM9 [48] completely abolishes the presence in tRNAs of both mcm^5U_{34} and $mcm^5s^2U_{34}$. This situation is embryo lethal in *Drosophila melanogaster* (drosophila) and mouse [49,50], which results in developmental defects in *Caenorhabditis elegans* (nematode) and plants [51,52] and generates stress hypersensitive phenotypes in yeast [37,42]. Alternatively, overexpressing Arabidopsis ELP3 or ELP4 in tomato and strawberry enhances resistance to pathogens [53,54]. In yeast, the loss of TRM9, drastically reduces the amount of polysomal mRNAs enriched in AGA and GAA codons, and corresponding protein production, without affecting global translation [37]. Deficiency in mcm^5U_{34} modification also leads to amino acid incorporation errors due to improper pairing of arginine tRNAs (UCU) with near-cognate serine codons in the ribosome [37]. Mutation of *URM11, URM12, CTU1* or *CTU2* prevents the formation of $mcm^5s^2U_{34}$ [55,56]. In yeast, this results in a slower propagation speed at high temperature of mutant stains compared to wild type (wt) [57]. More generally, the level of U_{34} thiolation is closely associated with thermotolerance properties of different yeast strains [34]. Dysfunction of CTU1 in nematodes also leads to a thermosensitive phenotype [46]. In Arabidopsis, the *urm11/urm12* double mutant is more sensitive to drought stress and produces leaf cells with altered ploidy levels and less chlorophyll content [55,56]. In rice, mutation of *CTU2* impairs the heat-stress response while its overexpression enhances tolerance to high temperature [58]. Also, in both rice and Arabidopsis, the *ctu1* or *ctu2* mutations are associated with a root-deficient phenotype [58–60].

In yeast, mcm^5U_{34} and $mcm^5s^2U_{34}$ levels vary in the presence of oxidative and alkylating agents or in heat and salt stress conditions [5,27–29]. Variations in the level of U_{34} modifications in the five concerned tRNAs have been shown to impact translation of stress-responsive mRNAs enriched in their cognate codons [37,61]. This is also true in *Schizosaccharomyces pombe* where, in response to H_2O_2, $mcm^5s^2U_{34}$-containing tRNA lysine (UUU) better translates AAA codon-rich stress-responsive genes [62]. In addition, a global reduction of yeast mcm^5U_{34} and $mcm^5s^2U_{34}$ levels in tRNA lysine (UUU), glutamine (UUG) and glutamic acid (UUC) induces ribosome pausing at cognate codons for a subpopulation of mRNAs and triggers the proteotoxic stress response [37,42]. The level of U_{34} thiolation is also critical in yeast to adjust translation and growth to the amount of sulphur amino acids [63]. Under nutritional stress, low levels of sulphur-containing amino acids directly impact negatively the thiolation status of U_{34}. This results in the reduced translation of key mRNAs coding for important translation and growth factors that are enriched in lysine, glutamine and glutamate codons. This, in turn, slows down cell growth and stimulates the synthesis and salvage of methionine and cysteine [63].

Interestingly, a crosstalk between the Target of Rapamycin (TOR) pathway and mcm^5U_{34} and $mcm^5s^2U_{34}$ tRNA levels has been observed in yeast, human and plants [60,64–67]. In yeast, the *urm11* mutant is hypersensitive to the TOR kinase inhibitor rapamycin and reduction in $mcm^5s^2U_{34}$ levels impacts the TOR pathway negatively by a yet-to-describe feedback mechanism [64,65]. In human cancer cells, higher expression of CTU1 is associated with increased cell growth and TOR activation [67]. In Arabidopsis, *ctu1* and *ctu2* mutants as well as the *urm11/urm12* double mutant phenocopy the root phenotype of hypomorph *tor* mutants [56,59]. Also, the Arabidopsis *ctu1* mutant is hypersensitive to TOR inhibitors [60]. These results suggest that stress-induced variations of mcm^5U_{34} and $mcm^5s^2U_{34}$ could also impact translation initiation through the TOR pathway. Overall, these observations indicate that mcm^5U_{34} and $mcm^5s^2U_{34}$ deposition is likely an evolutionarily conserved process that regulates translation initiation and elongation of a subset of transcripts involved in stress, nutrition and development.

Uridine in position 34 (as well as 6 other positions) can also be transformed to Ψ by the stand-alone pseudouridine synthase PUS1 [68,69]. In yeast, the *pus1* mutation causes a growth defect at high temperature. It also leads to synthetic lethality in combination with the loss of other pseudouridine synthases or in the presence of destabilized tRNA variants [69]. This suggests that PUS1-dependent modifications become essential when other aspects of tRNA biogenesis or modifications are disturbed. More specifically, Ψ in position 34 is proposed to stabilize codon-anticodon interactions, preventing the formation of unconventional pairs at non-synonymous near-cognate tRNAs [69]. The stabilizing property of Ψ is attributed to the presence of an extra NH moiety able to make more hydrogen bonds than uridine [70]. Also, Ψ in position 34 prevents 5-carmoylmethyluridine (mcm^5U_{34}) modification of intron-containing tRNA Isoleucine (UAU), a modification that would jeopardize its normal decoding capacity [71]. Although yeast tRNA Ψ levels have been shown to vary upon exposure to oxidative and alkylating agents [5,27] and during cold and heat stress [72], it is not clear at the moment if these modifications affect especially Ψ in position 34 (or other tRNA positions that are also converted to Ψ, see Table S1) and are used to reprogram translation in stress situations.

The nucleobase queuine (q) is a cyclopentendiol derivative of 7-aminomethyl-7-deazaguanine. This micronutrient, and its corresponding nucleoside queuosine (Q), cannot be synthesized by eukaryotes and must be acquired from the environment [73]. In most eukaryotes (with a few exceptions, such as *S. cerevisiae* and plants from the Brassicaceae family including Arabidopsis [74,75]), Q replaces G in position 34 of tRNAs having a GUN anticodon. The level of Q-tRNAs was shown to vary during development and the absence of Q-modified tRNAs is concordant with, and relevant to, the replicative undifferentiated cellular state. Accordingly, tRNAs of human primary tumours are hypomodified with respect to Q, with decreased levels correlating with disease progression

and poor patient survival such that Q hypomodification is proposed to be a deliberate and advantageous adaptation of cancer cells [76]. In mice, Q modification of tRNA is also required for normal tyrosine production such that animals made deficient in Q died within 18 days of withdrawing tyrosine from the diet [77]. Queuine deficiency also impacts the activity of several antioxidant systems [78] and phosphorylation levels of tyrosine phosphoproteins involved in cell signalling [79]. In plants other than Brassicaceae, the degree of Q-modification in $tRNA^{tyr}$(GUA) impacts the capacity of this tRNA to suppress UAG termination codons present on tobacco mosaic virus mRNAs [80]. All these observations suggest that Q modification of tRNA may affect severalf biological processes through broad changes in protein translation profiles.

The exact molecular impact of Q-tRNA on translation is still debated and possibly varies among species. In *Schizosaccharomyces pombe*, Q modifications enhance the translational speed of C-ending codons for aspartate (GAC), histidine (CAC), asparagine (AAC) and tyrosine (UAC), and reduce that of synonymous U-ending codons thus equilibrating the genome-wide translation of these codons [81]. Furthermore, Q prevents translation errors by suppressing second-position misreading of the glycine codon GGC. The absence of Q causes reduced translation of mRNAs involved in mitochondrial functions, and accordingly, lack of Q modification causes a mitochondrial defect [81]. In drosophila, the quantity of Q-containing tRNAs was shown to vary across development and translation to be adapted to these variations [82]. For example, at the embryonic stage, the level of Q-tRNA is high, and gene highly expressed at this stage are enriched in C-ending codons for fast and very accurate translation. However, at the larval and pupal stages, when low levels of Q-tRNAs are available, U-ending synonymous codons are favoured in highly expressed genes. In mammals, the translation of Q-decoded codons is slowed down in the absence of Q modifications [83]. This dysregulation of translation results in the accumulation of misfolded proteins and aggregates that triggers the activation of endoplasmic reticulum (ER) stress and the unfolded protein response. Consistent with reduced rates of protein translation, Q-deficient mice had a substantially reduced body weight. Overall, these observations reveal a route by which environment nutrients (including those generated by the gut microbiome of animals or the endophytic bacteria of plant tissues) can adapt protein translation. Furthermore, it is interesting to note that methylation by the DNMT2 methyltransferase of cytidine in position 38 of tRNAs to generate m^5C is strongly stimulated by the Q_{34} modification in *S. pombe* [84]. As m^5C_{38} has been shown to have a function in the control of tRNA cleavage (see below) and translational accuracy [85], this observation suggests another way by which nutritional factors could modulate mRNA decoding and translation. Also, drosophila lacking DNMT2 are more sensitive to heat and oxidative stress, further suggesting a role for Q_{34}, in combination with m^5C_{38}, in stress tolerance at least in this species [86].

In addition to uridine and guanosine, cytidine at the tRNA wobble position 34 is another stress-sensitive ribonucleotide. Cytidine in position 34 can be modified to m^5C by the action of TRM4(NSUN2). In yeast, m^5C_{34} increases following oxidative stress, leading to the selective translation of UUG-enriched mRNAs among which are represented ribosomal and stress responsive genes [87]. Accordingly, yeast *trm4* mutants are hypersensitive to H_2O_2 [87]. The global tRNA m^5C level also varies in yeast following exposure to alkylating agents and during heat and salt stress [5,27,28] and following plant exposure to cold, drought and salt [31]. However, since m^5C can be introduced in six tRNA positions (34, 38, 48–50, 72), it is not known to which proportion these variations affect position 34. In nematodes, m^5C_{34} modification of tRNA leucine (CAA) facilitates the translation of leucine UUG codons upon heat stress and supports the animal fitness at high temperature suggesting that this modification is involved in the adaptation to heat stress [88].

