



Review

Anticodon stem-loop tRNA modifications influence codon decoding and frame maintenance during translation

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ARTICLE INFO

Keywords:

RNA modification
tRNA
Decoding
Frameshifting
Translation

ABSTRACT

RNAs are central to protein synthesis, with ribosomal RNA, transfer RNAs and messenger RNAs comprising the core components of the translation machinery. In addition to the four canonical bases (uracil, cytosine, adenine, and guanine) these RNAs contain an array of enzymatically incorporated chemical modifications. Transfer RNAs (tRNAs) are responsible for ferrying amino acids to the ribosome, and are among the most abundant and highly modified RNAs in the cell across all domains of life. On average, tRNA molecules contain 13 post-transcriptionally modified nucleosides that stabilize their structure and enhance function. There is an extensive chemical diversity of tRNA modifications, with over 90 distinct varieties of modifications reported within tRNA sequences. Some modifications are crucial for tRNAs to adopt their L-shaped tertiary structure, while others promote tRNA interactions with components of the protein synthesis machinery. In particular, modifications in the anticodon stem-loop (ASL), located near the site of tRNA:mRNA interaction, can play key roles in ensuring protein homeostasis and accurate translation. There is an abundance of evidence indicating the importance of ASL modifications for cellular health, and in vitro biochemical and biophysical studies suggest that individual ASL modifications can differentially influence discrete steps in the translation pathway. This review examines the molecular level consequences of tRNA ASL modifications in mRNA codon recognition and reading frame maintenance to ensure the rapid and accurate translation of proteins.

1. Introduction

Transfer RNAs (tRNAs) are key adaptor molecules in protein synthesis, shuttling amino acids into the ribosome in an order dictated by the genetic code. The ability of tRNAs to “read” messenger RNA (mRNA) blueprints during translation is essential for ensuring that amino acids are linked together in the correct order to generate functional proteins. One feature that distinguishes tRNAs from other types of RNA molecules is the large number and variety of post-transcriptional modifications enzymatically incorporated into their scaffolds. Typically, 10–20% of tRNA nucleobases are modified, and the chemical diversity of these modifications range broadly in complexity from methyl additions (e.g.

1-methyladenosine) incorporated by a single enzyme, to complex ring additions requiring multiple enzymes to install (e.g. Wybutosine (yW) is added in a 7-step enzymatic pathway) [1]. Given the substantial effort that biology makes to incorporate and maintain modifications in all organisms, it is unsurprising that post-transcriptional modifications play essential roles in tRNA function – affecting their structure, stability, aminoacylation, and mRNA decoding capabilities [2].

The significance of modifications in tRNAs is underscored by widespread observations that alterations in tRNA modification status drastically impact protein homeostasis [3,4]. This is exemplified during cellular stress, when changes to the overall modification landscape of tRNAs can reprogram tRNAs to control selective translation, as well as

Abbreviations: ASL, Anticodon stem loop; aa-tRNAs, amino-acyl tRNAs; tRNAs, transfer RNAs; mRNA, messenger RNA; s²C, 2-thiocytidine; ψ , pseudouridine; ncm⁵U, 5-carbamoylmethyluridine; ncm⁵s², 5-carbamoylmethyl-2-thiouridine; mcm⁵U, 5-methoxycarbonylmethyl-uridine; mcm⁵s²U, 5-methoxycarbonylmethyl-2-thiouridine; mnm⁵U, 5-methylaminomethyluridine; mnm⁵s²U, 5-methylaminomethyl-2-thiouridine; cmnm⁵s²U, 5-carboxymethylaminomethyl-2-thiouridine; τ m⁵U, 5-taurinomethyluridine; xm⁵U, class of position 5 uridine modifications [τ m⁵U, ncm⁵U, mcm⁵U, mcm⁵s²U, mnm⁵U, mnm⁵s²U, cmnm⁵s²U] –; cmo⁵U, uridine 5-oxy-acetic acid; I, Inosine; f²C, 5-formylcytidine; k²C, lysidine; Q, queuosine; t⁶A, N6-threonylcarbamoyladenine; ct⁶A, cyclic N6-threonylcarbamoyladenine; ms²t⁶A, 2-methylthio-N6-threonylcarbamoyladenine; i⁶A, N6-isopentenyladenine; ms²i⁶A, 2-methylthio-N6-isopentenyladenine; ms²io⁶A, 2-methylthio-N6-(cis-hydroxyisopentenyl) adenine; m¹G, 1-methylguanine; imG, wyosine; yW, wybutosine.

