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concept

Bringing order to translation: the contributions of transfer RNA anticodon-domain modifications

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The biosynthesis of RNA includes its post-transcriptional modifications, and the crucial functions of these modifications have supported their conservation within all three kingdoms. For example, the modifications located within or adjacent to the anticodon of the transfer RNA (tRNA) enhance the accuracy of codon binding, maintain the translational reading frame and enable translocation of the tRNA from the A-site to the P-site of the ribosome. Although composed of different chemistries, the more than 70 known modifications of tRNA have in common their ability to reduce conformational dynamics, and to bring order to the internal loops and hairpin structures of RNA. The modified nucleosides of the anticodon domain of tRNA restrict its dynamics and shape its architecture; therefore, the need of the ribosome to constrain or remodel each tRNA to fit the decoding site is diminished. This concept reduces an entropic penalty for translation and provides a physicochemical basis for the conservation of RNA modifications in general.

Keywords: entropy; order; pre-structured; rRNA; tRNA

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Introduction

The efficient and exact translation of genomic information into proteins is of fundamental importance in biology. Some 40 different transfer RNAs (tRNAs) decode all of the messenger RNA (mRNA) codons into the 22 amino acids of proteins, with the exception of the translational stop codons (Fig 1; Ambrogelly *et al,* 2007; Szymański & Barciszewski, 2007). In his 'wobble hypothesis', Francis Crick proposed that the many anticodons of different tRNAs underwent an induced fit to conform to the needs of the ribosome for a uniform structure in binding to the mRNA codons (Crick, 1966). However, the remodelling of the anticodon architecture produces an entropic penalty that would necessitate the repeated investment of energy for each tRNA every time it responds to its codon; thereby the accuracy and speed of translation would be compromised. In the Gibbs–Helmholtz equation, $\Delta G = \Delta H - T\Delta S$, G represents the Gibbs free energy, H represents the enthalpy and T represents the absolute temperature. The entropy term ΔS is a measure of the motional energy—which is often equated with disorder—within a system (for example, tRNA). A decrease in entropy is affected by a decrease in motional energy. For more than 30 years, modifications have been known to restrict the motional dynamics of tRNAs, and the anticodon-domain modifications are most effective (Fig 2; Schmidt *et al,* 1987). Modified nucleosides also alter the architecture of the anticodon stem and loop domain (ASL) towards that of the canonical structure with a 'U-turn', as first seen in the X-ray crystallographic structure of yeast $tRNA^{Phe}$ (Fig 2; Kim *et al,* 1974). The crystallography structures were confirmed by solution nuclear magnetic resonance (NMR) and thermodynamic studies on the ASLs of *Escherichia coli* and mammalian tRNAs (Vendeix *et al,* 2008; Agris *et al,* 2007; Stuart *et al,* 2003; Cabello-Villegas *et al,* 2002; Sundaram *et al,* 2000). In restricting the conformational dynamics of the ASL of tRNA, modifications decrease the ΔS. Here, I propose that this lowering of entropic energy and shaping of the anticodon reduces the need for the ribosome continually to remodel each anticodon loop for codon binding.

Bifunctional tRNA architecture

In general, tRNA isoacceptors are aminoacylated with one amino acid with great accuracy; therefore, each aminoacyl-tRNA synthetase recognizes one or more tRNA species as being chemically and structurally distinct (Ibba & Söll, 2000). However, the accuracy is considerably improved by the ability of some synthetases to proofread and then to edit, if incorrect, the amino acid that is bound to the tRNA (Schimmel & Ribas de Pouplana, 2001). During aminoacylation, the distal portion of the amino-acid-accepting stem (AAS) of tRNA (Fig 2) is distorted with the 3'-terminal adenosine placed inside the active site of the enzyme (Fukunaga & Yokoyama, 2005). In recognizing their cognate tRNAs, some synthetases distort the anticodon loop (Nakanishi *et al*, 2005). This suggests a mechanism by which a transient change in structure communicates through the tRNA to the active site of the enzyme that the cognate anticodon has been recognized (Ghosh & Vishveshwara, 2007; Rogers *et al*, 1993). Once aminoacylated, the coaxial stems of the AAS and the ribothymidine stem and loop domain (TSL) of all tRNAs with the exception of the initiator methionyl-$tRNA^{Met}$ are bound by elongation factor-GTP, and the tRNA transported to the decoding site of the ribosome, the A-site (Fig 3). The isoaccepting tRNAs then decode their appropriate amino-acid codons on the ribosome. Here, I focus on the modifications of the anticodon domain and their physicochemical contributions to codon binding.