Finally, adenosine in position 34 can be deaminated to inosine (I) by the TAD2(ADAT2)/TAD3(ADAT3) complex. Inosine at position 34 (I_{34}) expands the tRNA decoding capacity as it can pair with U-, C- and A-ending codons [89]. Lack of I_{34} modifications is associated with several human diseases [90] and, in plants, to slower chloroplast translation, thereby affecting development [91]. In yeast, the global tRNA inosine level varies in the presence of oxidative or alkylating agents [5,27,29,30], but it is not clear if these variations impact positions 34, 37 or both and how they affect translation.

Stress-responsive modifications of tRNA position 37

Position 37, with position 34, are the two major modified positions in tRNA anticodon loops. All tRNAs harbour a purine at position 37 that is often modified into more complex derivates. A modified base in position 37 is proposed to stabilize, by base stacking, weaker (A:U) interactions between tRNA's position 36 and mRNA's first codon position [92]. Also, modification of position 37 impairs interactions with position U_{33} that would otherwise negatively affect the anticodon loop [92]. Modifications at position 37 are mainly known to be important for proper mRNA decoding and to prevent frameshift during translation [93].

N6-threonylcarbamoyladenosine (t^6A), N6-isopentenyladenosine (i^6A), as well as related ribonucleotides [1], are universally conserved stress-sensitive modifications found at position 37 that are crucial to translational accuracy. t^6A_{37} is found in nearly all tRNAs that decode ANN codon [94] and its general level was shown to vary following yeast exposure to oxidative and alkylating agents [5,27], as well as during heat stress [28]. In drosophila, variations in the amount of t^6A_{37} impact protein synthesis homoeostasis and can favour or inhibit translation of specific open reading frames [93]. In human cell lines, the amount to t^6A_{37} is regulated by the intracellular levels of CO_2 and bicarbonate, and hypomodification of t^6A_{37} in mitochondrial tRNAs was shown to downregulate mitochondrial translation in a codon-specific manner [95]. Variations in tRNA t^6A_{37} content in relation to CO_2 levels are proposed to regulate oxidative phosphorylation under hypoxic conditions, a process particularly important for solid tumour cell proliferation [95]. As such, t^6A_{37} is used as a prognostic marker for breast cancer [24]. Surprisingly, lowering the proportion of t^6A_{37}-modified

initiator methionyl-tRNA ($tRNAi^{Meti}$) in drosophila was shown to downregulate TOR kinase activity, inhibiting translation and growth [96]. This led to the suggestion that t^6A_{37}-modified $tRNAi^{Meti}$ could be a limiting factor for growth that is under the control of external stimuli [96].

i^6A_{37} is another important tRNA stress-sensitive modification, helping to properly decode the first codon position forming an A:U or U:A base pair [92]. In yeast, i^6A_{37} levels vary following salt and heat stress and exposure to oxidative and alkylating agents [5,27,28,30] and strains deficient in i^6A_{37} fail to sporulate [97]. In *S. pombe*, strains deficient in i^6A production present a mitochondrial dysfunction, mainly related to a lower content of i^6A-modified cytosolic tRNA tyrosine [98], and a reduced amount of several polysomal mRNAs enriched in i^6A-dependent codons [99]. Also, these strains are hypersensitive to rapamycin suggesting again, as for t^6A, mcm^5U and mcm^5s^2U, the existence of a crosstalk between the level of i^6A-containing tRNA and the TOR pathway [99]. Accordingly, in nematodes, the loss of i^6A results in slower growth and development [100].

In plants, tRNA isopentenyltransferase 2 and 9 (IPT2 and IPT9) can generate i^6A in position 37 of tRNA recognizing codons beginning with U [101]. AtIPT2 and 9 are indispensable for the biosynthesis of *cis*-zeatin, a stress-regulating plant cytokinin [101]. This is due to the fact that *cis*-zeatin can only be produced by the degradation of tRNAs containing the hydroxylated form of i^6A (io^6A) [101]. *Cis*-zeatin is important to maintain minimal cytokinin activity under growth-limiting conditions, including abiotic stress [102–104]. In these conditions, *cis*-zeatin replaces *trans*-zeatin for a lower promotion of cell division activities and an efficient set-up of the stress-responsive genetic programme. Heat, cold, drought and salt stress as well as nitrogen deficiency all lead to peaks of *cis*-zeatin with a strong decrease in *trans*-zeatin [102–104]. Accordingly, plants with higher levels of i^6A and io^6A in their tRNAs are proposed to be more resistant to abiotic stress as they can produce higher amounts of free *cis*-zeatin following stress-induced tRNA turnover [102]. Based on these observations, it has been suggested that plant stress tolerance could potentially be improved by increasing the content of endogenous i^6A/io^6A tRNAs [103].

It is interesting to note that the amount of tRNAs containing t^6A and i^6A (including their derivates) can also vary in relation to nutritional signals. The formation of t^6A (and derivates) uses threonine, an essential amino acid that must be salvage from bacteria in eukaryotes. Therefore, as for Q, this is another way by which environmental nutrients can feedback on the regulation of protein translation. The biosynthesis of i^6A (and derivates) depends on dimethylally pyrophosphate that itself is derived from acetyl-CoA [1]. Since acetyl-CoA levels depend on glycolysis or fatty acid beta-oxidation, this suggests that modifying tRNA i^6A levels could be a way to adjust the translation of specific mRNAs to the cell metabolic status. Taking into consideration that Q_{34}, $mcm^5s^2U_{34}$ and m^1A_{58} (see below) levels are also controlled by the cell nutrient status, a picture is emerging in which connections between tRNA modifications and the cell translational output is not limited to environmental exceptional situations but is also part of the cell's normal metabolism and growth programmes [105].

Wybutosine (yW) is a complex stress-responsive guanine modification found only in position 37 of eukaryotes tRNA phenylalanine (GAA) [1]. In yeast, this modification requires the retrograde nuclear import of tRNA phenylalanine to be synthetized [106]. In yeast and plants, the level of yW depends on cell growth conditions [107,108] and, in yeast, varies upon exposure to oxidative and alkylating agents as well as following heat and salt stress [5,27,28,30]. These observations suggest that the level of yW is modulated in response to environmental conditions and the cell's metabolomic requirements. yW is important to limit frameshifting particularly at 'U' stretches [107] and its loss results in a fourfold increase in −1 frameshift in yeast and animals [109,110]. It remains to be seen if the translation of some stress-responsive mRNAs could benefit from 'programmed' frameshifting as this is the case for many viruses that use this strategy to generate multiples viral proteins [110]. This 'frameshifting potential' that depends on the amount of yW37-modified tRNA phenylalanine was proposed as the evolutionary driving force behind the emergence of this modification from the m^1G_{37} platform [105].

Deamination of adenosine in position 37 by TAD1 (ADAT1) results in the formation of inosine (I) that can be further transformed to 1-methylinosine (m^1I) by TRM5. TRM5 can also directly modify guanosine in position 37 to generate 1-methylguanosine (m^1G). The I, m^1I and m^1G tRNA content was shown to vary upon yeast exposure to oxidative and alkylating agents [5,27,29,30] and, for m^1I, in heat and salt stress conditions [28]. In plants, the *tad1* mutant is hypersensitive to heat and cold stress [111] suggesting that I_{37} and/or m^1I_{37} are playing a role in plant acclimation to these temperature variations. Also, in Arabidopsis, the *trm5* mutant is slow-growing, late flowering, has reduced lateral roots, and accumulates fewer proteins involved in photosynthesis and ribosome biogenesis [112]. At the molecular level, the loss of AtTRM5 leads to aberrant protein translation and disturbed hormone homoeostasis. Since TRM5 is responsible for m^1G_{37} and m^1I_{37} methylation, it is not known if the loss of one, the other or both modifications are leading to the observed phenotypes. Nevertheless, based on the impact of m^1G/m^1I deficiencies at position 37, it was suggested that hypomodified tRNA would be unable to efficiently decode their cognate codons or induce frameshifts, resulting in a global reduction of protein output [113]. In yeast, TRM5 is downregulated by exposure to alkylating agents [30] and m^1I was shown to oscillate throughout the cell cycle [114], suggesting a role for m^1I in cell cycle regulation and response to at least this stress condition.

Stress-responsive modifications of tRNA position 32

In the anticodon-loop region, 3-methylcytidine (m^3C) in position 32 was recently shown to be an important stress-sensitive modification directly regulating mRNA translation in yeast and mouse [30,115]. m^3C occurs at position 32 of all tRNAs decoding serine and threonine and of two tRNAs decoding arginine, and its global level was found to vary upon yeast exposure to oxidative and alkylating agents as well as in heat

and salt stress conditions [5,27,28,30]. The upregulation of m^3C content upon yeast exposure to alkylating agents led to the selective translation of mRNAs enriched in four specific threonine codons, suggesting that these codons are differentially recognized by m^3C-modified $tRNA^{Thr}$ [30,116]. TRM140(METTL2) and TRM141(METTL6) are responsible for the m^3C_{32} modification [115,117]. In human, METTL6 was identified as a crucial regulator of tumour cell growth, and its deletion in mouse stem cells results in changes in mRNA ribosome occupancy and impairs pluripotency [115]. These results suggest that m^3C_{32} is a key modification required to adapt translation to various cell growth and stress conditions. Interestingly, the m^3C_{32} modification of three *S. pombe* tRNAs is dependent on the synthesis of i^6A_{37} [118], suggesting that these two modifications can be interconnected. Also, in *Trypanosoma brucei*, m^3C_{32} can be further converted to m^3U_{32} by the action of the TAD2/TAD3 complex [119].

