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Can protein expression be regulated by modulation of tRNA modifications profiles?

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Abstract

tRNAs are the central adaptor molecules in translation. Their decoding properties are influenced by post-transcriptional modifications, particularly in the critical anticodon-stem-loop (ASL) region. Synonymous codon choice, also called codon usage bias, affects both translation efficiency and accuracy, and ASL modifications play key roles in both these processes. In combination with a handful of historical examples, recent studies integrating ribosome profiling, proteomics, codon-usage analyses and modification quantifications show that levels of tRNA modifications can change under stress, during development or in specific metabolic conditions, and can modulate the expression of specific genes. Deconvoluting the different responses (global or specific) to tRNA modification deficiencies can be difficult because of pleiotropic effects but, as more cases emerge, it does seem that tRNA modifications changes could add another layer of regulation in the transfer of information from DNA to protein.

Introduction

The genetic code is degenerate as, with the exceptions of Met and Trp, all amino acids are encoded by two and up to six codons. Codon usage choice (which codon in a synonymous set is used to encode a given amino acid) is not only driven by neutral processes such as mutation biases or GC percent but is also molded by selection (1). The mechanistic and evolutionary forces in play are extremely complex, involve multiple factors and, even if these are not yet fully understood, general principles are emerging as recently discussed and debated in numerous reviews (2–9).

tRNA abundance has long been proposed as a key factor in shaping codon choice and it is clear that highly expressed proteins in all organisms are biased towards codons decoded by abundant tRNAs (the specific sets varying with every organism) (3). However, many other factors are in play in codon choice, both across genes and within genes (3, 7). Not only different organisms have different codon biases, but the bias can actually reflect specific phenotypic traits such as thermophily or photosynthesis (10). In addition, tRNA pools can change during stresses to favor the expression of proteins with “stress adapted codons” (11).

Codon choice greatly influences translation speed and consequently ribosome density on mRNAs, affecting mRNA degradation, splicing, translation accuracy, protein folding and structure (9). Hence, codon choice influences both the final protein levels and the function of a given protein, leading to dramatic phenotypes caused by specific “silent” mutations (12–14). One key component in modulating translation speed, and therefore in shaping codon choice, is the presence or absence of tRNA modifications in the Anticodon Stem Loop (ASL). Indeed, the function of tRNA molecules is modulated by modifications that have different roles depending on the specific modification and position in the tRNA molecule (15–17). Some affect tRNA stability (18), others act as determinants or anti-determinants for different components of the translation apparatus (15). ASL modifications mold the set of tRNA molecules used in any given organism (19) and influence both decoding efficiency (20–22) and accuracy (23–25). The presence of modifications explains previously observed discrepancies between tRNA levels and codon bias of highly expressed proteins (26) or differences in codon usage between specific species (27, 28).

Because of the central role of ASL modifications in decoding, a possible regulatory role was proposed in the early 90s (29). In the proposed model, a stress or changing environment would affect the level of a specific modification, triggering a change in expression of specific gene (or gene set) leading to a homeostatic response and allowing an adaption to the given perturbation (Fig. 1). In the last 10 years, more quantitative methods have emerged to measure tRNA modifications (30, 31). In addition, genome-wide approaches to analyze codon usage (32), to measure translation speed by ribosome profiling (21, 22, 33) and to quantify protein levels by proteomics (34) are now available. In combination, these technical improvements have led to several new examples of modulation of protein expression by tRNA modifications (Table 1). More generally, models for regulatory roles for tRNA modifications and the existence of Modification Tunable Transcripts (MoTT) are gaining traction (35–38). However, because of the complexity of their function in translation and the pleiotropic effects of tRNA modification deficiencies, the cases where the regulatory feedback loop shown Fig. 1 have been fully understood are rare, and most examples would require additional work to demonstrate and elucidate the regulatory mechanisms in play. Another added complexity is the cross-correlation between codon effect and parameters related to RNA composition. For example, replacement of codons by iso-codons change codon recognition but also, inevitably, the RNA bases composition. This can lead to cross-correlations between codon effect and RNA structural effects that are difficult to untangle (4).

