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tRNA's Modifications Bring Order to Gene Expression

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Summary

The posttranscriptional modification of RNA is a significant investment in genes, enzymes, substrates, and energy. Advances in molecular genetics and structural biology indicate strongly that modifications of tRNA's anticodon domain control gene expression. Modifications at the anticodon's wobble position are required for recognition of rarely used codons, and restrict or expand codon recognition depending on their chemistries. A shift of the translational reading frame occurs in the absence of modifications at either wobble position-34 or the conserved purine-37, 3'-adjacent to the anticodon, causing expression of alternate protein sequences. These modifications have in common their contribution of order to tRNA's anticodon.

Introduction

The naturally occurring modifications of RNA have been recognized for half a century, yet we are just beginning to learn how important these modifications are for gene expression. The more than 100 modification chemistries are analogous to the side chains of amino acids in their contribution of hydrophobic or hydrophilic properties to RNA structure [1•]. Cells devote a great deal of resources to the modification of RNA. In fact, considerably more genetic information is allocated to tRNA modifications than to tRNA genes [2]. Of the seventy-five modifications found in tRNAs, those occurring in the physically and functionally separable anticodon stem and loop domain (ASL) are best understood (Figure 1) [1•]. Here, we focus on the control of gene expression associated with the physicochemical contributions of the wobble position-34 and purine-37 modifications that introduce order into tRNA's anticodon loop. This order influences the recognition of codons and maintenance of the translational reading frame.

Modifications select and enable codon recognition

The genetic code is degenerate in that most amino acids have more than one codon. (Figure 2). In all organisms and organelles, codons outnumber the tRNAs required to translate them. Modifications of nucleosides within the anticodon loop modulate binding to certain codons, and therefore imbue specific tRNAs with the ability to regulate gene expression [1•;3•]. One particularly interesting mechanism of gene regulation is the use of codon bias [4•]. Codon bias is the disparity among synonymous codon usage. Among genomes, synonymous codons are not used in equal frequencies. Surprisingly, the use of rare codons in mRNAs is associated with a protein folding that differs in three-dimensional structure from that derived of mRNAs

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with common synonymous codons [5•]. As would be expected, codon bias is linked to tRNA isoacceptor abundance in the cell [6]. tRNA abundance, in turn, is correlated to environmental responses [7]. In *E. coli*, usage of the six codons of arginine varies from 1.4 to 24.1 per 1000 codons [8]. The most commonly used arginine codons are CGU and CGC. The other four, CGA, CGG, AGA, AGG, are rare codons found in low frequency in *E. coli* mRNAs. Rare codon clustering or positioning near initiation or termination codons, such as with the rare arginine codons AGA or AGG, cause ribosome stalling and frameshifting related to the low abundance of the tRNAs that read these codons [9–11]. AGA and AGG are found in DNA replication and other regulatory genes [12•] in *E. coli* and are responsible for fimbriae (pili) formation in *Salmonella* [13]. *E. coli* has five tRNA isoacceptors that read the six arginine codons [14•]. The tRNA^{Arg}_{UCU} isoacceptor decodes the two regulatory codons AGA and AGG [12•]. tRNA^{Arg}_{UCU} has a 2-thiocytidine (*s*²C₃₂) modification at position-32, 5-methylaminomethyluridine (mnm⁵U₃₄) at wobble position 34, and N⁶-threonylcarbamoyladenine at position-37 (t⁶A₃₇) (Table 1) [14•]. These modifications may be properties of certain tRNAs responding to rare codons AGA and AGG. The *s*²C₃₂ influences the decoding of AGG [15]. In a clear distinction from the tRNA isoacceptor that reads the rare codons AGA and AGG, the isoacceptors that read the two common codons CGU and CGC contain the modified nucleoside inosine (I₃₄) at the wobble position-34 (Table 1). The difference in modifications between tRNAs that read rare codons and those that recognize common codons implicates tRNA modifications in regulation via rare codon usage. Though AGA and AGG are the most used arginine codons in humans and other eukaryotes [16•], the modification-dependent recognition of these codons is regulatory when the codons appear in significantly greater numbers in some proteins than in others. The yeast tRNA^{Arg}_{UCU} isoacceptor with 5-methoxycarbonylmethyluridine at wobble position-34 (mcm⁵U₃₄) is linked to regulating the translation of numerous of these codons in the DNA damage response factors (Table 1) [17•]. In expression of eukaryotic proteins in *E. coli*, a low yield of protein from codon bias is alleviated by altering codon content of the gene or over expressing the tRNA responsible for recognizing rare codons [18]. The fifth rarest codon in *E. coli* is that of isoleucine, AUA (Figure 2). Isoleucine has three isoacceptors, but only the tRNA^{Ile}_{CAU} isoacceptor has a lysine modification of C₃₄ (lysidine) (Table 1) [1•;14•]. The other two have an unmodified G₃₄ [14•]. The unmodified tRNA^{Ile}_{CAU} would incorrectly recognize the methionine codon AUG, but is modified for decoding AUA [19]. Thus, again a tRNA modification is responsible for recognition of the rare codon of isoleucine. Therefore, in family codon boxes, specific tRNA wobble modifications may control expression by recognizing synonymous codons that are rarely used and only for particular genes. In controlling gene expression, the *s*²-, mnm⁵-, t⁶A and other modifications of these tRNAs restrict nucleoside conformation, structure the anticodon, and reduce anticodon domain conformational dynamics in recognition of specific codons [1•].