Stress-responsive modifications outside of the tRNA anticodon-loop region

Not all stress-sensitive tRNA modifications directly impacting translation occur in the anticodon-loop region. One example is the modification by the TRM61/TRM6 complex of $tRNAi^{Meti}$ adenosine 58 to generate m^1A. In all eukaryotes, this modification is critical to ensure the stability of $tRNAi^{Meti}$ and is a way to control translation initiation [4,5,120,121]. In mammals, the ALKBH1 demethylase can demethylate m^1A_{58} in response to variations in nutritional conditions [122]. For example, in glucose deprivation condition, *ALKBH1* expression is up-regulated leading to a reduction of m^1A_{58}, $tRNAi^{Meti}$ and translation [122]. On the contrary, the knockdown of ALKBH1 results in higher m^1A_{58} levels of specific tRNAs (including $tRNAi^{Meti}$) and favour translation initiation and elongation from corresponding codons [122]. In addition of being more stable, m^1A_{58}-methylated tRNAs are preferentially recognized and delivered to actively translating ribosomes [122]. In eukaryote, m^1A can also be present at two other tRNA positions (9, and 14) [1]. In yeast, global m^1A levels vary upon exposure to oxidative and alkylating agents [5,27] and in plants upon exposure to cold, drought and salt stress [31]. It is not known if m^1A in position 58 is mainly affected in these conditions, but if this is the case, then translation could also be efficiently modulated by m^1A_{58} levels following stress.

In addition to its presence in position 37, m^1G is also found in position 9 of many cytosolic and mitochondrial tRNAs. In human, the TRM10A methylase is responsible for installing this modification on a large number of tRNAs (21) [123]. Recently, a very interesting link was established between tRNA m^1G_9 and mRNA m^6A modifications [123]. TRM10A and the m^6A demethylase FTO were shown to collaborate to target a specific subset of m^6A-containing mRNAs whose efficient translation requires the presence of m^1G_9-containing tRNAs. In the presence of both FTO and TRM10A, the m^6A level of targeted mRNAs is maintained low, preventing efficient binding of the m^6A reader protein YTHDF2. This situation contributes to maintain the targeted mRNAs stable while their translation is favoured by the presence of m^1G_9-modified tRNAs. In the absence of TRM10A, FTO would less efficiently demethylate these mRNA targets, leading to hyper m^6A methylation, YTHDF2 binding and mRNA instability. In addition, these unstable hypermethylated mRNAs would be poorly translated due to the absence of m^1G_9-modified tRNAs. Recently, the lack of m^1G_9 methylation was also shown to cause a decrease in the steady-state amount of human $tRNAi^{Meti}$ suggesting that this modification, as for m^1A_{58}, could be involved in regulating translation initiation [124]. Global m^1G levels are known to vary in different environmental situations (see earlier) and it remains to be determined if these modifications affect positions 9, 37 or both.

The modification of guanosine to m^7G in position 46 is one of the most prevalent tRNA modifications found in eukaryotes and concerns a large number of different tRNAs (11 in yeast, 22 in human) [125]. The m^7G_{46} level was found to vary in yeast upon oxidative and alkylating treatments [5,27] and, in plants, in cold, drought and high salt conditions [31]. TRM8/TRM82 in yeast and METTL1/WDR4 in human are responsible for this modification. m^7G_{46} is important to stabilize tRNAs, accordingly the *trm8* or *trm82* mutants have an increased sensitivity to high temperature [126]. In human cells, depletion of METTL1 results in the loss of m^7G_{46} and causes a global reduction in translation. Furthermore, in this mutant, mRNAs having low translation efficiency (TE) compared to wt have a significantly higher frequency of codons decoded by m^7G_{46}-modified tRNAs [125]. A ribosome occupancy study in the *mettl1* mutant also revealed an increase ribosome pausing at codons whose translation is dependent on the presence of m^7G_{46}-containing tRNAs [125]. These results suggest that the amount of m^7G_{46} in tRNAs can regulate the translation of a subpopulation of mRNAs in response to various environmental conditions.

Several other common tRNA modifications are stress-sensitive, like 2-methylguanosine (m^2G) and N^2,N^2-dimethylguanosine ($m^{2,2}G$) (in positions 10 and 26), 5-methyuridine (m^5U) (in position 54), dihydrouridine (D) (in positions 16–20) and (Ψ in 14 possible tRNA positions apart from position 34) [5,27,31]. These modifications are mainly associated with the stabilization of the tRNA tertiary structure and to prevent tRNA misfolding [1]. Dihydrouridine is more specifically important to maintain tRNA conformational flexibility, especially in low-temperature conditions [127]. Increased D levels are also observed in several cancer cell types [25]. On the contrary, Ψ is able to stabilize RNA, improving base-stacking by forming additional hydrogen bonds with water through its extra imino group [128]. For example, Ψ in position 55 was proposed to stabilize the tertiary structure of tRNA, particularly in extremely high-temperature conditions [1]. Ψ as D, has been found to increase in some cancers [129]. It remains to be seen if stress-induced variations of these modifications can adapt translation to specific cellular and environmental conditions.

Chemical modifications affecting the production of biologically functional tRNA fragments: an indirect way to adapt translation to stress?

tRNAs are generally considered as stable molecules, but this high stability greatly relies on the acquisition of a proper tertiary structure. Miss-folded tRNA molecules are targeted for degradation by the nuclear surveillance pathway or by the rapid tRNA decay (RTD) pathway [130,131]. Several chemical marks are important for tRNA proper folding (see above for numerous examples), so that hypomodified tRNAs are preferential targets of tRNA decay pathways [132–134]. For example, precursors of initiator tRNA methionine lacking m^1A_{58} are rapidly turned over by the nuclear surveillance pathway [135], while mature tRNAs valine (AAC) lacking both m^7G and m^5C (in position 34 and/or 48 and 49) are degraded by the RTD pathway at high (37°C) temperature in yeast [132].

While the nuclear surveillance and RDT pathways are expected to completely degrade tRNAs, relatively stable tRNA-derived RNA fragments (tRFs) of different sizes have been found in many different organisms [130,134]. Half-tRNAs (5′-halves (tRF5A) and 3′-halves (tRF3A)) are produced primarily upon different stresses by an endonucleotidic cleavage in the anticodon loop. Also, in normal physiological conditions, numerous short tRFs (around 15–25 nucleotides) are produced from mature tRNAs, mainly by cleavage in the D (tRF-5D) and T loops (tRF-3 T). tRFs have many proposed biological activities, one of which is to modulate translation [134]. Mechanisms by which such regulation can be achieved are still under study, but they could involve blocking access of eIF4F to the mRNA cap structure [136], directly interacting with the small ribosomal subunit to inhibit translation [137] or more specifically acting on specific mRNAs, in a microRNA-like manner [138,139]. We focus in the following on the few known situations in which key tRNA chemical decorations were shown to influence the biogenesis and/or function of tRFs and, doing so, potentially regulate translation.

Angiogenin (ANG) is a stress-inducible, vertebrate-specific, endonuclease of the RNase A family that can cleave the anticodon loop of a subset of tRNAs (i.e. those with a CA-motif in the anticodon) to generate tRF5A and tRF3A fragments [140,141]. ANG cleavage is inhibited by the presence of m^5C in position 38 (deposited by DNMT2) or in position 34 and/or 48 and 49 (deposited by TRM4 (NSUN2)) [134]. Therefore, stress conditions leading to variations in tRNA m^5C levels can not only directly adapt ribosome mRNA decoding properties (see above) but, by influencing ANG cleavage activity, also produce variable amounts of tRFs. In turn, this stress-specific tRF population could potentially contribute to adapt translation to the situation. Another enzyme responsible for generating tRFs is RNase L, a mammalian-specific endonuclease that can cleave the anticodon loop of tRNA histidine depending on the presence of a specific tRNA modification [142]. RNase L is activated upon detection of double-stranded RNA, a hallmark of viral infection, and cleaves single stranded viral and cellular RNAs at the very promiscuous recognition site UNN. The presence of Q in position 34 is protecting most tRNAs from RNase L cleavage, except for tRNA histidine where, on the contrary, it stimulates cleavage at position 37, leading to the synthesis of stable tRNA histidine fragments and to a general decrease in protein synthesis [142]. Modifications in tRNA Q_{34} levels could therefore affect this situation, leading to the synthesis of new populations of tRNA fragments with different impacts on translation. tRNAs decorated with specific chemical marks can also be the target of eukaryotic toxin ribonucleases for competitive purposes. For example, the subunit of the zymocin toxin from *Kluyveromyces lactis* is a ribonuclease that, once introduced in competitor yeast cells, can down-regulate translation by cleaving several tRNAs presenting the mcm^5s^2U modification in position 34 [143]. In response, adjusting tRNA $mcm^5s^2U_{34}$ levels in yeast could potentially dampen this negative influence on translation. RNases from the T2 and (to a lesser extent) DICER families are also responsible for generating long and short stable tRNA fragments [139,144,145]. Certain tRNAs are more susceptible than others to cleavage by these enzymes and one largely understudied determinant of this selectivity could be the nature of chemical decorations on each tRNAs. Whether tRFs biological activity could be influenced by the nature of chemical marks they contain, is also largely unexplored. Indeed, in a single case, the presence of a modified ribonucleotide (Ψ in position 8) on small tRFs, issued from three different tRNAs, was shown to be essential to exert translation inhibition in a human stem cell line [146]. Clearly, more work is needed to establish the global impact of tRNA epitranscriptomic marks on the production and biological activity of tRFs and their impact on translation.

