REVIEW

A rationale for tRNA modification circuits in the anticodon loop

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ABSTRACT

The numerous post-transcriptional modifications of tRNA play a crucial role in tRNA function. While most modifications are introduced to tRNA independently, several sets of modifications are found to be interconnected such that the presence of one set of modifications drives the formation of another modification. The vast majority of these modification circuits are found in the anticodon loop (ACL) region where the largest variety and highest density of modifications occur compared to the other parts of the tRNA and where there is relatively limited sequence and structural information. We speculate here that the modification circuits in the ACL region arise to enhance enzyme modification specificity by direct or indirect use of the first modification in the circuit as an additional recognition element for the second modification. We also describe the five well-studied modification circuits in the ACL, and outline possible mechanisms by which they may act. The prevalence of these modification circuits in the ACL and the phylogenetic conservation of some of them suggest that a number of other modification circuits will be found in this region in different organisms.

Keywords: 3-methylcytidine; wybutosine; 5-methylcytidine; 2'-O-methylguanosine

tRNAs are heavily post-transcriptionally modified, and are modified at different stages of tRNA biogenesis (Nishikura and De Robertis 1981; Jiang et al. 1997; Phizicky and Hopper 2010; Ohira and Suzuki 2011), and these modifications are important for tRNA function in translation. While most modifications are introduced to tRNA independently, several modification circuits have been identified in which one or more modifications stimulates formation of a subsequent modification. All of the well-studied examples of this ordered modification occur in the anticodon loop (ACL) region of the tRNA, but it is not known why ordered modification occurs, or why it is seemingly more prevalent in the ACL region. Here we propose that this propensity for ordered modification evolved in the ACL region because of the requirement for specificity of these modifications, combined with the relative lack of distinctive information in the ACL region.

Of the numerous tRNA modifications found in different organisms, the largest variety of modifications and the highest modification density occurs in the ACL region. For example, of the 25 chemically distinct modifications found in cytoplasmic tRNAs in the yeast *Saccharomyces*

cerevisiae, 15 are found in the 9 nucleotides (nt) of the ACL region, comprising loop residues N₃₂–N₃₈ and the closing base pair N₃₁–N₃₉, while 16 are found in the remaining 67 or more nucleotides in the main body of the tRNA, comprising the acceptor stem, D-stem-loop, the bulk of the anticodon stem (from pairs N₂₇–N₄₃ to N₃₀–N₄₀), the T-stem-loop, and the variable arm (Fig. 1A,B). Six of these modifications are found in both regions. A similar biased distribution of tRNA modifications is widely found in other organisms: Of the 28 distinct tRNA modifications in *Escherichia coli*, 21 are found in the ACL region, while eight are found in the main body; and of the 28 distinct modifications in cytoplasmic tRNAs in humans, 17 are in the ACL region and 17 are in the main body (Machnicka et al. 2014). Overall modification density is also heavily biased toward the ACL region in all organisms. Thus, among sequenced eukaryotic cytoplasmic tRNAs, on average 30% of the ACL region residues are modified, while only 14.8% of the residues in the main body are modified (29.8% and 14.7% in yeast), and these percentages

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FIGURE 1. Schematic of tRNA and the biochemically distinct modifications found in the *S. cerevisiae* ACL region. (A) The secondary structure of tRNA. Each circle represents a residue and is color-coded based on subdomains as indicated. (B) Schematic of modifications found in each residue of the *S. cerevisiae* ACL region.

are similarly skewed in bacteria (15.0% in the ACL region and 5.7% in the main body) and in archaea (12.8% and 9.6%, respectively) (Machnicka et al. 2014).