Modification levels affect the translation of specific regulators: showcase examples

A historical example of a regulatory response mediated by an ASL modification is the role of ms²i⁶A37 in iron homeostasis (39). In the late 60's, it was discovered that *Escherichia coli* cells grown in iron deficient media contained tRNAs harboring lower levels of ms²i⁶A (40). These unmodified tRNAs were showed to translate less efficiently synthetic polynucleotide *in vitro* and led to the up regulation of several amino acid synthesis operons, all regulated by leader peptide/attenuation mechanisms, *in vivo* (41, 42), as well as of the

enterocholin synthesis operon (43). These findings led to the first regulatory model where low iron would lower $\text{ms}^2\text{i}^6\text{A}$ levels, which would lead to a homeostatic response, the increase in synthesis of an iron chelator molecule (29). It took 25 more years to understand the underlying molecular mechanisms for this regulatory loop. First, the discovery that the $\text{ms}^2\text{i}^6\text{A}37$ synthesis protein MiaB was an iron-sulfur-cluster dependent enzyme (44) explained how levels of iron can regulate the levels of this modification. Second, the discovery that the translation of the gene encoding the major regulator of iron homeostasis in *E. coli*, Fur, was directly coupled to the translation of the leader peptide gene *uof* (45) allowed to close the circle (Fig. 1). Indeed, *uof* contains a Ser-UCA codon at position 6 that is decoded by $\text{tRNA}^{\text{Ser}}\text{-UGA}$, which contains the $\text{ms}^2\text{i}^6\text{A}37$ modification followed by a rare Arg-AGA codon (45). The dependency on the Ser-UCA codon for iron-regulated translation of the Uof peptide was confirmed by recoding of this gene. When Ser-UCA was replaced by Ser-UCU, whose translation is not dependent on the $\text{ms}^2\text{i}^6\text{A}37$ modified $\text{tRNA}^{\text{Ser}}\text{-UGA}$ (Fig. 2), iron limitation resulted in decreased synthesis of Uof (45).

A more recent example is illustrated by the role of the tRNA modification $\text{m}^1\text{G}37$ in Mg^{2+} homeostasis in *Salmonella enterica* serovar Typhimurium (46). Expression of *mgtA*, the gene encoding the Mg^{2+} transporter, is regulated by attenuation dependent on the speed of translation of the leader peptide gene *mgtL*. The leader peptide contains several Pro encoding codons, which are decoded by tRNAs that harbor $\text{m}^1\text{G}37$ (Table 1). The insertion of this modification in tRNAs is catalyzed in *S. Typhimurium* by TrmD, an unusual methyltransferase that requires Mg^{2+} (most methyltransferases require no metal for catalysis) (47). In the proposed regulatory model [(47) and Fig 1], low Mg^{2+} levels affect the proline dependent translation of the leader peptide MgtL, leading to the formation of an antitermination structure which allows the transcription of the downstream Mg^{2+} transporter gene *mgtA*. A mutated version of TrmD resulted in higher expression of *mgtA*, indicating that a decrease in levels of $\text{m}^1\text{G}37$ modified tRNAs, and thus in translation of Pro codons, was responsible for the increase in expression of *mgtA*. However, the identity of the specific Mg^{2+} sensor, if TrmD or other components of the translation apparatus such as RpmA and RpmE ribosomal proteins or EF-P translational factor, is yet to be confirmed.