Variations in rRNA modifications and impact on translation

Diverse ribosome populations exist within cells and this heterogeneity can be due to variations in their rRNA and protein compositions, but also to post-transcriptional/translational modifications of these components (reviewed in Guo [147]). To what extent these variations in composition influence ribosome properties, thereby changing the output of translation, with some specialized ribosomes displaying differential affinities for particular mRNAs, is the subject of intense debates (reviewed in Ferretti and Karbstein [148]). We specifically review here evidence for ribosome functional specialization linked to variations in their rRNA chemical composition. Since rRNA modifications are generally installed during ribosome assembly and are considered to be irreversible [6,13], ribosome turnover would be needed to remove an existing ribosome population to the profit of a different one. This implies that ribosome functionalization by changing rRNA chemical composition would be a slow process, potentially useful as a long-term strategy of cell adaptation but not pertinent for rapid acclimation processes [148,149].

Variation in rRNA 2′-*O*-methylation and pseudouridylation levels and impact on translation

In certain situations, 2′-*O*-methylated nucleotides and pseudouridines are deposited by snoRNAs in substochiometric amounts, generating heterogeneous ribosome populations [6,38,150]. In human, the recent remapping of all 2′-*O*-methylated nucleotides allowed the identification of 'vulnerable' sites, particularly affected by fibrillarin and anti-tumoural p53 levels, and most likely to undergo specific regulation [150]. In human, snoRNAs are differentially expressed in cancers and some of them have been associated with oncogenesis [151,152]. Accordingly, levels of 2′-*O*-methylated nucleotides and Ψ are altered in cancer lines [17,18], although it is not clear at the moment if these changes always result of corresponding changes of snoRNA expression. Also, whether 2′-*O*-methylation and pseudouridylation rRNA profiles can vary under non-pathological conditions remains to be determined. Interestingly, ribosomes with reduced amount of 2′-*O*-methylation levels present a fourfold reduction in their capacity to initiate translation using internal ribosome entry sites (IRES) [17,39]. Reducing the amount of pseudourydilated rRNAs in ribosomes was also shown to impact IRES-dependent translation, either by increasing or reducing its efficiency depending on the studied mRNA [18,153,154]. IRES-dependent translation concerns many important cellular mRNAs, including growth factors and receptors, apoptosis regulators, oncogenes and tumour suppressors [155], and modulating its efficiency, by increasing or reducing 2′-*O*-methylation and pseudouridylation levels, could represent a way to adapt translation to specific cellular conditions. In addition to IRES-containing mRNAs, modulating the amount of 2′-*O*-methylation was also shown to impact (positively or negatively) the cap-dependent TE of several mRNAs [39]. However, since removing 2′-*O*-methylation over a certain threshold can impact ribosome biogenesis [156], it is possible that these effects are simply due to a general reduction of ribosome availability and not to ribosome specialization per see [148]. Therefore, it is unclear at the moment if global variations in 2′-*O*-methylation and pseudouridylation contribute or not to generate specialized ribosomes affected in their capacity to initiate cap-dependent translation on mRNA subsets.

A key evolutionary conserved Ψ present on 18S rRNA (position 1248 in human and 1191 in yeast) and structurally located at the ribosome P site, can be further modified by a methyltransferase and an aminocarboxyl propyl transferase (respectively named EMG1 and TSR3 in human) to generate 1-methyl-3-amino-carboxyl-propyl Ψ (m^1acp^3 Ψ) [19]. This modification is involved in 18S rRNA processing [13]. Also, by interacting with the 40S ribosomal protein RPS16 and tRNA, it can directly impact the ribosome P site function [157]. Recently, a large subset of human tumours was found to possess hypo-m^1acp^3Ψ-modified 'onco-ribosomes'[19]. In these tumour cells, while global protein translation was unaffected, the TE of a subset of mRNAs coding for ribosomal proteins increased leading to the higher accumulation of corresponding proteins [19]. The same observation was made for *tsr3* mutant cell lines presenting low m^1acp^3Ψ levels [19]. This suggests that ribosomes lacking m^1acp^3Ψ can specifically promote the translation of a subset of mRNAs. It remains to be determined if non-pathological conditions can also result in the production of specialized m^1acp^3Ψ-free ribosomes.

m^5C rRNA variations and impact on translation

The yeast RNA methyltransferase RCM1 is responsible for converting the cytidine in position 2278 of 25S rRNA in m^5C [149]. The nematode-corresponding position (m^5C_{2381}) is also targeted by NSUN5, the ortholog of RCM1. In these two organisms, as well as in drosophila, the loss of RCM1(NSUN5) confers increased lifespan and resistance to different types of stress [149]. In *RCM1*-knockout cells, ribosome lacking m^5C_{2278} are more efficient to translate several stress-responsive genes [149]. Since RCM1 is localized in the nucleoli [10], a stress-mediated response involving the down-regulation of RCM1 enzymatic activity is likely to be slow, involving the exchange of methylated ribosomes for unmethylated ones so that this mechanism would preferentially modulate long-term chronic stress [149]. In human, high NSUN5 expression promotes the progressing of cancer cells through cell cycle regulation [158]. Accordingly, the epigenetic silencing of NSUN5 in human glioma cells generates ribosomes lacking 28S C_{3761} methylation (the equivalent of yeast 25S C_{2278}) that promote the selective translation of stress-responsive genes and limit global protein synthesis and cell growth [159]. Also, NSUN5 knockout in mice leads to reduce body weight and reduced protein synthesis in many tissues [160].

Another conserved methyltransferase, NOP2(NSUN1), is involved in converting a second large subunit rRNA cytidine (in position 2870 for yeast, 2982 for nematodes and 4417 for human) in m^5C. In yeast and human, NOP2(NSUN1) is essential for rRNA processing and synthesis of the large ribosome subunit, a function that is independent of its m^5C modification activity [161–163]. Accordingly, NOP2 (NSUN1) is essential for yeast growth and mammalian embryo development [161,162]. This is not the case for nematodes, as *nsun1* worms are viable and not significantly affected in ribosome biogenesis nor in global translation [164] (a situation analogous to what is observed in *nsun5* worms [149]). *nsun1* worms have a longer lifespan and were shown to remodel the translation of specific mRNA transcripts [164]. Therefore, the level of NSUN1 in nematodes is likely important for the synthesis of specialized ribosomes more or less adapted to the translation of different mRNA subsets.

At the moment, it is not clear exactly what environmental signals could regulate NOP2(NSUN1) and RCM1(NSUN5) levels. The fact that the human NSUN5 CpG island promoter can be epigenetically regulated [159] may be a way environmental cues could achieve such regulation. Overall, these observations suggest that RCM1(NSUN5) and NOP2 (NSUN1) (at least in nematodes), by adjusting ribosome m^5C levels, adapt translation to different physiological conditions such that, in fast growth conditions, highly m^5C-modified ribosomes are preferred

while m^5C-hypomodified ribosomes are favoured in stress situations.

m^6A rRNA variations and impact on translation

Another important rRNA modification that affects ribosome translation in animals (globally and/or on mRNA subsets) is m^6A [165–167]. In human and nematodes, 18S and 28S/26S rRNAs are decorated each by one m^6A, installed by METTL5(METL5) on 18S [12,165,166] and by ZCCHC4 on 28S/26S [12,167,168]. In nematodes, the loss of METL5 does not globally affect translation while the TE of cyp-29A3, a transcript coding for a cytochrome P450, is reduced 10-fold, suggesting that ribosome 18S m^6A methylation levels can regulate the translation of specific mRNAs [166]. *metl-5* worms have an increased lifespan and are more resistant to several abiotic stresses [166]. These phenotypes are proposed to be the direct result of reduced CYP-29A3 translation and CYP-29A3-dependent synthesis of eicosanoid lipids in the mutant. In contrast to nematodes, in human cells, knocking out METTL5 results in a general decrease in TE, but whether some specific mRNAs are more affected than others has yet to be investigated [165]. In human cells, knocking out ZCCHC4, also results in a general decrease in TE (of about 25%), but in that case, a subset of 311 mRNAs were shown to be much more affected than others, including transcripts coding for membrane protein targeting, mRNA catabolic process, ER localization and translation initiation, here again suggesting that 28S m^6A levels can affect the translation of specific mRNAs [167]. The identification of physiological and/or environmental conditions leading to the accumulation of ribosome population with substochiometric amount of m^6A-modified rRNA is still needed to firmly established rRNA m^6A level as a new regulation layer of the animal stress response.

m^1A rRNA variations and impact on translation

Another key rRNA modifying enzyme is the methylase RRP8 that generates m^1A in position 645 of yeast 25S rRNA [169]. The loss of m^1A_{645} results in the production of ribosomes altered in their general ability to initiate translation, possibly linked to a reduced competence for the 60S subunit lacking m^1A_{645} to bind to the 40S subunit [169]. Surprisingly, despite having a reduced translation initiation efficiency, most proteins are produced in similar amounts in wt and *rrp8* mutant lines. Exceptions to this rule concern several enzymes involved in carbohydrate metabolism that are either up or down regulated in *rrp8* mutant compared to wt, suggesting that ribosomes lacking m^1A_{645} translate corresponding mRNAs more or less efficiently [169]. This suggests that, under some growth conditions, ribosomes lacking m^1A_{645} could be synthesized to specifically regulate the translation of mRNAs involved in producing key carbohydrate metabolism enzymes. In human, m^1A-modified nucleotides are elevated in the urine of cancer patients [20] and lowering the level of 28S m^1A_{1309} (the equivalent of yeast 25S m^1A_{645}) leads to the downregulation of cell proliferation in a p53-dependent manner [170]. These results suggest that methylation at this position is a way to control cell proliferation in mammals. Finally, in nematodes, T07A9.8 (the orthologue of the yeast RRP8 enzyme) methylate position A_{674} of 26S rRNA (the equivalent of yeast 25S m^1A_{645}) [171]. Impairing this function leads to an extending life span for nematodes, again linking m^1A modification at this position to cell cycle regulation [171].