The enrichment of the variety and density of modifications in the ACL region is consistent with their important and varied roles during translation (Phizicky and Hopper 2010; Gu et al. 2014; Grosjean and Westhof 2016). A number of ACL region modifications affect mRNA decoding or reading frame maintenance, by modulating codon:anticodon interactions and fine-tuning local structure during translation (Björk et al. 1989, 2007; Urbonavicius et al. 2001; Lecoite et al. 2002; Murphy and Ramakrishnan 2004; Waas et al. 2007; Weixlbaumer et al. 2007; Johansson et al. 2008; El Yacoubi et al. 2011; Maehigashi et al. 2014; Lorenz et al. 2017). Specifically, to ensure the efficiency and accuracy of translation, all tRNAs adopt a canonical U-turn structure in the ACL to promote a stable codon–anticodon interaction in the ribosome A-site (Auffinger and Westhof 2001), and many ACL region modifications reinforce formation of this conserved loop structure by preventing base-pairing between nucleotides in the ACL, and by improving stacking interactions with neighboring residues (Murphy et al. 2004; Agris 2008). In addition, some ACL region modifications have a crucial role in ensuring charging fidelity by serving as charging determinants or anti-determinants (Muramatsu et al. 1988; Pütz et al. 1994). The importance of ACL modifications relative to body modifications is underscored by their preferential phylogenetic retention. For example, in the human unicellular endosymbiont *Candidatus Riesia*

pediculicola, which has a streamlined genome and likely a minimal tRNA modification set, the ACL modifications have been retained, whereas the body modifications have been lost (de Crécy-Lagard et al. 2012).

Many modification enzymes target a single base at specific positions

Most tRNA modification enzymes that modify body residues in the tRNA target a specific base, albeit by different mechanisms. For example, the yeast tRNA pseudouridine (Ψ) synthase Pus4 modifies U₅₅ by recognizing the identity of all the universally conserved nucleotides in the T-loop and its proximal stem as well as the structure of a portion of the T-arm (Becker et al. 1997), and consistently modifies mRNAs with a similar stem–loop motif (Lovejoy et al. 2014). Similarly, the yeast tRNA 5-methyluridine (m⁵U) methyltransferase modifies U₅₄ by recognizing several conserved nucleotides in the T stem–loop as well as the stacked T-stem and acceptor stem (Becker et al. 1997); the yeast 7-methylguanosine (m⁷G) tRNA methyltransferase Trm8/Trm82 recognizes the local structure around the variable loop and especially the D arm and T arm to modify residue G₄₆ in the third residue of variable loops of 5 nt (Leulliot et al. 2008), similar to the *Aquifex aeolicus* m⁷G MTase (Okamoto et al. 2004); and the *E. coli* U₂₀ dihydrouridine synthase DusC interacts with the D and T stem–loops to orient tRNA and U₂₀ near its catalytic site (Byrne et al. 2015). However, for some body modifications, the specificity of the enzymes is less clear. For example, the yeast 1-methylguanosine (m¹G) methyltransferase Trm10 modifies 13 of 19 tRNA species that have a G₉ residue, with no obvious common sequence element, although Trm10 substrate recognition seems to depend on an as yet undefined structural conformation, since tRNAs with an extended variable loop are consistently unmodified (Swinehart et al. 2013). Similar arguments may explain the specificity of Trm3 for 2'-O-methylation of G₁₈ on a subset of yeast tRNAs (Cavaillé et al. 1999), and of archaeosine tRNA-guanine transglycosylase, which modifies G₁₅ on a subset of tRNAs through an alternative λ-form tRNA conformation (Ishitani et al. 2003).

In the ACL region, some modification enzymes also only require the presence of the correct nucleotide at the residue to be modified. One such example is yeast Tad2/3, which in the context of tRNA catalyzes inosine (I) formation in all eight sequenced tRNA with A₃₄ (Gerber and Keller 1999). Similarly, *E. coli* TruA recognizes and pseudouridylates U₃₈, U₃₉, and U₄₀ of the anticodon stem–loop, using the elbow and the D-stem of the tRNA to establish the orientation of tRNA and position the modification sites near the catalytic center (Hur and Stroud 2007), and its family member yeast Pus3 appears to modify all tRNAs with U₃₈ or U₃₉ (Machnicka et al. 2014). Slightly different recognition mechanisms involving relatively simple rules

are used by some other ACL region enzymes. For example, enzymes responsible for t^6A_{37} (N^6 -threonylcarbamoyl adenosine) specifically recognize tRNAs with U_{36} residues in all domains of life (Deutsch et al. 2012; Miyauchi et al. 2013). Another possible example in this category is the $tRNA^iA_{37}$ (N^6 -isopentenyladenosine) synthase, which requires A_{36} – A_{37} – A_{38} and likely some other secondary elements (Persson et al. 1994; Motorin et al. 1997).

Consistent with their lack of discrimination among different tRNA species, many modifications are usually made independently of other modifications, since deletion of one modifying enzyme often does not alter levels of other modifications in the cell (Huang et al. 2005; Wilkinson et al. 2007; Kotelawala et al. 2008; Guy et al. 2012; Han et al. 2015).