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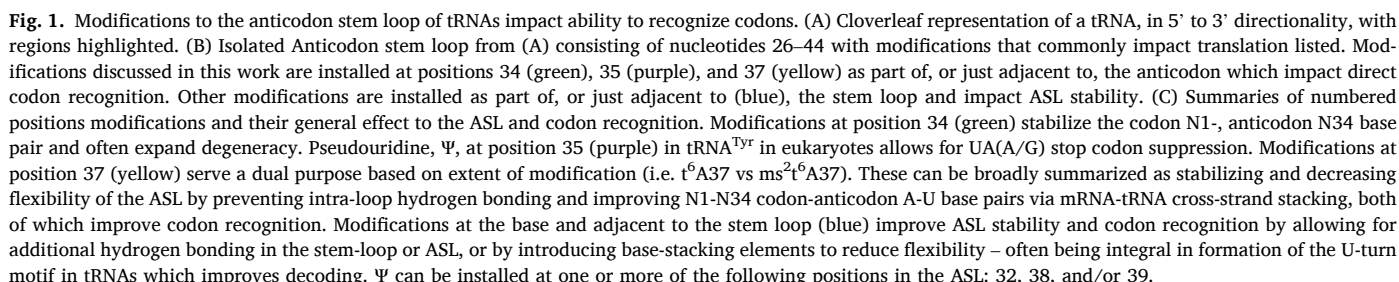
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<https://doi.org/10.1016/j.semcdb.2023.06.003>

Received 25 May 2022; Received in revised form 6 June 2023; Accepted 7 June 2023

Available online 28 June 2023

1084-9521/© 2023 Published by Elsevier Ltd.



The ASL contains the highest density of modifications within tRNA sequences. Over 20 varieties of modifications have been reported within tRNA ASLs, and tRNA positions 32, 34, 37, 38, and 39 are most commonly modified (Fig. 1). These modifications typically enhance the loop stability and mRNA recognition, though their identity and role at each ASL position varies between organisms and tRNA isoacceptors (Fig. 1B) [2,14,15]. Positions 34 and 37 tend to have the most chemically complex modifications on tRNAs (and more broadly within RNA biology), and generally enhance crucial steps in the translation elongation pathway. The significance of these modifications is underscored by the observation that some of the enzymes that incorporate them are essential for cell viability – as illustrated by the requirement of human cells for the tRNA-specific adenosine deaminase 2 (ADAT2)-ADAT3 complex that catalyzes the conversion of adenosine to inosine at position 34 [9]. However, while genetic studies reveal the importance of many ASL modifications to cellular and human health, our understanding of

precisely how individual modifications assert their function rely on molecular-level biochemical and/or structural investigations. Below we examine how modifications in tRNA ASL regions impact amino acid addition, codon recognition, and mRNA:tRNA interactions, with an emphasis on their mechanism of action.

2.1. Position 34 modifications enhance (and sometimes expand) codon decoding by tRNAs

Position 34 in tRNAs base pairs with the 3rd nucleotide, or “wobble position” in an mRNA codon (Fig. 1 C). A wider variety of interactions are permitted between nucleotides at tRNA position 34 and the wobble base, including several types of non-Watson–Crick base pairs. While position 34 is not always modified, non-canonical wobble:tRNA interactions are largely facilitated by modifications to all four nucleotides at tRNA position 34. Uracil bases at this position possess some of the largest, most diverse modifications and are often essential for mRNA decoding. For example, at U34, tRNAs are commonly post-transcriptionally modified with xm⁵: 5-methylaminomethyl [mnm⁵] and 5-methylaminomethyl-2-thio [mnm⁵s²] in bacteria, and 5-carbamoylmethyl [ncm⁵], 5-carbamoylmethyl-2-thio [ncm⁵s²], 5-methoxycarbonylmethyl [mcm⁵], 5-methoxycarbonylmethyl-2-thio [mcm⁵s²] in yeast and higher eukaryotes [16,17]. The xm⁵ class of modifications is important for tRNA association and accommodation in the ribosome A site, and cells lacking U34 modifications become dysfunctional as a consequence of changes in mRNA decoding rates that alter protein homeostasis [10,18,19]. *In vitro* and cell-based studies of tRNA^{Lys(UUU)}, tRNA^{Gln(UUG)} and tRNA^{Glu(UUC)} demonstrate that mcm⁵ and s² at U34 stabilize tRNA binding and are indispensable for efficient translation on mRNAs enriched with AAA, CAA, or GAA codons [19–22]. Although mcm⁵, mcm⁵s², or ncm⁵s² promote the recognition of codons ending in A or G, these modifications do not appear to be required (at least in yeast) for U-G wobble decoding of the AAG, CAG, and GAG codons synonymous to AAA, CAA and GAA [22–24].