m^7G rRNA variations and impact on translation

BUD23 is an important protein involved in the biosynthesis of the translational apparatus, firstly by processing the pre-18S RNA into its mature form and secondly by modifying an 18S rRNA guanosine (in position 1639 for human and 1575 for yeast) to m^7G [172,173]. Since the methyltransferase activity is not needed to process pre-18S RNA, these two functions are considered to be independent of each other [173,174]. In human cell lines, 18S m^7G has been proposed to be present in substochiometric amount in ribosome populations, which may indicate a selective role in ribosome function [174]. Furthermore WBSCR22, the human ortholog of BUD23, is found to be overexpressed in breast cancer and has been proposed as a cancer biomarker [174]. In human cells, 48 h after knocking down BUD23, no global impact on protein translation rate was observed. Yet, the TE of more than 700 mRNAs was affected in this condition, including a strong TE decrease for mRNAs coding for mitochondrial proteins [172]. Also, mRNAs with contrasting GC 5′UTR content were differentially translated following BUD23 knockdown. Transcripts having a low GC 5′UTR content also had a low TE, while the opposite was true for transcripts having a high GC 5′UTR content [172]. These results suggest that variations in 18S m^7G levels could generate ribosomes with different affinities for mRNAs having contrasting GC 5′UTR content. However, it is not clear for the moment if cell translation occurring 48 h after knocking down BUD23 mainly results from ribosomes lacking m^7G or if the loss of BUD23 could impact translation independently of ribosome m^7G levels.

Conclusion

A large and convincing body of evidence exists to conclude that tRNA and rRNA chemical modifications are critical for the biogenesis, stabilization and proper decoding functions of the constitutive translation apparatus (reviewed in Sloan et al. and Sharma and Lafontaine [6,13]). However, whether variations in the level of these modifications, in some cell and environmental conditions, can be used to adapt this apparatus to target physiologically relevant mRNA subsets, possibly still await further experiments. At the molecular level, demonstrations that changing the nature and stoichiometry of many tRNA modifications, in yeast, nematodes, drosophila, plants and mammals, can indeed favour the translation of specific mRNAs are more and more numerous and convincing. Although less numerous, reports of heterogeneous ribosome populations, specialized to target specific mRNAs due to changes in the stoichiometry of one or several rRNA chemical marks, have been lately published using different eukaryotic systems. So, what is missing to firmly establish tRNA and

rRNA epitranscriptomic variations as a new layer of eukaryote gene regulation? First, it is not always clear in which (non-pathological) physiologically relevant conditions these variations can occur and what could be the impact of these changes at the organism level. In other words, can these variations significantly impact nutritional, developmental or stress-responses leading to acclimation and/or adaptation of individuals? Also, studies of these variations in natural populations coming from contrasting environments are clearly missing to ensure that this regulatory process is indeed under selection *in natura* and therefore meaningful as a regulatory process. Plants as complex organisms that can be studied at the physiological and molecular levels, as well as in natural environments, may represent good systems to try to solve these issues.

Acknowledgments

We thank Cécile Bousquet-Antonelli, Rémy Merret and Julio Saez-Vasquez for critical reading of the manuscript. This work was supported by the CNRS, the University of Perpignan (UPVD) and the Institut Universitaire de France (IUF). This study is set within the framework of the "Laboratoires d'Excellences (LABEX)" TULIP (ANR-10-LABX-41) and of the "École Universitaire de Recherche (EUR)" TULIP-GS (ANR-18-EURE-0019). AD is the recipient of a PhD grant from the Occitanie Region. This paper was based upon work from COST Action CA16120 EPITRAN, supported by COST (European Cooperation in Science and Technology).

Disclosure of conflicts of interest Statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Centre National de la Recherche Scientifique; Région Occitanie; École Universitaire de Recherche TULIP-GS [ANR-18-EURE-0019]; LabEx TULIP [ANR-10-LABX-41]; Institut Universitaire de France (IUF); and Université de Perpignan Via Domitia EPITRAN Cost Action (CA16120).

References

[1] McCown PJ, Ruszkowska A, Kunkler CN, et al. Naturally occurring modified ribonucleosides. Wiley Interdiscip Rev RNA. 2020;11:e1595.
[2] Roignant JY, Soller M. m(6)A in mRNA: an ancient mechanism for fine-tuning gene expression. Trends Genet. 2017;33:380–390.
[3] Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. Nat Rev Mol Cell Biol. 2019;20:608–624.
[4] Barraud P, Tisne C. To be or not to be modified: miscellaneous aspects influencing nucleotide modifications in tRNAs. IUBMB Life. 2019;71:1126–1140.
[5] Huber SM, Leonardi A, Dedon PC, et al. The versatile roles of the tRNA epitranscriptome during cellular responses to toxic exposures and environmental stress. Toxics. 2019;7(1):17.
[6] Sloan KE, Warda AS, Sharma S, et al. Tuning the ribosome: the influence of rRNA modification on eukaryotic ribosome biogenesis and function. RNA Biol. 2017;14:1138–1152.
[7] Polikanov YS, Melnikov SV, Soll D, et al. Structural insights into the role of rRNA modifications in protein synthesis and ribosome assembly. Nat Struct Mol Biol. 2015;22:342–344.
[8] Taoka M, Nobe Y, Yamaki Y, et al. Landscape of the complete RNA chemical modifications in the human 80S ribosome. Nucleic Acids Res. 2018;46:9289–9298.
[9] Bachellerie JP, Cavaille J, Huttenhofer A. The expanding snoRNA world. Biochimie. 2002;84:775–790.
[10] Sharma S, Yang J, Watzinger P, et al. Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. Nucleic Acids Res. 2013;41:9062–9076.
[11] Peifer C, Sharma S, Watzinger P, et al. Yeast Rrp8p, a novel methyltransferase responsible for m1A 645 base modification of 25S rRNA. Nucleic Acids Res. 2013;41:1151–1163.
[12] van Tran N, Ernst FGM, Hawley BR, et al. The human 18S rRNA m6A methyltransferase METTL5 is stabilized by TRMT112. Nucleic Acids Res. 2019;47:7719–7733.
[13] Sharma S, Lafontaine DLJ. 'View from a bridge': a new perspective on eukaryotic rRNA base modification. Trends Biochem Sci. 2015;40:560–575.
[14] Popis MC, Blanco S, Frye M. Posttranscriptional methylation of transfer and ribosomal RNA in stress response pathways, cell differentiation, and cancer. Curr Opin Oncol. 2016;28:65–71.
[15] Barbieri I, Kouzarides T. Role of RNA modifications in cancer. Nat Rev Cancer. 2020;20:303–322.
[16] Yanas A, Liu KF. RNA modifications and the link to human disease. Methods Enzymol. 2019;626:133–146.
[17] Belin S, Beghin A, Solano-Gonzalez E, et al. Dysregulation of ribosome biogenesis and translational capacity is associated with tumor progression of human breast cancer cells. PLoS One. 2009;4:e7147.
[18] Jack K, Bellodi C, Landry DM, et al. rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. Mol Cell. 2011;44:660–666.
[19] Babaian A, Rothe K, Girodat D, et al. Loss of m(1)acp(3)Psi ribosomal RNA modification is a major feature of cancer. Cell Rep. 2020;31:107611.
[20] Itoh K, Mizugaki M, Ishida N. Preparation of a monoclonal antibody specific for 1-methyladenosine and its application for the detection of elevated levels of 1-methyladenosine in urines from cancer patients. Jpn J Cancer Res. 1988;79:1130–1138.
[21] Krogh NA, F, Côme C, Fibiger Munch-Petersen H, et al. Profiling of ribose methylation in ribosomal RNA from diffuse larger B-cell lyphoma patients for evaluation of ribosomes as drug targets. NAR Cancer. 2020;2:1–11.
[22] Marcel J, Marchand V, Natchiar KS, et al. Ribosome RNA 2′ O-methylation as a novel layer of inter-tumor heterogneity in breast cancer. NAR Cancer. 2020;2:1–12.
[23] Dong C, Niu L, Song W, et al. tRNA modification profiles of the fast-proliferating cancer cells. Biochem Biophys Res Commun. 2016;476:340–345.
[24] Vold BS, Kraus LE, Rimer VG, et al. Use of a monoclonal antibody to detect elevated levels of a modified nucleoside, N-[9-(beta-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine, in the urine of breast cancer patients. Cancer Res. 1986;46:3164–3167.
[25] Kato T, Daigo Y, Hayama S, et al. A novel human tRNA-dihydrouridine synthase involved in pulmonary carcinogenesis. Cancer Res. 2005;65:5638–5646.
[26] Endres L, Dedon PC, Begley TJ. Codon-biased translation can be regulated by wobble-base tRNA modification systems during cellular stress responses. RNA Biol. 2015;12:603–614.
[27] Chan CT, Dyavaiah M, DeMott MS, et al. A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. PLoS Genet. 2010;6:e1001247.
[28] Rose RE, Pazos MA 2nd, Curcio MJ, et al. Global epitranscriptomics profiling of RNA post-transcriptional modifications as an effective tool for investigating the epitranscriptomics of stress response. Mol Cell Proteomics. 2016;15:932–944.
[29] Dedon PC, Begley TJ. A system of RNA modifications and biased codon use controls cellular stress response at the level of translation. Chem Res Toxicol. 2014;27:330–337.
[30] Chan CT, Deng W, Li F, et al. Highly predictive reprogramming of tRNA modifications is linked to selective expression of codon-biased genes. Chem Res Toxicol. 2015;28:978–988.