Both these examples (Uof or MgtL) invoke a regulatory system that is very sensitive to changes in translation speed of a specific protein and where the presence of just one codon decoded by the modified tRNA can drive the regulation. Another common feature is that the activity of the modifying enzyme appears to be directly dependent on the sensed molecule. These examples also show the complexity of discovering and validating these types of regulations using classical methods, and explain why the integration of multiple “omics” datasets has allowed to unearth possible new cases as discussed below.

tRNA modification levels can change with stress, environment or metabolism

With the exception of specific methylations such as m^1A that can be demethylated (48), tRNA modifications cannot be eliminated without degradation of the whole tRNA molecule. It was generally thought that, given the half-life of tRNA molecules (from nine hours to up

to days)(49), modification levels would not change quickly enough to be used as regulatory mechanisms. Yet, recent improvements in tRNA modification quantification methods have shown that tRNA modification levels can fluctuate (30, 50). Also, as tRNAs have been shown to rapidly turn over under stress (51, 52), there might be conditions under which the modification profiles of the tRNA pools can be altered.

In the two examples presented above, we discussed how changes in micronutrient levels could affect tRNA modification levels. Other metabolic signals include sulfur amino acid availability that influence levels of modification s²U34 in yeast (53) or bicarbonate concentration that can alter t⁶A levels in mammalian mitochondrial tRNAs (54). Competition for the precursor dimethylallyl pyrophosphate can affect levels of i⁶A modification in yeast (55), and taurine levels in mitochondrial tRNAs are dependent on folate (56). In general, as the synthesis of most modifications requires building blocks or cofactors derived from primary metabolism, one can envision regulatory interplays between metabolism and translation through the modulation of modification levels (57).

Numerous other factors such as physical and chemical stresses, stages in development, or diseases have also been shown to influence tRNA modifications levels. To cite a few: mild hypoxia increases cmo⁵U levels in *Mycobacteria bovis* BCG (50) and oxidative stress or exposure to alkylation agents, respectively, leads to m⁵C (58) or m³C (59) induction in yeast.

The main question in the context of this review is which of these factors found to modulate tRNA modifications are components of regulatory feedback loops. Listed in Table 1 are published cases of MoTTs beyond the two discussed above, and a few of these cases will now be discussed.

Codon bias analyses reveal modification tunable transcripts

In organisms with small genomes, the number of tRNAs has been reduced and one tRNA can decode all synonymous codons (60, 61). However, this is not the rule in most organisms, where different tRNAs can be used to introduce the same amino acid in the elongating polypeptide chain (62). This is the case for half of the amino acids in *Escherichia coli* (Fig. 2). Codon usage analyses can point to genes biased for codons decoded by tRNAs harboring a specific modification, which is absent from the tRNA decoding other synonymous codons. Hence, the expression of these genes could be dependent on the level of a specific modification. The identification of MoTTs often starts by codon usage analysis and, using this approach, many cases of MoTTs have been confirmed experimentally (Table 1). Sometimes, the expression of specific genes can be monitored using antibodies or translational fusions, as shown in the case of the *E. coli* *rpoS* encoding the stress sigma factor, which contains several LeuUUX (UUA/UUG) codons that are decoded by tRNAs modified with i⁶A37 and U/C34_m (63, 64) (Fig. 2). The absence of these modifications leads to low levels of RpoS translation, an effect that is suppressed by mutating the LeuUUA/UUG codon to CUN codon (Fig. 2) (63). It has been postulated that heat-shock and/or leucine starvation are the signals sensed in this system, but the molecular mechanisms are still to be elucidated.

Most of the examples of MoTTs given in Table 1 were identified by combining codon usage and/or proteomic analyses with ribosomal profiling experiments. In a recent elegant study by the Dedon group (50), it was shown that in *Mycobacterium bovis*, cmo⁵U levels increased under mild hypoxia conditions. In parallel, levels of proteins in the Dos dormancy regulon increased, including of the master regulator protein DosR. Codon usage analysis showed that the *dosR* gene is enriched in Thr-ACG codons decoded by tRNA^{Thr}-UGU, the only tRNA-Thr modified by cmo⁵U. Codon re-engineering as well as proteomics data showed that levels of the cmo⁵U modifying enzyme CmoA are increased in hypoxic conditions, further supporting the regulatory model proposed by the authors for hypoxia in *M. bovis*. The only missing link in this model is how low O₂ levels lead to changes in CmoA levels.