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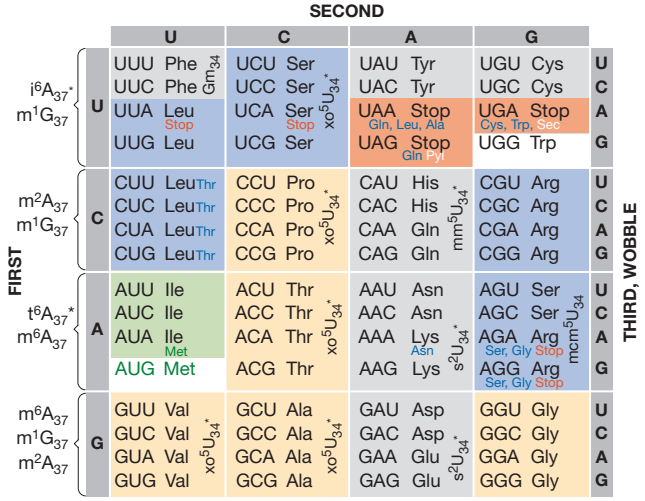

Fig 1 | Universal genetic code. The 64 codes are associated with the transfer RNA (tRNA) modifications that are important for decoding and/or translocation. Twofold degenerate amino-acid codes are highlighted in grey and fourfold degenerate codes are highlighted in tan. Amino acids with six codons are highlighted in blue. The threefold degenerate codons of Ile are highlighted in green, whereas the single codons of Met and Trp are highlighted in white. The three stop codons are highlighted in orange. Non-canonical codon use by some organisms and the mitochondrion is shown by using a small font for the amino acids (blue) or translational stop codons (red). The modified nucleoside abbreviations are defined in the text. Selenocysteine (Sec) and pyrrolysine (Pyl) codons are denoted in white. In the mitochondrion, $tRNA^{Met}$ responds to AUG and AUA, which is not used as an Ile codon (Agris *et al*, 2007; Szymański & Barciszewski, 2007; Björk *et al*, 1987).

