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 N_{32}–N_{38} and the closing base pair N_{31}–N_{39}, 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 N_{27}–N_{43} to N_{30}–N_{40}), 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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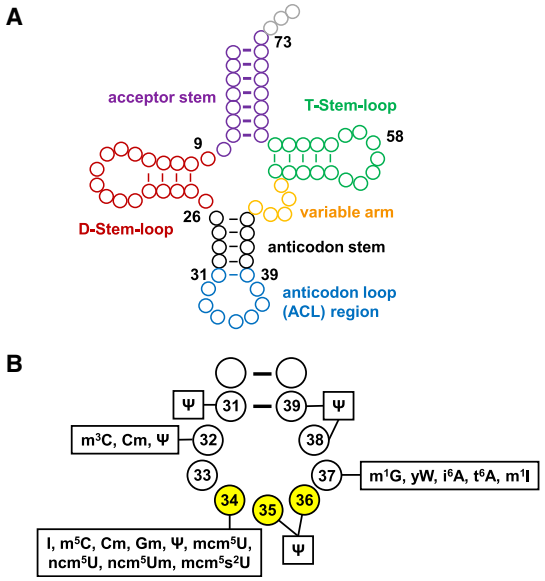

FIGURE 1. Schematic of tRNA and the biochemically distinct modifications found in the *S. cerevisiae* ACL region. (*A*) The secondary structure of tRNA. Each circle represents a residue and is color-coded based on subdomains as indicated. (*B*) Schematic of modifications found in each residue of the *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; Lecointe 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 U_{55} 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 (m^5U) methyltransferase modifies U_{54} 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 (m^7G) tRNA methyltransferase Trm8/Trm82 recognizes the local structure around the variable loop and especially the D arm and T arm to modify residue G_{46} in the third residue of variable loops of 5 nt (Leulliot et al. 2008), similar to the *Aquifex aeolicus* m^7G MTase (Okamoto et al. 2004); and the *E. coli* U_{20} dihydrouridine synthase DusC interacts with the D and T stem–loops to orient tRNA and U_{20} 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 (m^1G) methyltransferase Trm10 modifies 13 of 19 tRNA species that have a G_9 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 G_{18} on a subset of yeast tRNAs (Cavaillé et al. 1999), and of archaeosine tRNA-guanine transglycosylase, which modifies G_{15} 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 A_{34} (Gerber and Keller 1999). Similarly, *E. coli* TruA recognizes and pseudouridylates U_{38}, U_{39}, and U_{40} 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 U_{38} or U_{39} (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 i^6A_{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-methycytidine (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-methycytidine (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

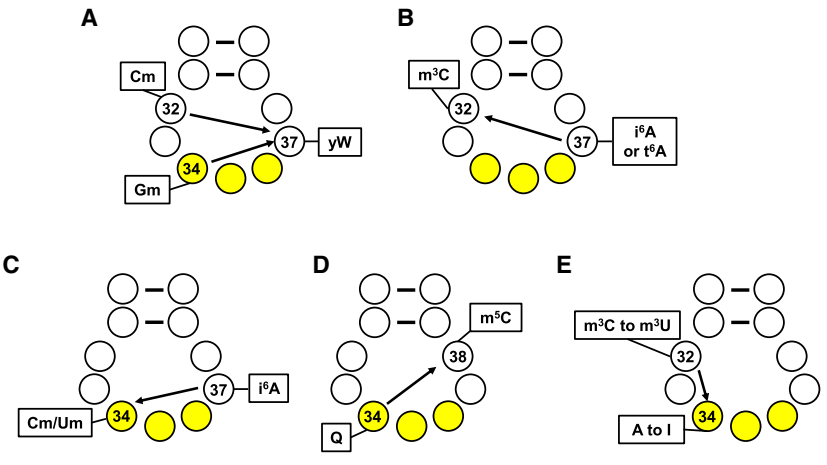

FIGURE 2. Schematic of the five anticodon loop circuits discussed in this review. (*A*) Cm_{32} and Gm_{34} drive yW_{37} formation in $tRNA^{Phe}$ of *S. pombe*, *S. cerevisiae*, and humans. (*B*) i^6A_{37} drives m^3C_{32} formation in $tRNA^{Ser}$ of *S. pombe* and *S. cerevisiae*, and t^6A_{37} drives 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*.