High resolution NMR and X-ray structural studies support biochemical and cellular findings that tRNA^{Lys(UUU)} xm⁵s²U34 modifications increase the ability of human and *E. coli* tRNA^{Lys(UUU)} to decode both AAA and AAG. In humans, tRNA^{Lys(UUU)} mcm⁵s² undergoes a tautomerization to form a U-G Watson–Crick like base-pair, while the mnm⁵s² in *E. coli* tRNA^{Lys(UUU)} similarly adopts a zwitterionic form [25–27]. The s² modification appears to be particularly important in tRNA^{Lys} decoding as it is involved in hydrogen bonding when pairing with G (in AAG codon), and increases binding affinity of tRNA^{Lys} when pairing with A (in AAA codon) [27–29]. In addition, kinetic studies of the translation pathway indicate that tRNA^{Lys(UUU)} possessing hypo-modified mcm⁵U34 (lacking s²) have slower EF-Tu rearrangement and P_i release following GTP hydrolysis (~6-fold), exhibit faster dissociation between the codon-recognition complex (~5-fold), and increase the rate constant for tRNA^{Lys(UUU)} rejection (~3-fold). Furthermore, s²-depletion from tRNA^{Lys(UUU)} also modestly impedes ribosome translocation when compared to the natively mcm⁵s²U34 modified tRNA^{Lys(UUU)} [29,30]. Similarly, the s² modification within *E. coli* tRNA^{Gln(UUG)} mnm⁵s²U34 enhances binding affinity to cognate codons (CAA and CAG), and increases the rate constant for GTP hydrolysis by EF-Tu during translation (~5-fold) [31]. Furthermore, xm⁵s²U34 modifications are essential in frameshifting/frame maintenance (see Section 2.1).

U34 modifications beyond xm⁵U have similar effects, enhancing weaker mRNA:tRNA basepairing interactions to allow for expanded codon decoding by a single tRNA species. Examples include one of the most prevalent U34 modifications in tRNA, cmo⁵U34, and the mitochondrial *E. coli* tRNA^{Leu(UUR)} tm⁵U34 modification. Mitochondrial *E. coli* tRNA^{Leu(UUR)} reads both UUA and UUG codons. UUG (but not UUA) decoding strictly requires the tm⁵U34 modification because it enables the tRNA to form a non-wobble Watson–Crick like U-G base pair [32–34]. cmo⁵U34 also facilitates tRNA interactions with a wide array of codons, as highlighted by the ability of *E. coli* and *S. typhirium* tRNA

species possessing cmo⁵U34 to recognize all four codons in their four-fold degenerate codon boxes (tRNA^{Ala}, tRNA^{Ser}, tRNA^{Thr}, tRNA^{Pro}, tRNA^{Val}, with tRNA^{Leu} having six codon degeneracy), while tRNAs from other species lacking the modification do not [35,36]. *In vitro* studies in *E. coli* demonstrate the tRNA^{Ala}_{1B}(CGU) cmo⁵U34 modification permits the efficient recognition of both the cognate codon (GCA) and non-cognate Ala (GCG) codons, with the U-G pairing treated as an almost-correct base-pair versus a mismatch [28,37,38]. NMR studies further reveal that cmo⁵U34 is important to pre-order anticodon stem loops, promoting the binding of *E. coli* tRNA^{Val3}(UAC) to all four valine codons (instead of one codon, like the un-modified tRNA^{Val3}(UAC)), further suggesting that cmo⁵U34 is important for both stabilizing and expanding codon reading [39].

Non-uridine modifications at position 34 can also act to expand the genetic code. The 5-formylcytidine (f⁵C34) modification in human mitochondrial, but not cytosolic, tRNA^{Met(CAU)} (hmt-tRNA^{Met(CAU)}) is a classic example of this [40]. In the mitochondria, hmtRNA^{Met(CAU)} decodes both initiator AUG codons, and incorporates Met amino acids onto the universal Ile codon, AUA, during elongation with high efficiency; 80% of Met residues installed during elongation occur on AUA codons [41]. The ability of f⁵C34-containing hmtRNA to decode codons at both the ribosome A- and P-sites is accomplished through a prototropic tautomerization. This tautomerization permits the formation of Watson–Crick f⁵C-A base-pair, along with the ASL U-turn needed for A-site binding on AUA codons [42]. Notably, *E. coli* tRNA^{Ile(CAU)} possesses a modified lysidine (k²C34) at the same position that seemingly functions in the opposite manner of hmtRNA^{Met(CAU)} f⁵C34, enhancing Ile-decoding AUA and restricting AUG decoding [43,44]. In conjunction with t⁶A37, k²C34 facilitates base stacking to enhance ASL stability and shifts codon recognition from AUG to AUA [43,45]. The opposing impacts of f⁵C and k²C modifications at position 34 on codon recognition demonstrate the significance tRNA ASL nucleotide variations can have on adaption and the continued evolution of the genetic code.