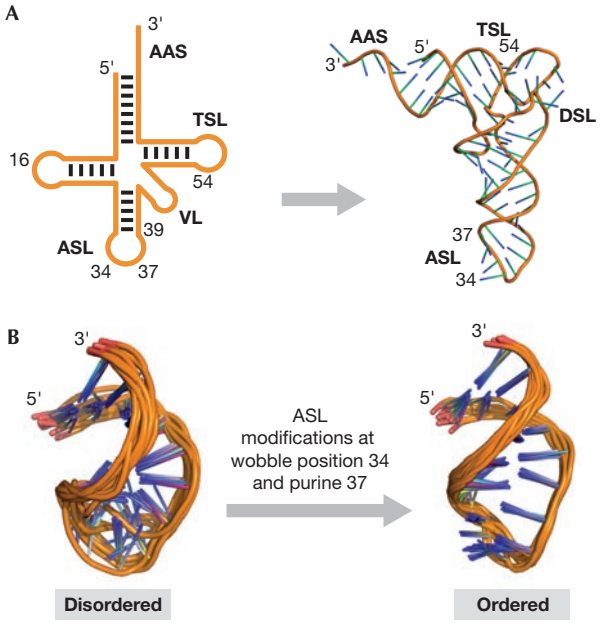

Fig 2 | Modifications order the anticodon stem and loop domain of transfer RNA. (**A**) The secondary structure and tertiary folding of cytoplasmic transfer RNAs (tRNAs). The physical and functional domains of the tRNA structure are the amino-acid-accepting stem (AAS), and the stem and loop domains designated dihydrouridine (DSL), anticodon (ASL), variable (VL) and thymidine (TSL). tRNA can fold into its tertiary structure before modification. pre-tRNA will fold with the help of Mg^{2+} and the aid of the most conserved of the modifications (Helm, 2006; Nobles *et al*, 2001). These modifications occur outside of the anticodon domain. (**B**) Modification of the anticodon stem and loop domain (ASL) of tRNA and its effect on dynamics. The ASL (left) is unmodified and disordered. Extensive modifications at wobble position 34 and purine 37 restrain the dynamics of the anticodon loop, and direct its conformation towards that of the canonical structure shown on the right.

ASLs vary in sequence and modification; yet, every anticodon domain entering the ribosomal A-site must have a similar global conformation. This architecture conforms to the structural restraints of the ribosome, and thereby maintains a rapid and consistent processivity for translation. The ribosome sustains a notable rate of synthesis (20–40 peptide bonds per second; reviewed in Lovmar & Ehrenberg, 2006). In addition, mRNA is translated with high fidelity (1 error per 1×10^{3}–1×10^{4} amino acids incorporated; Kurland, 1992). The accuracy of protein synthesis coupled with its speed requires a constant evaluation of the precision with which a tRNA anticodon is selected (Cochella *et al,* 2007). The precision of the anticodon–codon interaction is evaluated by kinetic and induced-fit mechanisms of proofreading in the decoding site of the ribosome. On entering the A-site of the ribosome and binding to the codon, the canonical shape of the ASL of the tRNA remains relatively unchanged (Valle *et al*, 2003; Steitz, 2008; Vendeix *et al*, 2008). There is little difference in anticodon conformation between the solution structures of the ASLs with their natural modifications and the same ASLs on the ribosome in response to their synonymous codons (Vendeix *et al*, 2008; Weixlbaumer *et al,* 2007; Murphy *et al,* 2004). Therefore, it seems that the architecture of the seven-member anticodon loop of all tRNAs is pre-structured and ordered by modification to conform to the A-site requirements of the ribosome. Posttranscriptional modifications can structure the ASL and place a restraint on its dynamics that reduces its entropic energy. However, the anticodon retains a uniqueness of chemistry, including that contributed by modifications, which enables it to respond to its respective codons.

The cognate anticodon readily forms a minihelix with the codon. The minihelix is evaluated and stabilized by the eight or nine hydrogen bonds between A1492, A1493 and G530 of 16S ribosomal RNA (rRNA) and the backbones of the codon and anticodon (Ogle *et al,* 2001). As A1492, A1493 and G530 are unable to form many of these hydrogen bonds when the non-cognate anticodon interacts with the codon, the non-cognate tRNA is released (Fig 3). When these interactions are successful, the small subunit converts from an 'open' to a 'closed' conformation. However, near-cognate anticodons—for example, those with a single mismatch in formation of the second or third base pair with the codon—might still trigger a conformational change in the small subunit. After the anticodon–codon minihelix is proofread, the conformation of tRNA is altered above the ASL—that is, above nucleosides 27 to 43 (Fig 3; Valle *et al*, 2003; Frank *et al,* 2005). A second proofreading event and substantial barrier is surmounted in the conformational change of the ternary complex, GTP hydrolysis and the release of the elongation factor with GDP (Fig 3; Cochella *et al*, 2007). For