DNA damage is another type of stress correlated to a regulatory response dependent on a tRNA modification tunable transcript. After DNA damage caused by methylmethane sulfonate (MMS) in *S. cerevisiae*, levels of the Trm9-dependent modification mcm⁵U34 increase and promote translation of the Arg(AGA)-enriched RNR1 protein (65). RNR1 is a component of the ribonucleotide reductase (RNR) complex, which is required for cell cycle progression into S-phase and for DNA damage response. While a *trm9* mutant showed delayed transition to S-phase after DNA damage, a codon optimized version of the RNR1 gene increased Rnr1 protein levels and rescued the DNA damage-induced cell cycle phenotype.

Interesting examples linked altered modification levels in cancer cells to tumor survival and metastasis. In melanoma cells expressing a mutant version of protein kinase B-RAF (BRAF^{V600E}) up regulation of U34 modification enzymes leads to an increase of mcm⁵s²U34 levels, promoting the translation of the hypoxia-induced factor 1 (HIF1), enriched in codons that require U34 tRNA modifications (66). HIF1 then contributes to tumor survival by increasing the glycolysis gene expression program, increasing glucose uptake and lactate production, thus reducing dependency on TCA cycle. The same modification effect is observed upon chronic exposure to BRAF inhibitors, such as vemurafenib and dabrafenib, leading the melanoma cells to acquire resistance to these drugs (66). Increased levels of other U34 tRNA-modifying enzymes were also observed in PyMT-induced breast cancer. In this case, increased translation of the DEK protein, enriched in mcm⁵s²U34 sensitive codons, regulated the translation of the oncogenic Lymphoid Enhancer binding Factor 1 (LEF-1) mRNA to promote tumor metastasis (67). The requirement of mcm⁵s²U34 for both HIF1 and DEK was confirmed by recoding of these genes using cognate synonymous codons whose translation do not depend on mcm⁵s²U34.

The absence of many tRNA modifications leads to similar pleiotropic phenotypes indicative of proteotoxic stress

One of the difficulty in identifying regulatory circuits that use tRNA modifications as sensors is that many ASL modifications have a role in fine-tuning translation by increasing or reducing elongation speed or accuracy (33). It is known that perturbations of translation speed can lead to protein misfolding directly by affecting co-translational folding (9, 21) or indirectly through misincorporation of erroneous amino acids (68). It became quickly

apparent, at least in yeast, that defects in $\text{mcm}^5\text{s}^2\text{U34}$ and $\text{t}^6\text{A37}$ synthesis gave rise to very similar phenotypes such as activation of the Gcn4 response independently of Gcn2 (20, 69, 70). Activation of the Gcn4 response was also observed in the absence of seven other modifications (33). These similarities could be explained with the model of the Leidel group that showed that the absence of $\text{mcm}^5\text{s}^2\text{U34}$ in yeast and other eukaryotes such as *C. elegans* led to defect in protein homeostasis and aggregation of endogenous proteins (21). In the developing mouse brain, lack of $\text{mcm}^5\text{s}^2\text{U34}$ led to the induction of the unfolded protein response (UPR). Since the UPR acts as a differentiation signal during mouse corticogenesis, this leads to defects in the differentiation of neural precursors (71).

Aggregation phenotypes were also observed in the absence of Queuosine (Q34) (22) or $\text{m}^2\text{G26}$ (52) in mammals. Another example of the pleiotropic phenotypes caused by lack of tRNA modification is the activation of the general amino acid control (GAAC) response, acting through Gcn2 in a yeast *trm7* mutant lacking $\text{C}_{\text{m}}32/\text{G}_{\text{m}}34$, because the absence of these modifications leads to poor charging of tRNA^{Phe} (72) and Trm7 also methylates rRNA (33).

Another type of observed indirect effect was seen in a study showing that the loss of 2-thiolation in U34 observed in yeast under heat stress was due to a strain-specific thermosensitivity of the thiolation enzymes (73). Generally, one should remain cautious in interpreting the consequences of tRNA modification deficiencies as pleiotropic and indirect effects complicate greatly the interpretation of the observed phenotypes.