Inosine (I), formed by the deamination of adenosine, was the first ASL modification discovered. The addition of inosine into tRNA at position 34 expands codon degeneracy by enabling tRNAs to productively decode three different nucleosides (A, U, and C) in an mRNA codon wobble position [46]. While inosine is widely considered a standard Crick nucleotide, it has only been reported in two *E. coli* tRNAs (tRNA^{Arg}₁(ACG) and tRNA^{Arg}₂(ACG)), and 8 cytosolic eukaryotic tRNAs [1,47,48]. When harboring just a single I34 modification, both *E. coli* tRNA^{Arg}(ACG) isoacceptors decode all three synonymous codons for arginine (CGU, CGA, and CGG), while their unmodified versions can only bind CGU [49]. Notably, tRNA^{Arg}₁(ACG) and tRNA^{Arg}₂(ACG) possess additional modifications at s²C32 and m²A37, respectively, within their ASLs, which drive them to prefer binding either CGU or CGC binding, and drastically reduce CGA decoding [13,49,50]. The combinatorial effect of modifications may help to explain codon bias against CGA in some organisms' mRNAs as well as begin to highlight the interplay that tRNA modifications have in the evolution of genetic code degeneracy [51,52]. When combined, structural and biochemical studies suggest that modifications, especially those at U34, are imperative in proper ASL formation and accurate decoding of mRNA (Fig. 1 C).

2.2. Position 37 modifications stabilize codon:anticodon interactions

Nucleotides at position 37 of tRNAs are adjacent to the 3' of the anticodon sequence, and not directly involved in making mRNA:tRNA hydrogen bonding interactions (Fig. 1). Nonetheless, these nucleotides, which are universally purines, are often modified and can modulate ASL structure to stabilize codon:anticodon interactions. N⁶-threonylcarbamoyladenosine (t⁶A) is among the modifications most commonly incorporated into A37 of tRNAs decoding ANN codons in all domains of life. t⁶A promotes tRNA binding and decoding of codons in the ribosome A site, as well as helps to maintain efficient translocation and the ribosome reading frame [53,54]. This is accomplished through a network of

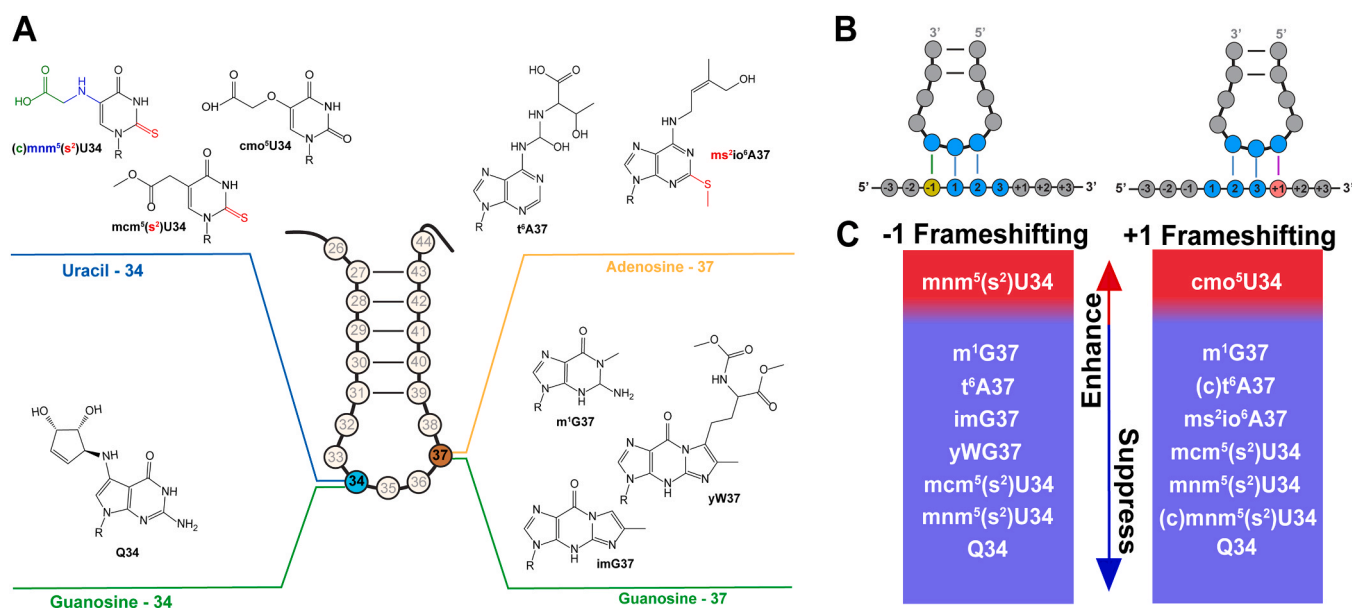


Fig. 2. Modifications at positions 34 and 37 are important in modulating -1 and +1 frameshifts (A) Isolated anticodon stem loop with nucleobase modifications involved in enhancing or suppressing frameshifting when installed at positions 34 or 37. Colored sections of nucleobases and their abbreviations indicate that various levels of complexity of that modification are involved in directing frameshifting events. (i.e. (c)mnm⁵(s²)U34 has three possible modification states which influence frameshifting - green: (c)mnm⁵(s²)U34, red: mnm⁵(s²)U34, blue: mnm⁵U34 - as shown in (B). (B) Visualization of basepairing between codon and anti-codon positions during -1 and +1 frameshifting. (C) Modifications at position 34 or 37 that enhance or suppress frameshifting. The modification mnm⁵(s²)U34 can enhance or suppress -1 frameshifting depending on the codon context in bacteria.