[31] Wang Y, Pang C, Li X, et al. Identification of tRNA nucleoside modification genes critical for stress response and development in rice and Arabidopsis. BMC Plant Biol. 2017;17:261.
[32] Torrent M, Chalancon G, de Groot NS, et al. Cells alter their tRNA abundance to selectively regulate protein synthesis during stress conditions. Sci Signal. 2018;11(546):eaat6409
[33] Su D, Chan CT, Gu C, et al. Quantitative analysis of ribonucleoside modifications in tRNA by HPLC-coupled mass spectrometry. Nat Protoc. 2014;9:828–841.
[34] Alings F, Sarin LP, Fufezan C, et al. An evolutionary approach uncovers a diverse response of tRNA 2-thiolation to elevated temperatures in yeast. RNA. 2015;21:202–212.
[35] Huang HY, Hopper AK. Multiple layers of stress-induced regulation in tRNA biology. Life (Basel). 2016;6(2):16.
[36] Gu C, Begley TJ, Dedon PC. tRNA modifications regulate translation during cellular stress. FEBS Lett. 2014;588:4287–4296.
[37] Deng W, Babu IR, Su D, et al. Trm9-catalyzed tRNA modifications regulate global protein expression by codon-biased translation. PLoS Genet. 2015;11:e1005706.
[38] Monaco PL, Marcel V, Diaz JJ, et al. 2′-O-methylation of ribosomal RNA: towards an epitranscriptomic control of translation? Biomolecules. Biomolecules. 2018;8(4):106.
[39] Erales J, Marchand V, Panthu B, et al. Evidence for rRNA 2′-O-methylation plasticity: control of intrinsic translational capabilities of human ribosomes. Proc Natl Acad Sci U S A. 2017;114:12934–12939.
[40] Penzo M, Rocchi L, Brugiere S, et al. Human ribosomes from cells with reduced dyskerin levels are intrinsically altered in translation. FASEB J. 2015;29:3472–3482.
[41] Michaud M, Cognat V, Duchene AM, et al. A global picture of tRNA genes in plant genomes. Plant J. 2011;66:80–93.
[42] Nedialkova DD, Leidel SA. Optimization of codon translation rates via tRNA modifications maintains proteome integrity. Cell. 2015;161:1606–1618.
[43] Carpentier MC, Deragon JM, Jean V, et al. Monitoring of XRN4 targets reveals the importance of cotranslational decay during arabidopsis development. Plant Physiol. 2020;184:1251–1262.
[44] Karlsborn T, Tukenmez H, Mahmud AK, et al. Elongator, a conserved complex required for wobble uridine modifications in eukaryotes. RNA Biol. 2014;11:1519–1528.
[45] Kalhor HR, Clarke S. Novel methyltransferase for modified uridine residues at the wobble position of tRNA. Mol Cell Biol. 2003;23:9283–9292.
[46] Dewez M, Bauer F, Dieu M, et al. The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. Proc Natl Acad Sci U S A. 2008;105:5459–5464.
[47] Abbassi NE, Biela A, Glatt S, et al. How elongator acetylates tRNA bases. Int J Mol Sci. 2020;21.
[48] Leihne V, Kirpekar F, Vagbo CB, et al. Roles of Trm9- and ALKBH8-like proteins in the formation of modified wobble uridines in Arabidopsis tRNA. Nucleic Acids Res. 2011;39:7688–7701.
[49] Walker J, Kwon SY, Badenhorst P, et al. Role of elongator subunit Elp3 in Drosophila melanogaster larval development and immunity. Genetics. 2011;187:1067–1075.
[50] Chen YT, Hims MM, Shetty RS, et al. Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. Mol Cell Biol. 2009;29:736–744.
[51] Mehlgarten C, Jablonowski D, Wrackmeyer U, et al. Elongator function in tRNA wobble uridine modification is conserved between yeast and plants. Mol Microbiol. 2010;76:1082–1094.
[52] Chen C, Tuck S, Bystrom AS. Defects in tRNA modification associated with neurological and developmental dysfunctions in Caenorhabditis elegans elongator mutants. PLoS Genet. 2009;5:e1000561.
[53] Silva KJP, Brunings AM, Pereira JA, et al. The Arabidopsis ELP3/ELO3 and ELP4/ELO1 genes enhance disease resistance in Fragaria vesca L. BMC Plant Biol. 2017;17:230.
[54] Pereira JA, Yu F, Zhang Y, et al. The arabidopsis elongator subunit ELP3 and ELP4 confer resistance to bacterial speck in tomato. Front Plant Sci. 2018;9:1066.
[55] Nakai Y, Horiguchi G, Iwabuchi K, et al. tRNA wobble modification affects leaf cell development in Arabidopsis thaliana. Plant Cell Physiol. 2019;60:2026–2039.
[56] John F, Philipp M, Leiber RM, et al. Ubiquitin-related modifiers of Arabidopsis thaliana influence root development. PLoS One. 2014;9:e86862.
[57] Pedrioli PG, Leidel S, Hofmann K. Urm1 at the crossroad of modifications. 'protein modifications: beyond the usual suspects' review series. EMBO Rep. 2008;9:1196–1202.
[58] Xu Y, Zhang L, Ou S, et al. Natural variations of SLG1 confer high-temperature tolerance in indica rice. Nat Commun. 2020;11:5441.
[59] Philipp M, John F, Ringli C. The cytosolic thiouridylase CTU2 of Arabidopsis thaliana is essential for posttranscriptional thiolation of tRNAs and influences root development. BMC Plant Biol. 2014;14:109.
[60] Leiber RM, John F, Verhertbruggen Y, et al. The TOR pathway modulates the structure of cell walls in Arabidopsis. Plant Cell. 2010;22:1898–1908.
[61] Tyagi K, Pedrioli PG. Protein degradation and dynamic tRNA thiolation fine-tune translation at elevated temperatures. Nucleic Acids Res. 2015;43:4701–4712.
[62] Fernandez-Vazquez J, Vargas-Perez I, Sanso M, et al. Modification of tRNA(Lys) UUU by elongator is essential for efficient translation of stress mRNAs. PLoS Genet. 2013;9:e1003647.
[63] Laxman S, Sutter BM, Wu X, et al. Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. Cell. 2013;154:416–429.
[64] Goehring AS, Rivers DM, Sprague GF Jr. Attachment of the ubiquitin-related protein Urm1p to the antioxidant protein Ahp1p. Eukaryot Cell. 2003;2:930–936.
[65] Chan TF, Carvalho J, Riles L, et al. A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). Proc Natl Acad Sci U S A. 2000;97:13227–13232.
[66] Otsubo Y, Kamada Y, Yamashita A. Novel links between TORC1 and traditional non-coding RNA, tRNA. Genes (Basel). 2020;11(9):956
[67] Yousef GM, Borgono CA, Michael IP, et al. Molecular cloning of a new gene which is differentially expressed in breast and prostate cancers. Tumour Biol. 2004;25:122–133.
[68] Simos G, Tekotte H, Grosjean H, et al. Nuclear pore proteins are involved in the biogenesis of functional tRNA. EMBO J. 1996;15:2270–2284.
[69] Khonsari B, Klassen R. Impact of Pus1 pseudouridine synthase on specific decoding events in Saccharomyces cerevisiae. Biomolecules. 2020;10(5):729
[70] Davis DR. Stabilization of RNA stacking by pseudouridine. Nucleic Acids Res. 1995;23:5020–5026.
[71] Hayashi S, Mori S, Suzuki T, et al. Impact of intron removal from tRNA genes on Saccharomyces cerevisiae. Nucleic Acids Res. 2019;47:5936–5949.
[72] Schwartz S, Bernstein DA, Mumbach MR, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell. 2014;159:148–162.
[73] Fergus C, Barnes D, Alqasem MA, et al. The queuine micronutrient: charting a course from microbe to man. Nutrients. 2015;7:2897–2929.
[74] Boland C, Hayes P, Santa-Maria I, et al. Queuosine formation in eukaryotic tRNA occurs via a mitochondria-localized heteromeric transglycosylase. J Biol Chem. 2009;284:18218–18227.
[75] Chen P, Jager G, Zheng B. Transfer RNA modifications and genes for modifying enzymes in Arabidopsis thaliana. BMC Plant Biol. 2010;10:201.
[76] Hayes P, Fergus C, Ghanim M, et al. Queuine micronutrient deficiency promotes warburg metabolism and reversal of the mitochondrial ATP synthase in hela cells. Nutrients. 2020;12(3):871