Conclusion

Several cases of MoTTs have now been experimentally confirmed (Table 1). However, only a few MoTTs have been shown to be elements of a full regulatory cycle (Fig. 1). Indeed, in most cases, the molecular mechanisms leading to the regulation of the tRNA modifying enzymes by stress agents are not elucidated. It does seem, however, that several MoTTs are part of a more general response of specialized translation of stress proteins that integrate many changes in the translation apparatus such as changes in elongation factors or tRNA levels (11, 35, 74). In these cases, the changes in translation efficiency seen in the absence of modifications would be an indirect consequence of specialized isoacceptor usage.

It is clear however that, even if they do not always have regulatory roles, tRNA modifications have such essential roles in translation that the corresponding defects lead to many diseases (75–77) that had gone unnoticed until recently because many of the tRNA modification genes were unknown. As these genes get identified by the basic science community, the growing importance of tRNA modifications in cellular biology and medicine as well as their potential regulatory roles can be made.

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Figure 1. Model for homeostatic regulation by tRNA modifications.

In blue, steps present in the regulation of a homeostatic response to (1) a stress/metabolic signal, leading to (2) altered activity of a tRNA modifying enzyme and consequently to changes in levels of a specific tRNA modification, which will reflect in (3) alteration in the synthesis of a codon dependent MoTT (Modification Tunable Transcript), resulting in (4) a homeostatic response to the initial stress/metabolic signal. In gray, two cases with high potential for completing the full regulation circle are displayed: a, regulation of *uof-fur* translation under iron depletion (45); b, regulation of MgtL translation under Mg²⁺ limitation (46). V = C or G or A; B = G or C or U.

Figure 2. Decoding wheel for *E. coli*

Circular representation of the genetic code displaying modified nucleosides based on Grosjean and Westhof (79) and of the coding capacities of the corresponding tRNAs based on the on Bjork and Hagervall (17). . The codon sequence of each amino acid is read in the inside-out direction (1–2–3). The modifications present at positions 32, 34, 37 and 38 to 40 are shown. Red circles connected by a line, or a single circle, represent one tRNA species. Each red circle is displayed in the direction of a specific decoded codon. Amino acids displayed in blue can be decoded by different tRNAs harboring distinct modifications. Amino acids displayed in black are decoded by only one tRNA species.

Table 1.

Described cases of Modification Tunable Transcripts (MoTTs)