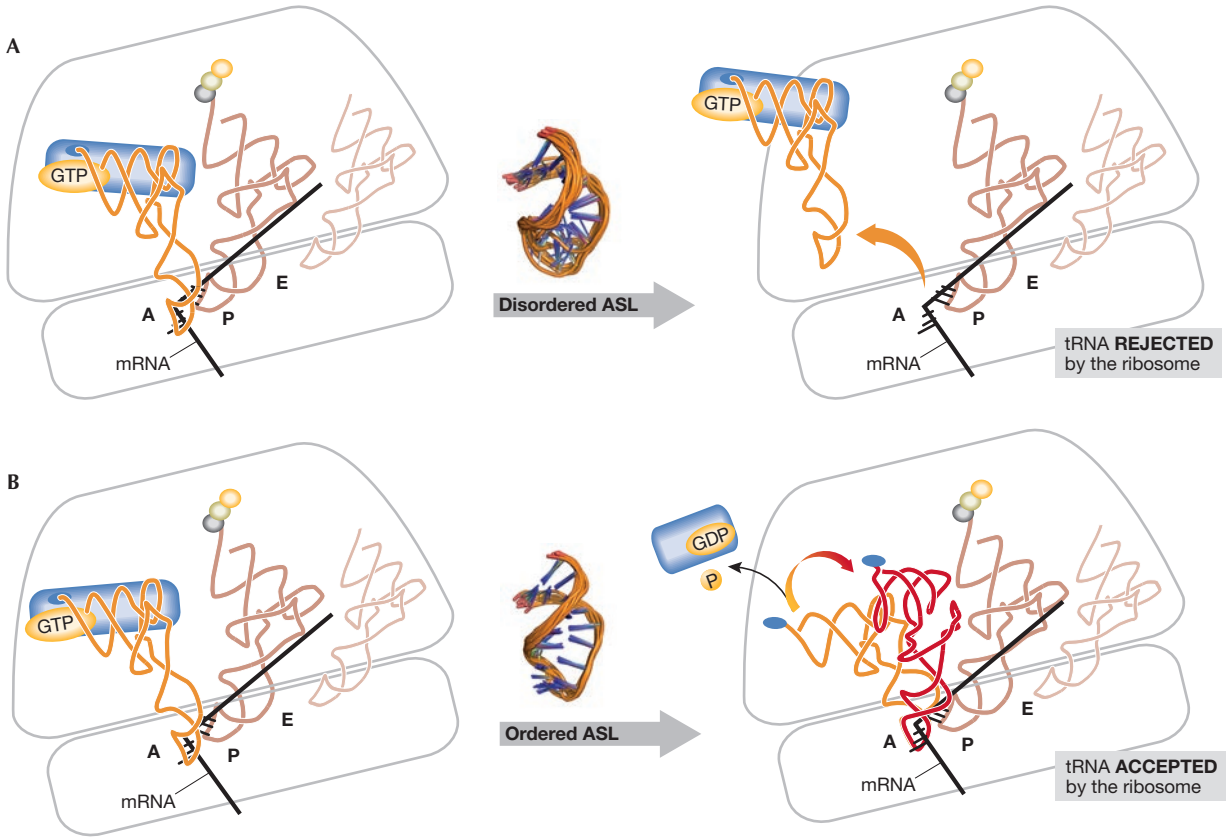

Fig 3 | Anticodon recognition and transfer RNA accommodation on the ribosome. (**A**) The complex of aminoacylated transfer RNA (tRNA) bound to elongation factor/GTP enters the A-site (left). The anticodon of the tRNA makes contact with the messenger RNA (mRNA) codon; however, the tRNA with a disordered anticodon stem and loop domain (ASL) cannot contact the codon correctly and is rejected by the ribosome (right). An aminoacyl-tRNA having successfully received the growing peptide now occupies the P-site, while the tRNA from which it received the nascent protein occupies the ribosome's exit or E-site. (**B**) Accommodation of aminoacyl-tRNA in the A-site. A complex of aminoacylated tRNA with elongation factor and GTP enters the A-site (left). Modifications have restricted the dynamics and shaped the architecture of the ASL of the tRNA. With recognition that the tRNA has the correct anticodon, the 16S ribosomal RNA (rRNA) nucleosides A1492, A1493 and G530 hydrogen bond to the backbones of the mRNA and the anticodon. The small ribosomal subunit and the tRNA undergo conformational changes. The conformation of the tRNA above the ASL changes, but the architecture of the ASL and its interaction with the codon remain unchanged (right). GTP is hydrolysed and the elongation factor-GDP leaves the ribosome.

this to occur, a correct interaction of the anticodon with the codon is communicated from the ASL to the structural neighbourhood of the 'hinge' region of the tRNA where the conformational change takes place and GTP is hydrolysed (Cochella *et al*, 2007).