intra-ASL hydrogen bonding interactions that enhance base-stacking and facilitate tRNA:mRNA associations [2,55]. t⁶A is incorporated alone or in conjunction with additional chemical moieties, as is the case in the human tRNA^{Lys(UUU)} which contains an ms² group (ms²t⁶A37) [56]. In either form (t⁶A or ms²t⁶A), this modification stabilizes A-U basepairs formed between the mRNA and tRNA (A1:U36 or U1:A36) in the A site by stacking over the codon:anticodon basepair. The stacking propagates up the 3' side of the ASL, and promotes an initial cross-strand stack that increases ASL flexibility. It should be noted that mnm⁵s²U34 (or mcm⁵s² in eukaryotes) is also required for the correct decoding of AAA and AAG lysine codons, as binding is weaker with the UUU anticodon when either modification is absent [25,57,58]. The ms² modification is not present on all t⁶A37 modifications in tRNAs, however it appears to improve decoding of AAG by tRNA^{Lys(UUU)} through dehydration of the ASL as well as the codon-anticodon interaction when it is installed [27,59,60]. In *E. coli* and several yeast species, t⁶A37 can also be further cyclized to an oxazolone ring (ct⁶A37) that ensures proper decoding by generating an additional hydrogen-bond with an A1 codon nucleotide in addition to the stacking effect the modification provides [61,62]. While t⁶A exists in all organisms and the enzymes that incorporate it are essential in many bacteria and eukaryotes, the orthologs function in distinct complexes and it is unclear how cells have evolved divergent strategies to maintain the modification [58,63].

Isopentyladenosine (i⁶A) and the (ms²)i⁶A modification are also frequently incorporated in tRNAs at A37. Much like t⁶A, i⁶A modifications stabilize U1:A36 base pairing during UNN codon decoding [64]. Lack of (ms²)i⁶A in *E. coli* tRNA^{Phe(GAA)}, tRNA^{Leu(UUR)}, tRNA^{Cys(GCA)} and tRNA^{Tyr(GUA)} decreases the ability of tRNAs to recognize their cognate codons [65–68]. In eukaryotes (ms²)i⁶A37 appears to ensure both accurate decoding in the manners detailed above, as well as the efficiency of suppressor tRNAs that decode the UGA stop codon [69,70]. Collectively, (ms²)t⁶A37 and (ms²)i⁶A37 help to maintain ASL structure by preventing hydrogen bonding between tRNA positions U33 and A37 and stabilizing the adjacent A-U Watson-Crick base pairs [2].

The other purine, guanosine, is commonly modified to N1-methylguanosine (m¹G), wyosine (imG) and wyosine derivatives at position 37 in tRNAs. These modifications facilitate proper decoding by

maintaining mRNA:tRNA interactions in the both the ribosome A and P sites [71–75]. m¹G is especially important for tRNA^{Pro}, where it stabilizes tRNA:mRNA interactions to increase the speed of peptide bond formation and modulate ribosome frameshifting [71–74] (see Section 2.2). The more chemically complicated wyosine modification enhances base stacking with adjacent bases to reduce ASL flexibility, further supporting the role that modification to purines at position 37 in tRNAs have in the pre-structuring and decoding capability of the ASL (Fig. 1 C).