The tRNA^{Lys} species of all organisms have the modifications mnm⁵*s*²U₃₄ and t⁶A₃₇, or their derivatives (Table 1) [14•]. The two modifications are both required for the tRNA to bind the two-fold degenerate, cognate AAA and wobble AAG, codons at the ribosomes A-site and to translocate from A- to P-site [20–22•]. For lysine codons, specificity resides in the third position or wobble position, because they share a ‘mixed codon box’ with the two asparagine codons (Figure 2). While they do not necessarily prevent the misreading of near-cognate codons, these modifications significantly increase the recognition of cognate lysine codons [23]. In yeast, genetic deletion of enzymes responsible for modifying tRNA wobble position of tRNA^{Lys} is lethal to the cells [23]. Thus, modifications that contribute structure and order serve to restrict codon recognition of a mixed codon box.

In contrast to lysine and asparagine codons, alanine, leucine, proline, serine, threonine and valine codons are all four-fold degenerate (‘family codon box’) (Figure 2). One isoaccepting tRNA species for each of these amino acids has the wobble position-34 modification, 5-

oxyacetic acid uridine modification, $\text{cmo}^5\text{U}_{34}$ (Table 1). In *S. enterica*, there are two tRNA^{Val} isoacceptors that decode the four valine codons. The ASL of *E. coli* tRNA^{Val}_{UAC}, with the anticodon UAC, decodes the complementary codon GUA in the absence of the modification $\text{cmo}^5\text{U}_{34}$ (F. A. P. Vendeix *et al.*, unpublished). However, the modification is required for decoding the other valine codons, including GUG [24•;25•]. *In vivo*, mutants having only the one tRNA^{Val} isoacceptor with $\text{cmo}^5\text{U}_{34}$ were viable, indicating that this wobble-modified tRNA^{Val} is capable of decoding all four valine codons [25•]. A genetic knockout of all tRNA^{Pro} isoacceptors except the isoacceptor with $\text{cmo}^5\text{U}_{34}$ allowed survival of *S. enterica*. Yet, the deletion of one of the enzymes in the biosynthesis of $\text{cmo}^5\text{U}_{34}$ in tRNAs resulted in a significant reduction of growth [26•].

The Role of Modification in Translational Frameshifting

In the course of protein expression, the mRNA is faithfully and accurately read by the ribosome according to the sequence of adjoining codon triplets that initiate at the methionine codon AUG and constitute the reading frame through to a termination code (Figure 3). However, two possible classes of errors have been identified. First there are missense errors which are due to the replacement of one amino acid by another. These types of errors include a termination codon read through [27•]. The second types of errors are in processivity. In addition to triggering a premature termination of the translation process, these errors have been found to induce frameshifting. Premature termination and missense errors are beyond the scope of this review and will not be discussed.