Modifications order the anticodon of tRNAs

Posttranscriptional modifications are one of the processing events that result in functional tRNA molecules. tRNAs have more than 70 chemically distinct modifications (Rozenski *et al*, 1999; Agris, 1996). tRNA modifications are found across the kingdoms and throughout evolution, although at the expense of maintaining a large amount of genomic information, and by means of the use of energy and materials (Agris, 1996, 2004). Modified nucleosides are found in all of the biological and structural domains of tRNA, the AAS, and the dihydrouridine stem and loop domain (DSL), ASL, variable loop domain (VL) and TSL (Fig 2). Most modified nucleosides are localized to internal and terminal loops (Sprinzl & Vassilenko, 2005). Modifications contribute to tRNA folding, Mg^{2+} binding, intron removal, protein recognition, codon recognition, fidelity of the translational reading frame and other functions in translation (Fig 1; Agris *et al*, 2007; Agris, 2004; Björk *et al*, 1987). In general, tRNA genes are transcribed, sized, spliced and modified into functional RNA. Some of the site-specific modifications of tRNA are highly conserved in type and location, and their syntheses precede the sizing of the tRNA; however, modification before sizing is not obligatory. By contrast, the synthesis of pseudouridine in the anticodon of $tRNA^{Tyr}$, Ψ_{35}, requires the presence of the intron (Johnson & Abelson, 1983). The precise order of the many nucleoside modifications in the processing of tRNAs is not known, but, with few exceptions, they contribute to folding, thermal stability and restricted dynamics (Helm, 2006).

Modifications at the anticodon wobble position 34 (such as inosine) and at the conserved purine 37, 3'-adjacent to the anticodon (such as N6-isopentenyladenosine) were the first to be associated with the recognition of specific codons by tRNA (Crick, 1966; reviewed in Nishimura & Watanabe, 2006). The

Table 1 | Modified nucleoside relative entropic contributions to transfer RNAs

Modified nucleoside[a]	$\Delta\Delta S_R$ [b]	References
$D_{17,18}$	–/– –	Sipa *et al*, 2007
Cm_{32}	–	Ashraf *et al*, 2000
f^5C_{34}	– –	H. Lusic, P.F. Agris & A. Deiters, unpublished data
Gm_{34}	+/–	Schmidt *et al*, 1987; Ashraf *et al*, 2000
Q_{34}	+	Morris *et al*, 1999
cmo^5U_{34}	++	Vendeix *et al*, 2008
s^2U_8	++	Testa *et al*, 1999; Sipa *et al*, 2007
$mcm^5s^2U_{34}$	+	Bajji & Davis, 2000
mcm^5U_{34}	++	Bajji & Davis, 2000
$mnm^5s^2U_{34}$	+	Durant *et al*, 2005; Vendeix *et al*, 2008[c]
mnm^5U_{34}	++	Yarian *et al*, 2000
i^6A_{37}	+	Cabello-Villegas *et al*, 2002; Kierzek & Kierzek, 2001
t^6A_{37}	+	Yarian *et al*, 2000; Vendeix *et al*, 2008[c]
$ms^2t^6A_{37}$	+/–	Durant *et al*, 2005; Vendeix *et al*, 2008[c]
m^1G_{37}	+	Ashraf *et al*, 2000
yW_{37}	+	Schmidt *et al*, 1987
Ψ_{39}	+	Yarian *et al*, 1999; Ashraf, 2000; Bajji & Davis, 2000; Tworowska & Nikonowicz, 2006
m^5C_{40}	–	Ashraf *et al*, 2000
m^5U_{54} (T_{54})	0	Schmidt *et al*, 1987; Sengupta *et al*, 2000
Ψ_{55}	0	Sengupta *et al*, 2000