2.3. Modifications at further positions within the ASL make diverse contributions to tRNA maturation and function

Direct structural and biochemical investigations of ASL modifications at tRNA positions beyond 34 and 37 remain limited, though interest in them is growing. Modifications at these positions can act synergistically to impact decoding (e.g. s²C32 and I34 of tRNA^{ACG}) (see Section 1.1)), direct the efficient installation of other nearby modifications (e.g. yeast tRNA^{Ser} where i⁶A37 appears as a pre- or co-requisite of successful m³C32 modification) and influence protein homeostasis [76–78]. Currently, pseudouridine (Ψ), an isomer of uridine, is among the most well studied modifications within tRNA ASLs. It can be incorporated throughout the ASL, and regardless of its location Ψ appears to be important for pre-structuring the ASL to enhance tRNA decoding. Indeed, Ψ modifications frequently incorporated into *E. coli* and eukaryotic tRNAs at positions U32, U38 and U39 are involved in maintaining proper intra-ASL base-pairing and structuring of the stem-loop (Fig. 1 C) [79,80]. For example, on *E. coli* tRNA^{Phe(GAA)} Ψ39 base-pairs with A31, strengthening the stability of stem-loop, while Ψ32 forms a bifurcated hydrogen-bond with A38 to stabilize the U-turn motif and open-loop structure required for efficient decoding at the ribosomal A-site [27,81–83]. More directly involved in codon recognition, Ψ35 in the tRNA^{Tyr(GΨA)} of multiple organisms is indispensable for both UAA and UAG stop codon suppression [84]. Strikingly, this modification does not change normal UAU and UAC decoding by tRNA^{Tyr(GΨA)}, suggesting that the purpose of Ψ35 is primarily to expand the genetic code. Similarly, Ψ35 in tRNA^{Asn(GΨU)} decodes a near cognate lysine codon (AAA) but does not affect normal asparagine decoding (AAC/U) (Fig. 1 C) [69,

Table 1

Summary of tRNA modifications in the anticodon stem loop of tRNAs and their impact on translation.

ASL Position	Modification	Enzyme(s)	Reported Roles in Frame Maintenance and/or Translation	References
32	s ² C	IscS ^a , TtcA ^a	Prevents I34 wobble with adenosine and limits CGA decoding of tRNA ^{Arg} (IGC)	[49,50,121]
	ψ	RluA ^a ; Pus9p ^b	H-bonding and U-turn formation for ASL stability and improved decoding	[82,122]
34	ncm ⁵ U	Elp complex ^b	Promotes decoding of NNA and NNG codons; Suppresses -1 frameshifting at split-box codons; Suppresses +1 frameshifting	[16,17,23]
	mcm ⁵ U	Elp complex ^b , Trm9 ^b	Promotes decoding of NNA and NNG codons; Suppresses -1 frameshifting at split-box codons; Suppresses +1 frameshifting	[16,17,23, 100,101]
	mcm ⁵ s ² U	Elp complex ^b , Trm9 ^b , Ncs2-6 ^b	Promotes decoding of NNA and NNG codons; Suppresses -1 frameshifting at split-box codons; Suppresses +1 frameshifting; Increases rates and association of translation	[16,25,57, 100,101, 123,124]
	mn ⁵ U	mn ⁵ E ^a	Promotes decoding of NNA and NNG codons; Suppresses -1 frameshifting at split-box codons; Suppresses +1 frameshifting	[25–27,92, 98]
	mn ⁵ s ² U	mn ⁵ A ^a , mn ⁵ E ^a	Promotes decoding of NNA and NNG codons; Suppresses and enhances -1 frameshifting at split-box codons in a sequence dependent manner; Suppresses +1 frameshifting; Increases rate of GTP hydrolysis of EF-Tu	[27,92,98, 124]
	(c)mn ⁵ s ² U	mn ⁵ A ^a , mn ⁵ E-G ^a	Promotes decoding of NNA and NNG codons; Suppresses -1 frameshifting at split-box codons; Suppresses +1 frameshifting	[31,125]
	cmo ⁵ U	CmoA ^a ⁺ ; CmoB ^b [*]	Expands codon degeneracy - pairing with NN(U/A/C/G); Pre-orders ASL; enhances +1 frameshifting (in absence of m ¹ G)	[36–38,114, 126]
	tm ⁵ U	Mto1 ^{c,†}	Improves decoding of UUA and UUG codons	[34,127]
	I	TadA ^a ; ADAT1-3 ^{b,c}	Expands codon degeneracy - pairing with NNU, NNA, and NNG codons	[47,48,128, 129]
	f ⁵ C	hmtRNA ^d [‡]	Allows hmtRNA ^{Met} (CAU) to decode AUG (Met) and AUA (Ile)	[41,42]

Table 1 (continued)