Some genes have evolved with sequences that allow the mRNA to re-program the ribosome to change the frame in which it initially was set to read. The frequency of this type of frameshifting occurring at a programmed site, has been estimated to be ~100% [28;29]. In contrast, lower frequency errors have been detected in the case of the spontaneous, or translational frameshift and in frameshift suppression by mutant tRNAs. The efficiencies of these events were in the order $>10^{-5}$ per codon and to a few percents, respectively [29]. The results of many studies have demonstrated that the fidelity and efficiency of the decoding of the genome is enhanced by modified tRNA [1•;30]. The modifications prevent frameshifting and thus, maintain the reading frame. Mechanistic models have incorporated the influence of modifications on frameshifting [31–35]. Among them the dual error model [36] was found to give a more plausible and accurate explanation on how +1 and –1 frameshifts take place under the influence of modification deficient tRNAs [37•] (Figure 3). More precisely, natural modifications in the anticodon suppress frameshifting. In the absence of the wobble-position modification queuosine (Q₃₄), tRNAs for tyrosine, histidine, asparagines, and aspartic acid induce +1 frameshifting and have a very limited effect on –1 frameshifting (Table 1) [37•; 38]. The absence of $\text{mm}^5\text{s}^2\text{U}_{34}$ found in *E. coli* tRNAs for lysine, glutamine, glutamic acid, arginine and leucine also promotes +1 frameshifting (Table 1) [37•;39]. Interestingly, $\text{mm}^5\text{s}^2\text{U}_{34}$ has been shown to play a crucial role in counteracting +2 ribosomal frameshifting (Table 1) [40].

The conserved and highly modified purine-37 plays a key role in the accuracy of translation. The modifications at position-37 negate translational frameshifting. The absence of 2-methylthio-*N*⁶-(*cis*-hydroxyisopentenyl) adenosine ($\text{ms}^2\text{io}^6\text{A}_{37}$) and N1-methylguanosine (m^1G_{37}) induce a +1 frameshift error (Table 1). However these two modified purines have minor effects on –1 frameshifting. The lack of the hypermodified nucleoside wybutosine (yW_{37}) that is specifically found in Eukaryea and Archaea tRNA^{Phe}, triggers +1, as well as –1, frameshifting [37•;38;41•]. The absence of yW_{37} and m^1G_{37} in tRNA^{Phe} initiates retroviral ribosomal frameshifting [42;43] which is essential for viral gene expression (Table 1).

Thus, the anticodon domain modifications have a profound effect on the reading frame maintenance, and their absence on programmed translational frameshifts. These same modifications pre-order the anticodon by restricting conformational space and dynamics [1; 44–46]. Thus, upon codon binding on the ribosome, tRNA's anticodon is substantially structured by position-34 and -37 modifications, and subjected to only subtle conformational changes [1;47]. In contrast, the ribosome 30S subunit undergoes a noticeable conformational change from an 'open' to a 'closed' state, and by hydrogen bonding interaction to the anticodon and codon backbones, the nucleosides A1492, A1493, and G530 of the 16S rRNA assess the correctness of the geometry of the minihelix formed during the codon base-pairing with the anticodon [1••]. All purine-37 modifications that have been studied negate intra-loop hydrogen bonding and preserve the open loop structure that is required for codon binding [1••]. The purine-37 modifications restrict conformational dynamics of the loop towards the structure of the canonical U-turn conformation and enhance base stacking especially in the case of the pyrimidine-rich anticodons [1••].

Thus, in the absence of modifications at positions-34 and/or -37, tRNA's anticodon domain is more dynamic. Apparently, a small fraction of tRNAs that lack modifications are accommodated at the ribosome's A-site by the kinetic and induced fit proofreading that exams the exactness of the anticodoncodon minihelix [48]. Following translocation, a frameshift occurs at the P-site [36].