[a]Contributions of modified nucleosides at their most common positions in the anticodon stem and loop domain (ASL), dihydrouridine stem and loop domain (DSL) or ribothymidine stem and loop domain (TSL) of transfer RNA (tRNA), and in short RNA duplexes (12–15 residues) with site-specific modifications. Stability measurements of the modified and unmodified RNAs were assessed by 13C-methyl-relaxation measurements, ultraviolet (UV) absorbance at 260 nm and nuclear magnetic resonance (NMR). UV analysis of D is problematic because of its saturated ring structure. Modifications are defined in the text. [b]The relative change in entropy, ΔΔSR, is taken from empirically recorded thermodynamic parameters, and from comparisons of NMR-derived families of modified and unmodified structures in solution from the Protein Data Bank (PDB). In general, the cited laboratories conducted NMR spectrometry under conditions of low salt (10–20 mM) and pH (5.8–6.8), and at temperatures of 10–25°C. The PDB structures were achieved using either AMBER or CHARMM-based software for molecular-dynamics calculations from NMR-derived torsion angle and distance restraints. Relative values of ΔΔS•T are in ranges of kcal/mol as follows: +, 1–4; ++, 5–10; 0, –1 to +1; –, –4 to –1; and – –, –4 to –10. [c]Some thermodynamic data on ms2t6A37 have been published (Durant *et al*, 2005), but these have been expanded by F.A.P. Vendeix, A. Malkiewicz & P.F. Agris, unpublished data.

anticodon of tRNA resides in a relatively large seven-residue loop. Modifications maintain an open-loop conformation for presentation of the anticodon to the codon by negating canonical and non-canonical intra-loop hydrogen bonding (Olejniczak & Uhlenbeck, 2006; Dao *et al*, 1994). Such an open seven-membered loop would be expected to be dynamic; however, several investigations (see Agris, 2004, and references therein) now support the hypothesis (Agris, 1991) that numerous modifications at wobble position 34 and conserved purine 37 of tRNA shape the architecture of the ASL and constrain its dynamics, decreasing the motional (entropic) energy (Table 1). The anticodon loops of many tRNAs are dynamic and disordered in solution, particularly those that are pyrimidine-rich (Agris, 1991). The dipole–dipole and hydrophobic interactions of pyrimidines are far less stable than those of purines (Saenger, 1984). When anticodon loop bases stack as a consequence of modifications at wobble position 34 and purine 37, the resulting structure is ordered and the conformational dynamics of the anticodon are restricted (Fig 2). Base stacking removes the hydrophobic nucleobase from the environment of the aqueous solvent and, in so doing, the surrounding water molecules are able to become more organized with hydrogen bonding to the ASL backbone. Therefore, modification results in an ordered ASL and a lowering of the entropy.