[77] Rakovich T, Boland C, Bernstein I, et al. Queuosine deficiency in eukaryotes compromises tyrosine production through increased tetrahydrobiopterin oxidation. J Biol Chem. 2011;286:19354–19363.
[78] Pathak C, Jaiswal YK, Vinayak M. Queuine promotes antioxidant defence system by activating cellular antioxidant enzyme activities in cancer. Biosci Rep. 2008;28:73–81.
[79] Pathak C, Jaiswal YK, Vinayak M. Queuine mediated inhibition in phosphorylation of tyrosine phosphoproteins in cancer. Mol Biol Rep. 2008;35:369–374.
[80] Beier H, Barciszewska M, Sickinger HD. The molecular basis for the differential translation of TMV RNA in tobacco protoplasts and wheat germ extracts. EMBO J. 1984;3:1091–1096.
[81] Muller M, Legrand C, Tuorto F, et al. Queuine links translational control in eukaryotes to a micronutrient from bacteria. Nucleic Acids Res. 2019;47:3711–3727.
[82] Zaborske JM, DuMont VL, Wallace EW, et al. A nutrient-driven tRNA modification alters translational fidelity and genome-wide protein coding across an animal genus. PLoS Biol. 2014;12: e1002015.
[83] Tuorto F, Legrand C, Cirzi C, et al. Queuosine-modified tRNAs confer nutritional control of protein translation. EMBO J. 2018;37:e99777.
[84] Muller M, Hartmann M, Schuster I, et al. Dynamic modulation of Dnmt2-dependent tRNA methylation by the micronutrient queuine. Nucleic Acids Res. 2015;43:10952–10962.
[85] Tuorto F, Herbst F, Alerasool N, et al. The tRNA methyltransferase Dnmt2 is required for accurate polypeptide synthesis during haematopoiesis. EMBO J. 2015;34:2350–2362.
[86] Schaefer M, Pollex T, Hanna K, et al. RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. Genes Dev. 2010;24:1590–1595.
[87] Chan CT, Pang YL, Deng W, et al. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. Nat Commun. 2012;3:937.
[88] Navarro IC, Tuorto F, Jordan D, et al. Translational adaptation to heat stress is mediated by RNA 5-methylcytosine in Caenorhabditis elegans. EMBO J (2021);40:e105496.
[89] Crick FH. Codon–anticodon pairing: the wobble hypothesis. J Mol Biol. 1966;19:548–555.
[90] Torres AG, Pineyro D, Rodriguez-Escriba M, et al. Inosine modifications in human tRNAs are incorporated at the precursor tRNA level. Nucleic Acids Res. 2015;43:5145–5157.
[91] Delannoy E, Le Ret M, Faivre-Nitschke E, et al. Arabidopsis tRNA adenosine deaminase arginine edits the wobble nucleotide of chloroplast tRNAArg(ACG) and is essential for efficient chloroplast translation. Plant Cell. 2009;21:2058–2071.
[92] Schweizer U, Bohleber S, Fradejas-Villar N. The modified base isopentenyladenosine and its derivatives in tRNA. RNA Biol. 2017;14:1197–1208.
[93] Rojas-Benitez D, Eggers C, Glavic A. Modulation of the proteostasis machinery to overcome stress caused by diminished levels of t6A-modified tRNAs in drosophila. Biomolecules : 2017; 7(1): 25
[94] Thiaville PC, Iwata-Reuyl D, de Crecy-Lagard V. Diversity of the biosynthesis pathway for threonylcarbamoyladenosine (t(6)A), a universal modification of tRNA. RNA Biol. 2014;11:1529–1539.
[95] Lin H, Miyauchi K, Harada T, et al. CO2-sensitive tRNA modification associated with human mitochondrial disease. Nat Commun. 2018;9:1875.
[96] Rojas-Benitez D, Thiaville PC, de Crecy-Lagard V, et al. The levels of a universally conserved tRNA modification regulate cell growth. J Biol Chem. 2015;290:18699–18707.
[97] Laten H, Gorman J, Bock RM. Isopentenyladenosine deficient tRNA from an antisuppressor mutant of Saccharomyces cerevisiae. Nucleic Acids Res. 1978;5:4329–4342.
[98] Lamichhane TN, Arimbasseri AG, Rijal K, et al. Lack of tRNA-i6A modification causes mitochondrial-like metabolic deficiency in S. pombe by limiting activity of cytosolic tRNATyr, not mito-tRNA. RNA. 2016;22:583–596.
[99] Lamichhane TN, Blewett NH, Crawford AK, et al. Lack of tRNA modification isopentenyl-A37 alters mRNA decoding and causes metabolic deficiencies in fission yeast. Mol Cell Biol. 2013;33:2918–2929.
[100] Lemieux J, Lakowski B, Webb A, et al. Regulation of physiological rates in Caenorhabditis elegans by a tRNA-modifying enzyme in the mitochondria. Genetics. 2001;159:147–157.
[101] Miyawaki K, Tarkowski P, Matsumoto-Kitano M, et al. Roles of Arabidopsis ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. Proc Natl Acad Sci U S A. 2006;103:16598–16603.
[102] Schafer M, Brutting C, Meza-Canales ID, et al. The role of cis-zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. J Exp Bot. 2015;66:4873–4884.
[103] Liu Y, Zhang M, Meng Z, et al. Research progress on the roles of cytokinin in plant response to stress. Int J Mol Sci. 2020; 21 (18):6574.
[104] Hai NN, Chuong NN, Tu NHC, et al. Role and regulation of cytokinins in plant response to drought stress. Plants (Basel) 2020; 9(4):422.
[105] Helm M, Alfonzo JD. Posttranscriptional RNA MODIFICATIONS: playing metabolic games in a cell's chemical Legoland. Chem Biol. 2014;21:174–185.
[106] Ohira T, Suzuki T. Retrograde nuclear import of tRNA precursors is required for modified base biogenesis in yeast. Proc Natl Acad Sci U S A. 2011;108:10502–10507.
[107] Urbonavicius JD L, Armengaud J, Grosjean H. Deciphering the complex enzymatic pathway for biosynthesis of wyosine derivates in anticodon of tRNAPhe. In: Grosjean H, editor. DNA and RNA modification enzymes: structure, mechanism, function and evolution: landes Bioscience, Austin, TX. 2009. p. 423–435.
[108] Hienzsch A, Deiml C, Reiter V, et al. Total synthesis of the hypermodified RNA bases wybutosine and hydroxywybutosine and their quantification together with other modified RNA bases in plant materials. Chemistry. 2013;19:4244–4248.
[109] Carlson BA, Lee BJ, Hatfield DL. Ribosomal frameshifting in response to hypomodified tRNAs in Xenopus oocytes. Biochem Biophys Res Commun. 2008;375:86–90.
[110] Waas WF, Druzina Z, Hanan M, et al. Role of a tRNA base modification and its precursors in frameshifting in eukaryotes. J Biol Chem. 2007;282:26026–26034.
[111] Zhou W, Karcher D, Bock R. Importance of adenosine-to-inosine editing adjacent to the anticodon in an Arabidopsis alanine tRNA under environmental stress. Nucleic Acids Res. 2013;41:3362–3372.
[112] Guo Q, Ng PQ, Shi S, et al. Arabidopsis TRM5 encodes a nuclear-localised bifunctional tRNA guanine and inosine-N1-methyltransferase that is important for growth. PLoS One. 2019;14:e0225064.
[113] Jin X, Lv Z, Gao J, et al. AtTrm5a catalyses 1-methylguanosine and 1-methylinosine formation on tRNAs and is important for vegetative and reproductive growth in Arabidopsis thaliana. Nucleic Acids Res. 2019;47:883–898.
[114] Patil A, Dyavaiah M, Joseph F, et al. Increased tRNA modification and gene-specific codon usage regulate cell cycle progression during the DNA damage response. Cell Cycle. 2012;11:3656–3665.
[115] Ignatova VV, Kaiser S, Ho JSY, et al. METTL6 is a tRNA m(3)C methyltransferase that regulates pluripotency and tumor cell growth. Sci Adv. 2020;6:eaaz4551.
[116] Noma A, Yi S, Katoh T, et al. Actin-binding protein ABP140 is a methyltransferase for 3-methylcytidine at position 32 of tRNAs in Saccharomyces cerevisiae. RNA. 2011;17:1111–1119.
[117] Xu L, Liu X, Sheng N, et al. Three distinct 3-methylcytidine (m(3) C) methyltransferases modify tRNA and mRNA in mice and humans. J Biol Chem. 2017;292:14695–14703.
[118] Arimbasseri AG, Iben J, Wei FY, et al. Evolving specificity of tRNA 3-methyl-cytidine-32 (m3C32) modification: a subset of tRNAsSer requires N6-isopentenylation of A37. RNA. 2016;22:1400–1410.
[119] Rubio MA, Gaston KW, McKenney KM, et al. Editing and methylation at a single site by functionally interdependent activities. Nature. 2017;542:494–497.