ASL Position	Modification	Enzyme(s)	Reported Roles in Frame Maintenance and/or Translation	References
	k ² C	TilS ^a	Restricts AUG (Met) decoding of tRNA ^{Ile} (CAU) in bacteria; Enhances base-stacking to stabilize ASL	[43–45]
	Q	Tgt ^a ; Qtrt1,2 ^c	Suppresses -1 frameshifting	[102,106, 130]
35	ψ	Pus7 ^b	Allows tRNA ^{Tyr} (GψA) to function as a UAA/UAG nonsense suppressor	[69,87]
37	t ⁶ A	TsaC ^a , TsaD ^a ; Tcs1-7 ^b	Stabilize A-U base pairs between N1 of codon and N34 of anticodon; suppress -1 frameshifting;	[63,106, 124]
	ct ⁶ A	TsaC ^a , TsaD ^a , TcdA ^a , Tcd1,2 ^b	Stabilize A-U base pairs between N1 of codon and N34 of anticodon	[61,62]
	(ms ²)t ⁶ A	TsaC ^a , TsaD ^a , TrmO ^a , Cdk1 ^c	Stabilize A-U base pairs between N1 of codon and N34 of anticodon; Improves decoding of AAG; stabilizes ASL	[27,59,60, 131]
	i ⁶ A	MiaA ^a , MiaB ^a , Mod5p ^b	Stabilize A-U base pairs between N1 of codon and N34 of anticodon; Enhance decoding of cognate codons	[65,67,69, 70]
	(ms ²)i ⁶ A	MiaA ^a , MiaB ^a	Stabilize A-U base pairs between N1 of codon and N34 of anticodon; Enhance decoding of cognate codons	[68,132]
	(ms ²)io ⁶ A	MiaA ^a , MiaB ^a , MiaE ^a	Stabilize A-U base pairs between N1 of codon and N34 of anticodon; Enhance decoding of cognate codons	[120]
	m ¹ G	TrmD ^a , Trm5 ^b	Suppress -1 frameshifting; Suppress +1 frameshifting; stabilizes ASL	[102–104, 116]
	imG	Trm5 ^b , Tyw1 ^b , Tyw3 ^b	Suppress -1 frameshifting; Enhance codon recognition; Enhance base-stacking to stabilize ASL	[133]
	yW	Trm5 ^b , Tyw1-4 ^b	Suppress -1 frameshifting; Enhance codon recognition; Enhance base-stacking to stabilize ASL	[133]
	ψ	TruA ^a ; Deg1 ^b	Increases ASL stability and improves decoding	[83,134]
38	ψ	TruA ^a ; Deg1 ^b	H-bonding with position 31 and stem-loop formation for ASL stability and improved decoding	[83,124, 134]

^{*} - Enzyme is part of a pathway for installation that has not been fully elucidated

[†] - Currently unknown what enzymes are involved in installation of modification.

^a - Known enzyme(s) for installation of modification in *Escherichia coli*

^b - Known enzyme(s) for installation of modification in *Saccharomyces cerevisiae*

^c - Enzyme(s) for installation in eukaryotes

^d - Modifications that have been chemically synthesized

84]. While it is clear that the inclusion of Ψ throughout the ASL is important for ASL structure and codon recognition, continued mechanistic studies of further modifications (e.g. m³C32, m³U32) will be required to understand the contributions of stem-loop stability and modification cross-talk to tRNA decoding.

3. tRNA modifications influence ribosome reading frame maintenance

Since mRNA codons are made up of 3 nucleotides, there are inherently 3 potential translation reading frames the ribosome can decode (Fig. 2). tRNA modifications play a critical role in helping the ribosome to maintain proper reading frame during elongation to ensure the correct synthesis of gene-encoded protein products. While the translation machinery is generally exceptional at maintaining reading frame, the ability to promote frameshifting can be situationally advantageous. Viruses exemplify this idea by robustly employing programmed shifting to move the ribosome into different reading frames in order to recognize alternative start sites and bypass or recode termination sites (Fig. 2) [85]. mRNA:tRNA interactions are integral to maintaining proper frame, and it stands to reason that tRNA ASL modifications affect not just decoding, but also frameshifting.

3.1. ASL modifications modulate – 1 frameshifting events

The ribosome spontaneously slips (shifts) into the – 1 reading frame once every 10⁴–10⁵ codons. However, some genes encode sequences that increase the frequency of – 1 frame slippage to produce multiple peptide products during translation [86,87] (Fig. 2B). These mRNAs typically possess structural elements, such as a hairpin or pseudoknot, and a “slippery” heptanucleotide sequence: X XXY YYZ (in which XXX and YYY are triplets of the same nucleotide, but different codons) [85,88]. In bacterial systems, a 5'-Shine Dalgarno sequence is also commonly found near the slippery sequence [86,89]. – 1 frameshifting occurs when the ribosome has the Y(YY/Z) split-box codon positioned in the A site, and the tRNA anticodon briefly dissociates before recognizing the new – 1 frame [90,91]. The propensity of a tRNA to dissociate from YYZ and instead decode the – 1 frame, YYY, can be influenced by the modification state of the tRNA's ASL at positions 34 and 37 (Table 1, Fig. 2 A) [16,17].