Some modification enzymes require prior modifications at other residues for efficient modification

In contrast to tRNA body residues, some residues in the ACL differ greatly in their modifications in different tRNAs. Thus, U_{34} has five different fates in different yeast tRNAs: It can be unmodified, or it is modified to one of four different derivatives (Fig. 1B). Similarly, C_{32} is unmodified or modified to either of two different derivatives in different tRNAs; A_{37} is unmodified or modified to three different derivatives; and G_{37} is modified to either of two derivatives.

This variability of modification of different ACL residues in different tRNAs requires unique mechanisms of recognition. As we document further below, a subset of these mechanisms involves prior modification of other residues (Fig. 2). Recent examples of such modification circuits include wybutosine (yW) formation at m^1G_{37} of $tRNA^{Phe}$ in *Schizosaccharomyces pombe*, *S. cerevisiae* and humans, which is greatly stimulated by 2'-O-methylated C_{32} (Cm) and G_{34} (Gm) (Guy and Phizicky 2015; Guy et al. 2012, 2015); 3-methylcytidine (m^3C) at C_{32} , which is greatly stimulated by prior i^6A_{37} formation in *S. pombe*, *S. cerevisiae*, and likely mouse (Arimbasseri et al. 2016; Han et al. 2017), or by prior t^6A_{37} (Han et al. 2017); Cm_{34} or Um_{34} modification of *E. coli* $tRNA^{Leu(CAA)}$ and $tRNA^{Leu(UAA)}$, which is stimulated by i^6A_{37} ; 5-methylcytidine (m^5C) at C_{38} in *S. pombe* and *Dictyostelium discoideum*, which is stimulated by prior queuosine (Q) formation at residue 34 (Muller et al. 2015); and A to I editing at N_{34} of

Trypanosoma brucei $tRNA^{Thr(AGU)}$, which is stimulated by prior m^3C_{32} formation and then deamination to form m^3U (Rubio et al. 2006, 2017). Note that some other complex modifications require a multistep reaction, and the dependence of a latter step reaction on the prior modification intermediates at the same residue (Grosjean et al. 1995; Morl et al. 1995) is not considered as a modification circuit by this review, and will not be further discussed.

Although a few modification circuits are reported to occur in the tRNA main body, all of them are found in *Thermus thermophilus*, an extreme thermophilic eubacteria of which tRNAs are adapted to high growth temperatures (Yokoyama et al. 1987). Examples include formation of 2-thioribothymidine (s^2T) from ribothymidine (rT) at U_{54} , which is stimulated by 1-methyladenosine (m^1A) at A_{58} (Shigi et al. 2006); Gm_{18} and m^1G_{37} , which are stimulated by m^7G_{46} at higher culture temperatures (Tomikawa et al. 2010); and 5-methyl-2-thiouridine (m^5s^2U) at U_{54} and m^1A_{58} , which are negatively regulated by Ψ_{55} at lower culture temperatures (Ishida et al. 2011). The latter two circuits are thought to be part of a network that maintains the proper balance of tRNA modifications and responds to temperature changes (Ishida et al. 2011).

A hypothesis to explain the prevalence of ACL region modification circuits

It is intriguing to consider why these modification circuits tend to occur in the ACL region, but not in other parts of a tRNA. One possible explanation is that ACL region modifications introduced first may act as additional recognition elements for other modifications in response to the requirement for modifications with great variation and high density

FIGURE 2. Schematic of the five anticodon loop circuits discussed in this review. (A) Cm_{32} and Gm_{34} drive yW_{37} formation in $tRNA^{Phe}$ of *S. pombe*, *S. cerevisiae*, and humans. (B) i^6A_{37} drives m^3C_{32} formation in $tRNA^{Ser}$ of *S. pombe* and *S. cerevisiae*, and t^6A_{37} drives m^3C_{32} formation in $tRNA^{Thr}$ of *S. cerevisiae*. (C) i^6A_{37} drives Cm_{34} and Um_{34} formation in $tRNA^{Leu(CAA)}$ and $tRNA^{Leu(UAA)}$ of *E. coli*. (D) Q_{34} drives m^5C_{38} formation in $tRNA^{Asp}$ of *S. pombe* and *D. discoideum*. (E) C_{32} to m^3C_{32} to m^3U_{32} drives I_{34} formation in $tRNA^{Thr(AGU)}$ of *T. brucei*.