[120] Tang J, Jia P, Xin P, et al. The Arabidopsis TRM61/TRM6 complex is a bona fide tRNA N1-methyladenosine methyltransferase. J Exp Bot. 2020;71:3024–3036.
[121] Zhang C, Jia G. Reversible RNA modification N(1)-methyladenosine (m(1)A) in mRNA and tRNA. Genomics Proteomics Bioinformatics. 2018;16:155–161.
[122] Liu F, Clark W, Luo G, et al. ALKBH1-mediated tRNA demethylation regulates translation. Cell. 2016;167:1897.
[123] Ontiveros RJ, Shen H, Stoute J, et al. Coordination of mRNA and tRNA methylations by TRMT10A. Proc Natl Acad Sci U S A. 2020;117:7782–7791.
[124] Vilardo E, Amman F, Toth U, et al. Functional characterization of the human tRNA methyltransferases TRMT10A and TRMT10B. Nucleic Acids Res. 2020;48:6157–6169.
[125] Lin S, Liu Q, Lelyveld VS, et al. Mettl1/Wdr4-mediated m(7)G tRNA methylome is required for normal mRNA translation and embryonic stem cell self-renewal and differentiation. Mol Cell. 2018;71:244–55 e5.
[126] Alexandrov A, Martzen MR, Phizicky EM. Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA. RNA. 2002;8:1253–1266.
[127] Dalluge JJ, Hamamoto T, Horikoshi K, et al. Posttranscriptional modification of tRNA in psychrophilic bacteria. J Bacteriol. 1997;179:1918–1923.
[128] Arnez JG, Steitz TA. Crystal structure of unmodified tRNA(Gln) complexed with glutaminyl-tRNA synthetase and ATP suggests a possible role for pseudo-uridines in stabilization of RNA structure. Biochemistry. 1994;33:7560–7567.
[129] Penzo M, Guerrieri AN, Zacchini F, et al. RNA pseudouridylation in physiology and medicine: for better and for worse. Genes (Basel). 2017; 8(11):301.
[130] Megel C, Morelle G, Lalande S, et al. Surveillance and cleavage of eukaryotic tRNAs. Int J Mol Sci. 2015;16:1873–1893.
[131] Wilusz JE. Controlling translation via modulation of tRNA levels. Wiley Interdiscip Rev RNA. 2015;6:453–470.
[132] Alexandrov A, Chernyakov I, Gu W, et al. Rapid tRNA decay can result from lack of nonessential modifications. Mol Cell. 2006;21:87–96.
[133] Chernyakov I, Whipple JM, Kotelawala L, et al. Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5′-3′ exonucleases Rat1 and Xrn1. Genes Dev. 2008;22:1369–1380.
[134] Lyons SM, Fay MM, Ivanov P. The role of RNA modifications in the regulation of tRNA cleavage. FEBS Lett. 2018;592:2828–2844.
[135] Anderson J, Phan L, Cuesta R, et al. The essential Gcd10p-Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA. Genes Dev. 1998;12:3650–3662.
[136] Ivanov P, Emara MM, Villen J, et al. Angiogenin-induced tRNA fragments inhibit translation initiation. Mol Cell. 2011;43:613–623.
[137] Gebetsberger J, Wyss L, Mleczko AM, et al. A tRNA-derived fragment competes with mRNA for ribosome binding and regulates translation during stress. RNA Biol. 2017;14:1364–1373.
[138] Wang Q, Lee I, Ren J, et al. Identification and functional characterization of tRNA-derived RNA fragments (tRFs) in respiratory syncytial virus infection. Mol Ther. 2013;21:368–379.
[139] Martinez G, Choudury SG, Slotkin RK. tRNA-derived small RNAs target transposable element transcripts. Nucleic Acids Res. 2017;45:5142–5152.
[140] Fu H, Feng J, Liu Q, et al. Stress induces tRNA cleavage by angiogenin in mammalian cells. FEBS Lett. 2009;583:437–442.
[141] Yamasaki S, Ivanov P, Hu GF, et al. Angiogenin cleaves tRNA and promotes stress-induced translational repression. J Cell Biol. 2009;185:35–42.
[142] Donovan J, Rath S, Kolet-Mandrikov D, et al. RNase L-driven arrest of protein synthesis in the dsRNA response without degradation of translation machinery. RNA. 2017;23:1660–1671.
[143] Lu J, Esberg A, Huang B, et al. Kluyveromyces lactis gamma-toxin, a ribonuclease that recognizes the anticodon stem loop of tRNA. Nucleic Acids Res. 2008;36:1072–1080.
[144] Thompson DM, Parker R. The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in Saccharomyces cerevisiae. J Cell Biol. 2009;185:43–50.
[145] Megel C, Hummel G, Lalande S, et al. Plant RNases T2, but not Dicer-like proteins, are major players of tRNA-derived fragments biogenesis. Nucleic Acids Res. 2019;47:941–952.
[146] Guzzi N, Ciesla M, Ngoc PCT, et al. Pseudouridylation of tRNA-derived fragments steers translational control in stem cells. Cell. 2018;173:1204–16 e26.
[147] Guo H. Specialized ribosomes and the control of translation. Biochem Soc Trans. 2018;46:855–869.
[148] Ferretti MB, Karbstein K. Does functional specialization of ribosomes really exist? RNA. 2019;25:521–538.
[149] Schosserer M, Minois N, Angerer TB, et al. Methylation of ribosomal RNA by NSUN5 is a conserved mechanism modulating organismal lifespan. Nat Commun. 2015;6:6158.
[150] Sharma S, Marchand V, Motorin Y, et al. Identification of sites of 2′-O-methylation vulnerability in human ribosomal RNAs by systematic mapping. Sci Rep. 2017;7:11490.
[151] Mei YP, Liao JP, Shen J, et al. Small nucleolar RNA 42 acts as an oncogene in lung tumorigenesis. Oncogene. 2012;31:2794–2804.
[152] Yoshida K, Toden S, Weng W, et al. SNORA21 - an oncogenic small nucleolar RNA, with a prognostic biomarker potential in human colorectal cancer. EBioMedicine. 2017;22:68–77.
[153] Yoon A, Peng G, Brandenburger Y, et al. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. Science. 2006;312:902–906.
[154] Rocchi L, Pacilli A, Sethi R, et al. Dyskerin depletion increases VEGF mRNA internal ribosome entry site-mediated translation. Nucleic Acids Res. 2013;41:8308–8318.
[155] Weingarten-Gabbay S, Elias-Kirma S, Nir R, et al. Comparative genetics. Systematic discovery of cap-independent translation sequences in human and viral genomes. Science 2016; 351 (6270):aad4939.
[156] Liang XH, Liu Q, Fournier MJ. Loss of rRNA modifications in the decoding center of the ribosome impairs translation and strongly delays pre-rRNA processing. RNA. 2009;15:1716–1728.
[157] Jindal S, Ghosh A, Ismail A, et al. Role of the uS9/yS16 C-terminal tail in translation initiation and elongation in Saccharomyces cerevisiae. Nucleic Acids Res. 2019;47:806–823.
[158] Jiang Z, Li S, Han MJ, et al. High expression of NSUN5 promotes cell proliferation via cell cycle regulation in colorectal cancer. Am J Transl Res. 2020;12:3858–3870.
[159] Janin M, Ortiz-Barahona V, de Moura MC, et al. Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. Acta Neuropathol. 2019;138:1053–1074.
[160] Heissenberger C, Liendl L, Nagelreiter F, et al. Loss of the ribosomal RNA methyltransferase NSUN5 impairs global protein synthesis and normal growth. Nucleic Acids Res. 2019;47:11807–11825.
[161] Wang H, Wang L, Wang Z, et al. The nucleolar protein NOP2 is required for nucleolar maturation and ribosome biogenesis during preimplantation development in mammals. FASEB J. 2020;34:2715–2729.
[162] Hong B, Brockenbrough JS, Wu P, et al. Nop2p is required for pre-rRNA processing and 60S ribosome subunit synthesis in yeast. Mol Cell Biol. 1997;17:378–388.
[163] Bourgeois G, Ney M, Gaspar I, et al. Eukaryotic rRNA modification by yeast 5-methylcytosine-methyltransferases and human proliferation-associated antigen p120. PLoS One. 2015;10: e0133321.
[164] Heissenberger C, Rollins JA, Krammer TL, et al. The ribosomal RNA m(5)C methyltransferase NSUN-1 modulates healthspan and oogenesis in Caenorhabditis elegans. eLife 2020;9:e56205.
[165] Rong B, Zhang Q, Wan J, et al. Ribosome 18S m(6)A methyltransferase METTL5 promotes translation initiation and breast cancer cell growth. Cell Rep. 2020;33:108544.
[166] Liberman N, O'Brown ZK, Earl AS, et al. N6-adenosine methylation of ribosomal RNA affects lipid oxidation and stress resistance. Sci Adv. 2020;6:eaaz4370.

[167] Ma H, Wang X, Cai J, et al. N(6-)Methyladenosine methyltransferase ZCCHC4 mediates ribosomal RNA methylation. Nat Chem Biol. 2019;15:88–94.

[168] Ren W, Lu J, Huang M, et al. Structure and regulation of ZCCHC4 in m(6)A-methylation of 28S rRNA. Nat Commun. 2019;10:5042.

[169] Sharma S, Hartmann JD, Watzinger P, et al. A single N(1)-methyladenosine on the large ribosomal subunit rRNA impacts locally its structure and the translation of key metabolic enzymes. Sci Rep. 2018;8:11904.

[170] Waku T, Nakajima Y, Yokoyama W, et al. NML-mediated rRNA base methylation links ribosomal subunit formation to cell proliferation in a p53-dependent manner. J Cell Sci. 2016;129:2382–2393.

[171] Yokoyama W, Hirota K, Wan H, et al. rRNA adenine methylation requires T07A9.8 gene as rram-1 in Caenorhabditis elegans. J Biochem. 2018;163:465–474.

[172] Baxter M, Voronkov M, Poolman T, et al. Cardiac mitochondrial function depends on BUD23 mediated ribosome programming. eLife 2020;9:e50705.

[173] White J, Li Z, Sardana R, et al. Bud23 methylates G1575 of 18S rRNA and is required for efficient nuclear export of pre-40S subunits. Mol Cell Biol. 2008;28:3151–3161.

[174] Haag S, Kretschmer J, Bohnsack MT. WBSCR22/Merm1 is required for late nuclear pre-ribosomal RNA processing and mediates N7-methylation of G1639 in human 18S rRNA. RNA. 2015;21:180–187.