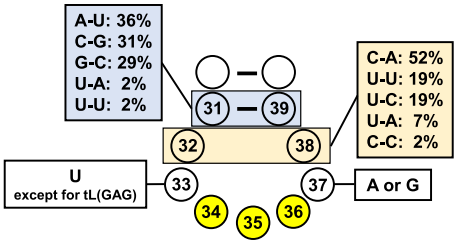

FIGURE 3. Schematic of the *S. cerevisiae* ACL landscape.

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: N_{33} is almost always a uridine in elongator tRNAs; N_{37} is almost always a purine; and the vast majority of N_{32}–N_{38} 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 N_{32}–N_{38} 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 N_{32}–N_{38} 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 subsequent 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 C_{35} of the encoded GCC anticodon to form a GUC anticodon, generating the $U_{33}G_{34}U_{35}$ 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 yW_{37} 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

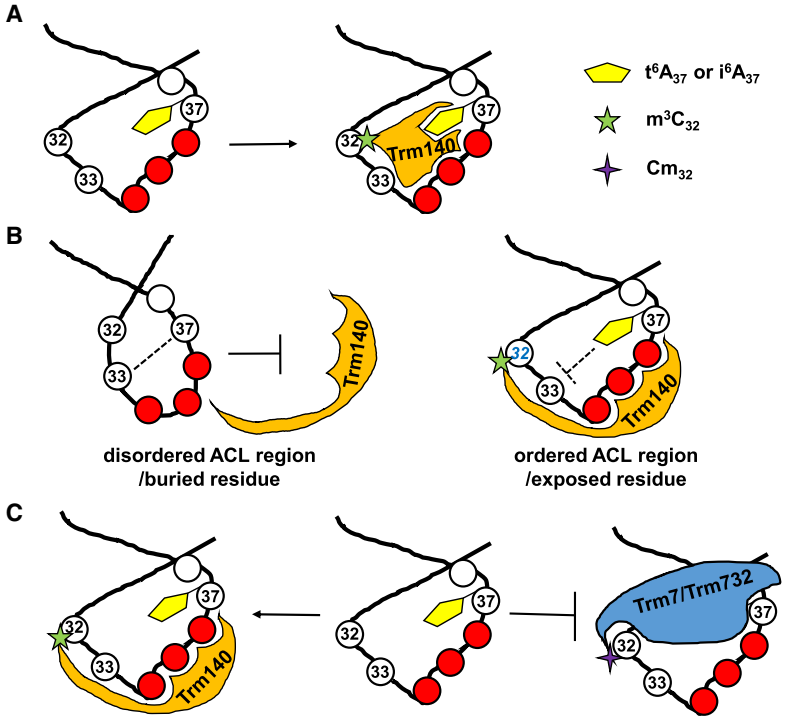

FIGURE 4. Three possible mechanisms to explain the ordered modification circuits in the ACL region, using the i^6A_{37}/t^6A_{37}-m^3C_{32} circuit as an example. (*A*) The initial modification (i^6A_{37} or t^6A_{37}) directly acts as a recognition element for the subsequent modification enzyme (Trm140 for m^3C_{32} modification). (*B*) The initial modification (i^6A_{37} or t^6A_{37}) alters the structure of the ACL to present a structure that is itself recognized or properly exposes the target residue (C_{32}) for the subsequent modification enzyme (Trm140). (*C*) The initial modification (i^6A_{37} or t^6A_{37}) prevents a subsequent modification from occurring (Cm_{32} by Trm7/732), 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 C_{32} and N_{34} 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 C_{32} and N_{34} 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 m^1G_{37}, reexport of the $tRNA^{Phe}$ to the cytoplasm, and then further modification of m^1G_{37} 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 m^1G_{37}, 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 yW_{37} formation on prior Cm_{32} and Gm_{34} 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 Cm_{32} and Gm_{34}; and Tyw1 recognition of the prestructured ACL region possibly including increased Tyw1 access to m^1G_{37}. Both models would be consistent with the partial modification of yW observed in *trm734Δ* mutants, which have the Cm_{32} modification but not Gm_{34} and *trm732Δ* mutants, which have Gm_{34}, but not Cm_{32} (Guy and Phizicky 2015; Guy et al. 2012, 2015).