Conclusion

Modifications are essential to tRNA folding, secondary and tertiary structure, and thus tRNA function [1••;49]. Some aminoacyl-tRNA synthetases recognize their cognate tRNA's identity through the structure and chemistry contributed by modified nucleosides, particularly within the anticodon domain (Table 1) [50•]. The modifications of the anticodon stem and loop regulate gene expression by ordering conformation and dynamics for recognition of codons, maintenance of the reading frame, and by their absence, allowing mRNA programmed frameshifts.

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Figure 1.

Modifications of the anticodon stem and loop (ASL) of tRNA order the loop preventing frameshifting and allowing for accurate codon selection. **(a)** Modifications such as s^2C at (■), s^2U_{34} , mnm^5U_{34} , cmo^5U_{34} and Q at wobble position-34 (■) and t^6A , m^1G , ms^2io^6A and yW at position-37 (■) structure the anticodon toward the canonical “U-turn (at invariant U_{33}) and increase stability by facilitating the stacking of the aromatic nucleoside bases of positions 34–37. Purine-37 modifications negate intra-loop base pairing (...). **(b)** Representative prokaryotic modified nucleosides that are involved in control of rare codon recognition and reading frame maintenance/frameshifting. The chemical structures and locations (positions 32, 34 or 37) of the modifications in tRNA’s anticodon domain are shown. Abbreviated names are defined in the text. Eukaryotic modifications with similar chemistries, such as mcm^5s^2U , can be found at the RNA Modification Database (URL: <http://library.med.utah.edu/RNAMods/>).

		SECOND									
		U		C		A		G			
FIRST	U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U	THIRD, WOBBLE
		UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C	
		UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop	A	
		UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp	G	
	C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U	
		CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C	
		CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A	
		CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G	
	A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U	
		AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C	
		AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A	
		AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg	G	
	G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U	
		GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C	
		GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A	
		GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G	

Figure 2.

The Universal Genetic Code. Figure. The 61 sense codons are color shaded according to extent of degeneracy: single codes are in grey (□); twofold degenerate codes, pink (□); threefold, yellow (□); fourfold, green (□); two- plus fourfold or 6-fold degenerate codes in blue (□). The stop codons are in **red**. Accurate and efficient recognition of codons are dependent on the specific modifications at the anticodon stem and loop (ASL) of tRNA and the modifications' ability to restrict or expand codon box recognition. Rare codons that are discussed are in **bold**.

Figure 3.

The dual error model of frameshifting (adapted from [44]). **(a)** In place of a hypomodified cognate tRNA, an unmodified near-cognate tRNA binds to the A-site of the ribosome causing a pause after translocation to the P-site. Following this, a -1 or $+1$ frameshift is triggered. **(b)** The hypomodified tRNA evolves at a slow pace and failed to bind to the A-site. As a result the cognate tRNA is paused and a $-1/+1$ frameshifting is induced. **(c)** As opposed to the first case **(a)**, the hypomodified tRNA successfully binds to the A site and after translocation to the P-site a frameshift is produced.

Table 1

Contribution of modifications to codon recognition, frameshifting and as aminoacyl-tRNA synthetase identity determinants.

Modification	Codon Reading	Frameshifting	aminoacyl-tRNA Synthetase (aaRS)
Q ₃₄	NNU	+1	gluRS, lysRS
mm ⁵ s ² U ₃₄	NNA/G	+1/+2	
cmo ⁵ U ₃₄	NNA/C/G/U	ND	
ms ² io ⁶ A ₃₇	NNU/C	+1	
m ¹ G ₃₇	NNU /C/A/G	+1	ileRS
yW ₃₇	NNA	+1/-1	
t ⁶ A ₃₇	NNA/G	ND	
i ⁶ A ₃₇		ND	
mm ⁵ U ₃₄ ;mcm ⁵ U ₃₄ *	AGA/G	Codons in tandem,+1/-1	ileRS
k ² C ₃₄	AUA		
s ² C ₃₂	AGG	+1/-1	
I ₃₄	NNU/C/A		

ND Denotes Not Determined

* Denotes eukaryotic modification