FIGURE 3. Schematic of the *S. cerevisiae* ACL landscape.

in this region, combined with the lack of variability in the local sequence and structure. Indeed, the ACL sequence outside the anticodon itself has limited variation: N₃₃ is almost always a uridine in elongator tRNAs; N₃₇ is almost always a purine; and the vast majority of N₃₂–N₃₈ combinations are C-A, U-A, or U-U (Auffinger and Westhof 1999; Marck and Grosjean 2002). Of the 2726 tDNA gene sequences in the tRNA database, 48% of N₃₂–N₃₈ pairs are C-A, 18% are U-A, and 11% are U-U (Auffinger and Westhof 1999; Marck and Grosjean 2002); while in yeast 52% of N₃₂–N₃₈ pairs are C-A, 19% are U-A, and 19% are U-C (Fig. 3; Jühling et al. 2009). Thus, the majority of sequence variation in the ACL region comes from the anticodon sequence, which is a unique signature for each tRNA species and is often important for tRNA synthetase recognition (Kisselev 1985; Giegé et al. 1998). Moreover, as addressed above, the universal conservation of anticodon stem-loop structure is selected by the translation machinery, so structural information in this region is unlikely to be useful for individual enzyme specificity. The conserved sequence and structural similarities in the ACL region provide little room for substrate recognition of ACL modifying enzymes, if their specificities solely come from sequence elements around the modification site. Therefore, it is reasonable to speculate that evolution may have selected for modification circuits in which modifications introduced first positively or negatively regulate formation of other nearby modifications, adding another layer of complexity in the ACL region to enhance enzyme modification specificity.

Three mechanisms might explain the observed ordered modification circuits in ACLs (Fig. 4). First, the initial modification might directly act as a recognition element for the subse-

quent modification enzyme, much like bromodomains found in chromatin-associated proteins and nuclear acetyltransferases, which bind acetyl-lysine (Zeng and Zhou 2002). A classical example of this mechanism of ordered modification is queuosine formation at residue 34 in marsupial mitochondrial tRNA^{Asp}. This modification requires prior deamination of C₃₅ of the encoded GCC anticodon to form a GUC anticodon, generating the U₃₃G₃₄U₃₅ recognition sequence for tRNA guanine transglycosylase to catalyze queuosine formation (Morl et al. 1995; Börner et al. 1996; Xie et al. 2003). Second, the initial modification might alter the structure of the ACL to present a structure that is itself recognized or properly exposes the target residue for the subsequent modification enzyme. Third, an initial modification might prevent a particular subsequent modification from occurring, allowing a different modification enzyme to act in its stead. Although the precise mechanism by which any of the recently established ordered modification circuits is not yet known, we describe below what is known in each case.

The yW₃₇ modification, or derivatives of it, is almost universally found on tRNA^{Phe} in eukaryotes, and not on any other tRNA (Machnicka et al. 2013), and it is known

FIGURE 4. Three possible mechanisms to explain the ordered modification circuits in the ACL region, using the i⁶A₃₇/t⁶A₃₇–m³C₃₂ circuit as an example. (A) The initial modification (i⁶A₃₇ or t⁶A₃₇) directly acts as a recognition element for the subsequent modification enzyme (Trm140 for m³C₃₂ modification). (B) The initial modification (i⁶A₃₇ or t⁶A₃₇) alters the structure of the ACL to present a structure that is itself recognized or properly exposes the target residue (C₃₂) for the subsequent modification enzyme (Trm140). (C) The initial modification (i⁶A₃₇ or t⁶A₃₇) prevents a subsequent modification from occurring (Cm₃₂ by Trm7/Trm732), allowing a different modification enzyme (Trm140) to act instead.