The modifications of uridine at wobble position 34 of tRNA are of particular interest because they contribute a degree of order (Table 1) and are crucial to the specificity of codon recognition (Fig 1). Few sequenced cytoplasmic tRNAs show an unmodified U_{34} (Sprinzl & Vassilenko, 2005). The 2-thiouridine (s^2U_{34}) derivatives reinforce base pairing to codons ending in A. They are strictly gauche$^+$, C3'-*endo* and *anti*, and are found in tRNAs with weakly stacked pyrimidine-rich anticodons, such as those for Lys, Glu and Gln. The 5-methylaminomethyluridine (mnm^5U_{34}) and 5-methoxycarbonylmethyluridine (mcm^5U_{34}) contribute order in the absence of the thio-moiety (Table 1) and are found in pyrimidine-rich anticodon domains. They restrict tRNA to recognition of codons ending in A and G, and are important for translocation (Phelps *et al*, 2004; Agris, 2004). Uridine 5-oxyacetic acid (cmo^5U_{34}) restricts motional dynamics within the anticodon domain (Table 1); however, its function contrasts greatly with that of the other uridine modifications. The cmo^5U_{34}, and presumably other 5-oxy-derivatives (xo^5U_{34}, where x is H, methyl or acetic acid), enables the one isoaccepting tRNA species for each of Ala, Leu, Pro, Ser, Thr and Val that has the modification to read three and sometimes all four of the synonymous codons, NNA/G/U/C (Näsvall *et al*, 2004, 2007; Weixlbaumer *et al*, 2007; Vendeix *et al*, 2008). With the deletion of two of the three genes for the $tRNA^{Pro}$ isoacceptors in *Salmonella*, the one remaining $tRNA^{Pro}$ with a cmo^5U_{34} was able to maintain cell viability (Näsvall *et al*, 2004). However, in the presence of all three isoacceptors, the removal of one of the modification enzymes in the synthetic pathway for cmo^5U_{34} led to poor growth of the culture. In addition, this modification is crucial for the reading of the Ala, Pro and Val codons ending in G (Näsvall *et al*, 2007; Vendeix *et al*, 2008). Therefore, cmo^5U_{34} expands the ability of these six tRNAs to read theoretically 24 of the 61 amino-acid codes. In contrast to cmo^5U_{34}, which occurs in six different sets of isoaccepting tRNAs, 5-formylcytidine (f^5C_{34}) contributes disorder to the ASL of the one mitochondrial $tRNA^{Met}$ (Table 1; Païs de Barros *et al*, 1996). Mitochondrial $tRNA^{Met}$ decodes both the AUA and AUG codons. The f^5C_{34} might allow the mitochondrial $tRNA^{Met}$ to respond to the two codons in both the P-sites and the A-sites of the ribosome. The 2'-O-methylations of G and C (Gm_{34} and Cm_{32}) seem to introduce some dynamics to the 5'-side of purine-rich anticodon loops that already have considerable order owing to the stacking properties of the purines (Table 1). A polymer composed of the parent compound (7-deazaguanylic acid) of the modified nucleoside queosine, Q, showed few thermodynamic differences when compared to polyG (Seela *et al*, 1982). However, molecular-dynamics simulations of

the anticodon-loop structures of tRNAs containing Q (Asn, Asp, His and Tyr) indicated that the modification restricts anticodon dynamics (Morris *et al*, 1999).

Purine-37 modifications are 3'-adjacent to the anticodon and can be complex. They negate intra-loop base pairing and thereby ensure the correct width to the anticodon loop (Dao *et al*, 1994). In negating intra-loop base pairing, the purine-37 modification lowers the melting temperature of an ASL compared with that of the unmodified RNA (Stuart *et al,* 2000). However, the augmented stacking properties of these modifications order the 3'-side of the anticodon domain (Table 1). This is apparent not only in the X-ray crystallographic structures of tRNAs, but also in the solution-structure analyses of modified ASLs in comparison to their unmodified counterparts. The diverse chemistries of N6-methyladenosine, N6-isopentenyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonylcarbamoyladenosine, N1-methylguanosine and wyeosine (m^6A_{37}, i^6A_{37}, t^6A_{37}, $ms^2t^6A_{37}$, m^1G_{37} and yW_{37}) bring order to the ASL while negating intra-loop base pairing (Table 1). However, the purine 37 is the only nucleoside within the anticodon domain that shows any substantial movement from its geometry in the unbound ASL to that of the ASL bound to the codon in the A-site (Vendeix *et al*, 2008; Weixlbaumer *et al,* 2007; Murphy *et al,* 2004). The purine 37 nucleoside moves to a position above the third base of the anticodon and the first base of the codon (Weixlbaumer *et al,* 2007; Murphy *et al,* 2004). The position maintains the 3'-stack of the anticodon domain and, at the same time, becomes a hydrophobic platform that stabilizes the first base pair of the anticodon–codon interaction. Many of the modifications of purine 37 increase its hydrophobicity (Agris, 1996) and, therefore, enhance its ability to stabilize this crucial first base pair—particularly when the pair is an A•U or U•A. Almost without exception, tRNAs that have a A_{36} also have an i^6A_{37} or a derivative at position 37; tRNAs that have a U_{36} frequently have a t^6A_{37} or one of its derivatives at position 37. This observation holds for the tRNAs of Phe, Leu, Ser, Tyr, Cys and Trp, and of Ile, Met, Thr, Asn, Lys and Ser, respectively; this tendency is therefore related to decoding and not to the recognition of the tRNAs by cognate aminoacyl-tRNA synthetase. The modified purine 37 facilitates codon binding (Agris, 2004) and is important for maintaining the translational reading frame (Urbonavicius *et al,* 2001).