The m^3C_{32} modification is found in almost all sequenced $tRNA^{Ser}$ and $tRNA^{Thr}$ species with C_{32}, 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 m^3C_{32} 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 t^6A and/or i^6A: Trm140 recognizes the sequence element G_{35}–U_{36}–t^6A_{37} of $tRNA^{Thr}$ substrates, and this sequence element is necessary and sufficient for m^3C 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 t^6A_{37} or i^6A_{37} (Han et al. 2017). In both sets of substrates, A_{37} modifications (either i^6A or t^6A) are stimulatory, but not absolutely necessary for m^3C_{32} formation, and available data suggests that each element of $tRNA^{Thr}$ and $tRNA^{Ser}$ recognition contributes independently to Trm140 recognition, including t^6A and i^6A (Han et al. 2017). A similar result was previously observed in *S. pombe* in which i^6A_{37} stimulates m^3C_{32} formation for all three $tRNA^{Ser}$ species with the modification (Arimbasseri et al. 2016).

The role of t^6A_{37} or i^6A_{37} in stimulating m^3C_{32} 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 i^6A is much more hydrophobic than the acidic and polar threonyl group found in t^6A. A more plausible explanation is that both i^6A and t^6A facilitate formation of the proper structure of the tRNA ACL, allowing for Trm140 recognition and m^3C_{32} modification. Indeed, t^6A_{37} has been shown to have a role in preordering the ACL by preventing base-pairing between U_{33} and A_{37}, and by enhancing stacking interactions between A_{37} and A_{38} (Murphy et al. 2004). It is also possible that the bulky t^6A and i^6A modifications act as a negative recognition element for Trm7/Trm732, to prevent Cm_{32} 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 Cm_{34} and Um_{34} on prior i^6A_{37} modification in *E.coli*. The *E. coli* N_{34} 2′-*O*-methytransferase TrmL catalyzes this methyl transfer reaction on its two substrates, $tRNA^{Leu(CAA)}$ and $tRNA^{Leu(UAA)}$ (Liu et al. 2013). The presence of i^6A_{37}, which is catalyzed by MiaA (Soderberg and Poulter 2001), strongly stimulates formation of Cm_{34} and Um_{34}, 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 A_{36}–A_{37}–A_{38} was shown to be important for Cm_{34} and Um_{34} formation; however, whether this sequence element is necessary for recognition by TrmL itself or for the i^6A_{37} modification is unclear, since the A_{36}–A_{37}–A_{38} motif is the known determinant for MiaA (Soderberg and Poulter 2001).

For m^5C_{38} modification of $tRNA^{Asp}$ in *S. pombe*, the stimulatory role of Q_{34} 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 Q_{34}, or that Q_{34} 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 Q_{34} could alter the geometry of the ACL, allowing for better interactions between Dnmt2 and tRNA. Nonetheless, Q_{34} 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 m^5C_{38} is specific for $tRNA^{Asp}$.

Deamination of A_{34} to inosine in $tRNA^{Thr(AGU)}$ in *T. brucei* is stimulated by C_{32} deamination to uridine, based on the observation that in vitro transcribed $tRNA^{Thr(AGU)}$ with U_{32} is edited to I_{34} with higher efficiency and initial rate than transcripts with C_{32} (Rubio et al. 2006). Remarkably, recent results show that the initial C to U editing step is preceded by m^3C modification by a complex of the m^3C methyltransferase Trm140 and the deaminase ADAT2/3, which catalyzes both reactions (Rubio et al. 2017; McKenney et al. 2018). While formation of m^3C_{32} followed by formation of m^3U_{32} 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 m^3U_{32} stimulates the subsequent A_{34} 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 m^3C 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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