that the GAA anticodon sequence is necessary for yW formation in oocytes (Droogmans and Grosjean 1987). yW formation is the last step of tRNA^{Phe} maturation, and the critical 2'-O-methylation of C₃₂ and N₃₄ required for efficient yW formation (Guy et al. 2012) is part of an intricate tRNA^{Phe} maturation pathway in *S. cerevisiae* (Ohira and Suzuki 2011). These steps include initial export of unspliced pre-tRNA from the nucleus to the cytoplasm (Sarkar and Hopper 1998), tRNA splicing on the mitochondrial surface (Yoshihisa et al. 2007), 2'-O-methylation of C₃₂ and N₃₄ by Trm7/Trm732 and Trm7/Trm734 (Guy et al. 2012), retrograde transport of the tRNA back to the nucleus (Takano et al. 2005; Murthi et al. 2010), formation of m¹G₃₇, reexport of the tRNA^{Phe} to the cytoplasm, and then further modification of m¹G₃₇ by Tyw1, Tyw2, Tyw3, and Tyw4 to form yW (Noma et al. 2006; Ohira and Suzuki 2011). There is an apo structure of Tyw1, which catalyzes the first step of yW from m¹G₃₇, from *Methanococcus jannaschii* and *Pyrococcus horikoshii* (Goto-Ito et al. 2007; Suzuki et al. 2007); however, little insight is provided for the specificity of the enzyme.

The dependence of yW₃₇ formation on prior Cm₃₂ and Gm₃₄ modification in *S. cerevisiae*, *S. pombe*, and humans (Guy and Phizicky 2015; Guy et al. 2012, 2015) could be explained by one of the first two mechanisms mentioned above: direct Tyw1 recognition of Cm₃₂ and Gm₃₄; and Tyw1 recognition of the prestructured ACL region possibly including increased Tyw1 access to m¹G₃₇. Both models would be consistent with the partial modification of yW observed in *trm734Δ* mutants, which have the Cm₃₂ modification but not Gm₃₄ and *trm732Δ* mutants, which have Gm₃₄, but not Cm₃₂ (Guy and Phizicky 2015; Guy et al. 2012, 2015).

The m³C₃₂ modification is found in almost all sequenced tRNA^{Ser} and tRNA^{Thr} species with C₃₂, as well as in mammalian tRNA^{Arg}(CCU) and tRNA^{Arg}(UCU), but not in other tRNA species. In *S. cerevisiae*, a single Trm140 homolog catalyzes m³C₃₂ formation in all six of its tRNA^{Thr} and tRNA^{Ser} substrates (D'Silva et al. 2011; Noma et al. 2011), whereas in *S. pombe* there are two paralogs, one responsible for tRNA^{Ser} modification and the other for tRNA^{Thr} modification (Arimbasseri et al. 2016). *S. cerevisiae* Trm140 has two recognition modes for its substrates, and each involves t⁶A and/or i⁶A: Trm140 recognizes the sequence element G₃₅-U₃₆-t⁶A₃₇ of tRNA^{Thr} substrates, and this sequence element is necessary and sufficient for m³C modification of another tRNA species (Han et al. 2017). In contrast, Trm140 recognizes tRNA^{Ser} species through interaction with seryl-tRNA synthetase and the distinctive tRNA^{Ser} variable loop recognized by SerRS (Himeno et al. 1997), as well as by either t⁶A₃₇ or i⁶A₃₇ (Han et al. 2017). In both sets of substrates, A₃₇ modifications (either i⁶A or t⁶A) are stimulatory, but not absolutely necessary for m³C₃₂ formation, and available

data suggests that each element of tRNA^{Thr} and tRNA^{Ser} recognition contributes independently to Trm140 recognition, including t⁶A and i⁶A (Han et al. 2017). A similar result was previously observed in *S. pombe* in which i⁶A₃₇ stimulates m³C₃₂ formation for all three tRNA^{Ser} species with the modification (Arimbasseri et al. 2016).

The role of t⁶A₃₇ or i⁶A₃₇ in stimulating m³C₃₂ modification by Trm140 family members is unclear, but it seems unlikely that *S. cerevisiae* Trm140 directly recognizes both modifications, since they are chemically very distinct: The isopentenyl group of i⁶A is much more hydrophobic than the acidic and polar threonyl group found in t⁶A. A more plausible explanation is that both i⁶A and t⁶A facilitate formation of the proper structure of the tRNA ACL, allowing for Trm140 recognition and m³C₃₂ modification. Indeed, t⁶A₃₇ has been shown to have a role in preordering the ACL by preventing base-pairing between U₃₃ and A₃₇, and by enhancing stacking interactions between A₃₇ and A₃₈ (Murphy et al. 2004). It is also possible that the bulky t⁶A and i⁶A modifications act as a negative recognition element for Trm7/Trm732, to prevent Cm₃₂ modification in *S. cerevisiae* (Guy et al. 2012), although its recognition elements are not yet known.