The contribution of modified nucleosides to the ordering of RNA structure is evident for modifications in loops, whereas their enthalpic (ΔH) contributions are readily observed in the base-paired regions of the RNA. Investigations of RNA duplexes show that certain modifications enhance the ΔH contribution to the thermal stability of base pairing, such as the s^2U in pairing with A (Testa *et al*, 1999). However, significant changes in the enthalpy term of the free-energy determination are not always evident from the study of unpaired and naturally occurring modifications in the anticodon loop (Ashraf *et al*, 2000) or the unpaired terminus of a duplex (Kierzek & Kierzek, 2001). For example, when s^2U_{34} at the wobble position of ASL^{Lys} was studied, it did not contribute a significant change in enthalpy; however, it did reduce the conformational dynamics of the loop and enhanced the codon binding (Ashraf *et al*, 1999). Ψ occurs in the anticodon loop at position 38, in the anticodon stem at residue 39 and sometimes at 31, 35 or 40. The entropic contribution of Ψ seems to be dependent on location, stem or loop, and context. Ψ_{39}, in the ASL stem and adjacent to the loop (Fig 2), has been most extensively studied. It contributes to the thermal stability of the anticodon stem of tRNA and to the order of the anticodon domain (Table 1); however, in the absence of other modifications, it does not contribute to codon binding (Yarian *et al,* 1999). Ψ_{55} is commonly found in the TSL with T_{54}. Although extremely common, neither Ψ_{55} nor T_{54} results in a significant entropic contribution to the tRNA (Table 1). T_{54}, which makes a reverse Hoogsten pair with A_{58}—often N1-methyladenosine—across the TSL loop, modestly increased the thermal stability of the TSL (Sengupta *et al*, 2000). Consistent with our understanding of the contribution of the 2-thio group to the stability of U•A base pairs, tRNAs of thermophilic organisms are further modified from T_{54} to s^2T_{54} and show an increased thermal stability (Shigi *et al,* 2002). The modification 5-methylcytosine is found at position 40 (m^5C_{40}) within the ASL stem, and at position 49 of the TSL stem. The modifications do not seem to contribute order to either of these domains (Table 1). In contrast to the many modifications that constrain the molecular dynamics of tRNA, dihydrouridine located in the DSL (Fig 2) increases molecular motion (Table 1). D, which is the only non-aromatic nucleoside found in nucleic acids, is unable to stack and is so strongly C2'-*endo* that it transfers the conformation to nucleosides that are 3'-adjacent (Stuart *et al*, 1996). The tRNAs of the psychrophilic organisms growing at temperatures of −5 °C to 12 °C are supplemented with D in comparison to mesophiles (Dalluge *et al,* 1997), therefore taking advantage of the contribution of the nucleoside to dynamics.

Conclusion

The wobble position and purine-37 modifications reduce conformational dynamics and shape the anticodon-domain architecture. However, their distinctive chemistries either restrict codon recognition to one or two codons, or expand recognition to three or even four synonymous codons. With an ordered structure, the recognition and binding of the tRNA anticodon to cognate and wobble codons occurs accurately, rapidly and with a reduced entropic penalty to the ribosome. The saving of energy and an efficient use of time are important for the cell to respond as quickly as possible to signals and environmental changes. Modifications are not exclusive to tRNA, and their evolution and maintenance was an investment that reduced the need for a repetitive expenditure of energy in moulding the functional form of RNA.

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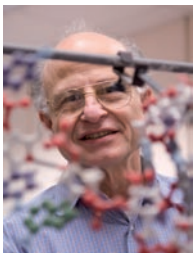

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