Organism	Enzyme	tRNA	Stress/Metabolic signal	Mod	Codon	MoTT	Homeostatic response	Ref.
<i>E. coli</i>	MiaA	tRNA ^{Leu} -UAA	*	t ⁶ A37	Leu-UUA Leu-UUG	RpoS IraP Hfq	Induction of general stress response	(63, 64)
<i>E. coli</i>	TrmL	tRNA ^{Leu} -C'UAA	*	C/U34 _m	Leu-UUA Leu-UUG	RpoS	Induction of general stress response	(64)
<i>E. coli</i>	TusA	tRNA ^{Leu} -UAA	*	s ² -U34	Leu-UUX	RpoS	Induction of general stress response	(64)
<i>M. bovis</i> BCG	CmoA (BCG_0612)	tRNA ^{Thr} -UGU	Hypoxia	cmo ⁵ U34	Thr-ACG	DosR	Increase in DosR levels induces Dos regulon and promote survival under hypoxic conditions	(50)
<i>S. cerevisiae</i>	Uba4p Urm1p	tRNA ^{Lys} -UUU tRNA ^{Glu} -UUC tRNA ^{Gln} -UUG	Sulfur amino acid (Met, Cys) availability	s ² U34	Lys-AAA Glu-GAA Gln-CAA	^	Sulfur amino acid starvation down regulates translation and cell growth, and increases Met/Cys/Lys synthesis and salvage	(53)
<i>S. cerevisiae</i>	Trm4	tRNA ^{Leu} -CAA	Oxidative stress (H ₂ O ₂)	m ⁵ C34	Leu-UUG	ε ⁵ RPL22a	RPL22a ribosomal protein translation contributes to oxidative stress survival response	(58)
<i>S. cerevisiae</i>	Trm9	tRNA ^{Arg} -UUC tRNA ^{Glu} -UUC	SN ₂ alkylating agents (MMS)	mcm ⁵ (s ²)U34	Arg-AGA Glu-GAA	ε ⁵ MPH1, RPL40A, DEF1 NBP1, YRB1, CMD1, MYO1 ²	MMS promotes translation of DNA damage repair genes1 and cell cycle control2	(59)
<i>S. cerevisiae</i>	Trm140	tRNA ^{Thr} -IGU	SN ₂ alkylating agents (MMS)	m ³ C32	Thr-ACC Thr-ACU	^	Up regulation of Thr enriched genes (membrane and cell wall genes are candidates).	(59)
<i>S. cerevisiae</i>	Trm9	tRNA ^{Arg} -UCU	DNA damage (MMS)	mcm ⁵ U34	Arg AGA	RNR1	Transition to S- phase and DNA damage response.	(65)
<i>E. coli</i>	MiaB	tRNA ^{Ser} -UGA	Iron depletion	ms ² t ⁶ A37	Ser-UCA	uof	Decrease in levels of Ferric uptake regulator (Fur)	(45)
<i>H. sapiens</i>	YRDC OSGEPL1	mt-tRNA ^{Ser} -AGY others: Asn, Thr, Lys, Ile	HCO ₃ levels/hypoxia	t ⁶ A37	t ⁶ A mt-tRNAs dependent codons	ε ⁵ ND2, ND5	Reduction in translation of Complex I. Might contribute to Warburg effect.	(54)
<i>S. typhimurium</i>	TrmD	tRNA ^{Pro} -CGG tRNA ^{Pro} -GGG tRNA ^{Pro} -AGG	Mg ²⁺ limitation	m ¹ G37	Pro-CCG Pro-CCC Pro-CCU	MgtL	Mg ²⁺ limitation affects translation of MgtL, increasing expression of Mg ²⁺ transporter (MgtA)	(46, 47)
<i>H. sapiens Mus musculus</i>	ELP1 ELP3 CTU1 CTU2	tRNA ^{Lys} -UUU tRNA ^{Gln} -UUG tRNA ^{Glu} -UUC	Melanoma BRAFV600E cells and tumor exposure to RAF inhibitors (vemurafenib, dabrafenib)	mcm ⁵ s ² U34	Lys-AAA Gln-CAA Glu-GAA	HIF1α	Increase in glycolysis gene expression program, increasing survival of the tumor and its resistance to drugs.	(66)
<i>Mus musculus</i>	IKABKAP/ELP1	tRNA ^{Lys} -UUU tRNA ^{Gln} -UUG tRNA ^{Glu} -UUC	PyMT-induced breast cancer	mcm ⁵ s ² U34	Lys-AAA Gln-CAA Glu-GAA	DEK	Increase in U34 modifying enzymes induces translation of the oncogenic LEF-1 mRNA promoting breast cancer cells motility and metastasis.	(78)
<i>Mus musculus</i>	IKABKAP/ELP1	tRNA ^{Lys} -UUU tRNA ^{Gln} -UUG tRNA ^{Glu} -UUC	*	mcm ⁵ s ² U34	Lys-AAA/AG Gln-CAA/CAG Glu-GAA/GAG	ε ⁵ BRCA2	# Absence of U34 modifying enzymes lead to reduction in protein levels of BRCA2, enriched in AA-ending codons, and involved in DNA repair.	(67)

presence of codon bias not confirmed

homeostatic response not specific to initial stress/metabolic signal

codon-dependent translation not confirmed by recoding. All other non-marked MoTTs were confirmed by recoding.

no specific MoTT identified

specific stress induction

no

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