Similarly, any of the three mechanisms might also be used to explain the dependence of Cm₃₄ and Um₃₄ on prior i⁶A₃₇ modification in *E. coli*. The *E. coli* N₃₄ 2'-O-methyltransferase TrmL catalyzes this methyl transfer reaction on its two substrates, tRNA^{Leu}(CAA) and tRNA^{Leu}(UAA) (Liu et al. 2013). The presence of i⁶A₃₇, which is catalyzed by MiaA (Soderberg and Poulter 2001), strongly stimulates formation of Cm₃₄ and Um₃₄, since in vitro transcribed tRNA^{Leu}(CAA) and tRNA^{Leu}(UAA) without modifications are not substrates of TrmL, while the same tRNA transcripts are efficient TrmL substrates if they are premodified by recombinant MiaA (Zhou et al. 2015). In the same study, the sequence A₃₆-A₃₇-A₃₈ was shown to be important for Cm₃₄ and Um₃₄ formation; however, whether this sequence element is necessary for recognition by TrmL itself or for the i⁶A₃₇ modification is unclear, since the A₃₆-A₃₇-A₃₈ motif is the known determinant for MiaA (Soderberg and Poulter 2001).

For m⁵C₃₈ modification of tRNA^{Asp} in *S. pombe*, the stimulatory role of Q₃₄ has been demonstrated both in vivo and in vitro with purified Pmt1 methyltransferase (Muller et al. 2015). This experiment suggests either that Pmt1 directly interacts with Q₃₄, or that Q₃₄ appropriately affects the ACL structure of tRNA^{Asp}. The crystal structures of several Dnmt2 homologs have been solved, but unfortunately without the tRNA substrate (Dong et al. 2001; Schulz et al. 2012; Li et al. 2013). Based on a modeled tRNA^{Asp}-Dnmt2 structure, it is also possible that Q₃₄ could alter the geometry of the ACL, allowing for better interactions between Dnmt2 and tRNA. Nonetheless, Q₃₄ cannot be the sole determinant, since the tRNA guanine transglycosylase (TGT) that exchanges G with Q in tRNAs acts on

all tRNAs with a GUN anticodon (Katze et al. 1982), whereas m⁵C₃₈ is specific for tRNA^{Asp}.

Deamination of A₃₄ to inosine in tRNA^{Thr(AGU)} in *T. brucei* is stimulated by C₃₂ deamination to uridine, based on the observation that in vitro transcribed tRNA^{Thr(AGU)} with U₃₂ is edited to I₃₄ with higher efficiency and initial rate than transcripts with C₃₂ (Rubio et al. 2006). Remarkably, recent results show that the initial C to U editing step is preceded by m³C modification by a complex of the m³C methyltransferase Trm140 and the deaminase ADAT2/3, which catalyzes both reactions (Rubio et al. 2017; McKenney et al. 2018). While formation of m³C₃₂ followed by formation of m³U₃₂ occurs in the nucleus prior to 5' leader removal and export of tRNA into cytoplasm, A to I editing at the wobble residue occurs in the cytoplasm (Gaston et al. 2007). As in the other cases, it is unclear how m³U₃₂ stimulates the subsequent A₃₄ deamination.

In summary, we have documented a large number of modifications in the ACL region that depend on prior modifications in the tRNA, and have proposed that the modification circuits may have evolved so that the second modification in the circuit can use additional recognition sites directly or indirectly from the first modification to achieve specificity. This helps resolve the dilemma in the ACL region of the need for different modifications at the same residue, combined with the lack of sufficient sequence variation or structural information to obtain the desired specificity. In principle, the anticodon sequence or set of anticodon sequences can provide some of the required specificity, but not always, such as in the case of m³C modification, which acts on tRNAs with very different anticodons. In all of these documented cases, lack of the first modifications in nonsubstrate tRNAs would also prevent the second modifications in these tRNAs, thereby improving overall specificity of modifications in the entire tRNA population. This set of five modification circuits within the ACL, and the phylogenetic conservation of two of them, suggest the existence of other modification circuits in the ACLs of different tRNA species or organisms, driven in part by the need for specific substrate recognition.

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