Xm⁵U34-containing tRNAs typically suppress – 1 frameshifts. For example, mcm⁵s²U34 modifications in tRNA^{Arg}(UCU), tRNA^{Gln}(UUG), and tRNA^{Glu}(UUC) limit – 1 ribosome movements on split-box codons (Y(YY/Z)) (Fig. 2 C) [92–94]. However, in bacteria, mnm⁵U34 and s²U34 modifications on tRNA^{Lys}(UUU) have different effects depending on the sequences that they are decoding. These modifications limit – 1 frameshifting in AAA/A split codon boxes, and increase – 1 frameshifting on analogous AAA/G sequences. These observed differences are due to the increased affinity of hypomodified tRNA^{Lys}(UUU) for U-G binding the A-site tRNA (Fig. 2 C) [95–97]. This suggests the possibility that one reason that eukaryotes possess two distinct isoacceptors for lysine codons (tRNA^{Lys}(UUU) and tRNA^{Lys}(UUC)) could be to limit such frameshifting events and more finely control translation fidelity [98].

Further modifications, including wybutosine (yW37)-related modifications, suppress – 1 frameshifts (Fig. 2 C) to different degrees (m¹G > imG > yW) [99–101]. Another complex G34 modification, Queuosine (Q34), also limits the “shiftiness” of tRNA^{Asn}, although the effects of Q depend on the modification status of nucleobases in further tRNA regions. [102,103]. This is in line with observations made on other tRNAs, which demonstrate that t⁶A37 can reduce – 1 frameshifting on lysine codons [104,105]. These data showcase the importance of the interplay of modifications across the tRNA ASL in frame maintenance.

3.2. ASL modifications generally suppress + 1 frameshifting events

In addition to moving backwards by a single nucleotide (–1 frameshifting), ribosomes can also slip forwards to alter their reading frame (+1 frameshifting) (Fig. 2B). Bacteria take advantage of this and use + 1 frameshifting to “fix” errors in their DNA sequences. In this process, specialized tRNAs allow bacteria to overcome insertion and deletion mutations in their DNA by causing the ribosome to skip ahead along an mRNA sequence by 1 nucleotide, thereby changing the decoding reading frame [106,107]. These tRNAs are collectively referred to as “+ 1 frameshift suppressors”, since they suppress DNA mutations. Classic examples of + 1 frameshift suppressors include tRNA^{SufD}, a derivative of tRNA^{Gly}(GGC), and tRNA^{SufA6}, a derivative of tRNA^{Pro}(GGG). tRNA^{SufD} and tRNA^{SufA6} possess an additional nucleotide inserted in their ASL adjacent to position 37, creating a “position 37.5” [108–111]. m¹G is required at 37.5 for tRNA^{SufA6} to promote + 1 frameshifting, as the modified m¹G37.5 base flips during elongation to weaken mRNA:tRNA interactions [111,71,74,112–114].

When canonical (i.e. not suppressor) tRNAs are translating, + 1 frameshifting events are generally repressed by ASL modifications (Fig. 2 C). The removal of modifications from ASL positions 34 and 37 increases + 1 frameshifting in a wide range of tRNAs. This is consistent with findings (see Sections 1.1, 1.2 and 2.1) that both of these ASL positions are generally important for tRNA decoding. In particular, xm⁵U34 modifications mitigate + 1 frameshifts [113,115,116] (Table 1, Fig. 2 C). Similarly, other modifications at position 34, such as Q34 in tRNA^{Tyr}(GUA) also limit + 1 frameshifting [117]. Examples of position 37 modifications that suppress + 1 frameshifts include ms²io⁶37 of tRNA^{Phe}(GAA) and m¹G37 in tRNA^{Pro} in *E. coli*, as well as t⁶A37 and (c)⁶A/t⁶A37 in yeast tRNAs [1,99,104,118,119]. The function of m¹G37 is particularly significant, as it is essential for mitigating + 1 and + 2 frameshifts promoted by a second tRNA^{Pro}(UGG) modification, cmo⁵U34, in its absence [112]. This example highlights the interplay of cross-tRNA modifications in expanding codon recognition (cmo⁵U34 allows tRNA^{Pro}(UGG) to recognize all 4 proline encoding codons) while guaranteeing proper frame maintenance. In general, tRNA ASL modifications are crucial for ensuring that ribosomes translate the desired mRNA reading frame during protein synthesis.

4. Conclusions

It is evident that tRNA modifications are vital maintainers of translation efficiency and fidelity across all domains of life. These modifications offer nature an atomic-level mechanism to fine-tune protein synthesis. The potential of a single tRNA modification to have an outsized physiological impact is tremendous given that modifications can influence intra- and intercellular signaling, and the incorporation of further tRNA modifications [77,120]. Gaining a molecular-level picture of how each of the chemically diverse tRNA modifications impact translation will be needed to uncover the roles of tRNA modifications in gene regulation, define the mechanisms of tRNA-mediated drug resistance, and develop therapeutic strategies to treat diseases arising from the dysregulation of tRNA modifications.

Declaration of Competing Interest

The authors declare no conflict of interests.

Acknowledgements

We thank the National Institutes of Health (NIGMS R35 GM128836) and National Science Foundation (CAREER Award 2045562) for their support.

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