Published in final edited form as:
*FEBS Lett*. 2010 January 21; 584(2): 265–271. doi:10.1016/j.febslet.2009.11.049.

# Do all modifications benefit all tRNAs?

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## Abstract

Despite the universality of tRNA modifications, some tRNAs lacking specific modifications are subject to degradation pathways, while other tRNAs lacking the same modifications are resistant. Here, we suggest a model in which some modifications have minor, possibly redundant, roles in specific tRNAs. This model is consistent with the low specificity of some modification enzymes. Limitations of this model include the limited assays and growth conditions on which these conclusions are based, as well as the high specificity exhibited by many modification enzymes with important roles in translation. The specificity of these enzymes is often enhanced by complex substrate recognition patterns and sub-cellular compartmentalization.

### Keywords

tRNA; modifications; degradation; methylation; thiolation; editing

## 1. Introduction

tRNA modifications are universal. All characterized tRNA species bear numerous modifications of their bases and of their corresponding ribose moieties. Modifications are found on 11.9 % of the residues of the 561 sequenced tRNAs, with a median of 8 modifications per tRNA [1]. This data set includes tRNAs from a wide range of organisms, including archaea (59 tRNAs), eubacteria (135), fungi (65), animals (111) and plants (44), as well as from chloroplasts (35), mitochondria (95) and viruses (17). Furthermore, this data set includes tRNAs with each different amino acid acceptor and each different anticodon within each of the phylogenetic domains, and multiple tRNAs from several organisms. For example, in the yeast *Saccharomyces cerevisiae*, 16.4% of the residues of the 34 sequenced cytoplasmic tRNA species bear modifications, with a range from 7 to 17 modifications per tRNA, and 9.5 % of the residues of the 17 sequenced mitochondrial tRNAs bear modifications, with a range from 6 to 9 modifications per tRNA. Thus, these results imply the universal occurrence of tRNA

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modifications. In support of this claim, tRNA modifications are also conserved in the smallest free-living organisms [2], and in organisms living in both extremely cold and extremely hot environments, although modifications are much reduced in some organisms from cold environments [3,4].

Many modification enzymes act on multiple tRNA substrates, catalyzing the same modification at a particular position, or a defined set of positions, in different tRNA species of a single organism. This is illustrated by analysis of the modifications found in cytoplasmic tRNAs of the yeast *Saccharomyces cerevisiae*. In many cases one enzyme catalyzes all of the modifications of a particular type, whether the modification is only found at one position, or is found at multiple positions in the tRNA. Thus, for example, Trm11/Trm112 is responsible for each of the 20 known occurrences of $m^2G$, which are all at position 10 [5], Trm6/Trm61 (also called Gcd10/Gcd14) is responsible for each of the 23 known occurrences of $m^1A$, which are at position 58 [6,7], the Elp-Kti complex is responsible for each of the 11 occurrences of the $cm^5U$ moiety of $mcm^5U$, $ncm^5U$, $ncm^5Um$, and $mcm^5s^2U$, which are found at position 34 [8-10] and Trm4 is responsible for all of the 30 known occurrences of $m^5C$, which are found at positions 34, 40, 48, and 49 [11]. In other cases, a group of two or more enzymes catalyzes formation of the same modification, and each enzyme is responsible for the subset of modifications that occur at a particular position or set of positions. Thus, for example, Trm5 and Trm10 each catalyze formation of the subset of the 18 characterized $m^1G$ modifications that occur at $G_{37}$ and $G_9$ respectively [12,13], Dus1, Dus2, Dus3, and Dus4 each catalyze the subset of the 100 characterized dihydrouridine modifications that occur at positions 16 and 17, 20, 20a and 20b, and 47 respectively [14], and six pseudouridylases catalyze formation of the 102 characterized pseudouridine modifications, each acting at a subset of the 15 different positions with this modification [15-20].

Whereas many of the modifications around the anticodon have significant effects on translation or translation fidelity [10,21-24], a large body of physical evidence supports the claim that modifications are also important for the folding and stability of tRNAs. When compared to native modified tRNA, completely unmodified tRNA has a reduced Tm of ~ 5°C, has reduced tertiary interactions at low $Mg^{++}$ concentrations, and is more dynamic [25-32]. The stabilization effects of modifications are almost certainly due to body modifications (those that are in the central core of the tRNA and remote from the anticodon), since residues in the anticodon region do not interact with the main body of the tRNA.

Examination of individual modifications supports the claim that modifications have a role in stabilizing tRNA structure and/or folding. Thus $T_{54}$ instead of $U_{54}$ leads to a 6 °C increase in the Tm of *E. coli* $tRNA^{fMet}$ [33] and a 2 °C increase in the Tm of an otherwise unmodified T-stem-loop oligonucleotide [34], and each of $T_{54}$, $\Psi_{55}$ and $m^5C_{49}$ in an otherwise unmodified $tRNA^{Phe}$ 3′ half-molecule contribute significantly to the binding affinity for the unmodified 5′ half of the tRNA [35]. Furthermore, model studies demonstrate that both pseudouridine and 2′-O methylation have stabilizing effects on helices [36] [37-42], and that $m^1A_9$ promotes correct folding of human mitochondrial $tRNA^{Lys}$ [43].

However, it is not clear from these studies if individual modifications have quantitatively similar stabilizing or folding effects on all tRNAs bearing the corresponding modifications. We describe below in vivo evidence demonstrating that lack of certain specific modifications in the body of tRNA has major effects on the stability or function of only a small number of tRNA species with the corresponding modifications, and only minor effects on other tRNAs with the same modifications. We also summarize evidence for lack of specificity for at least some modification enzymes. These studies thus suggest that perhaps the modification of multiple species of tRNA by a particular enzyme occurs as a result of overlapping substrate specificity, and not necessarily because of equal evolutionary demand for these modifications

in all tRNAs. Then we point out the caveats in these arguments, particularly, evidence suggesting that modifications with a role in translation generally have specific roles in each of their substrate tRNAs. We note, that in many of these cases, the modification activity itself is either highly specific or additional layers of control regulate substrate specificity.

## 2. Evidence suggesting that some modifications may have quantitatively minor roles in some tRNAs

### 2.1. Lack of $m^7G$ and $m^5C$ leads to specific degradation of mature $tRNA^{Val(AAC)}$

We have shown that yeast cells lacking $m^7G_{46}$ and $m^5C$ due to lack of Trm8 and Trm4 are temperature sensitive due to degradation and deacylation of mature $tRNA^{Val(AAC)}$ [44] by a rapid tRNA decay pathway that is mediated by the 5′-3′ exonucleases Rat1 and Xrn1, and by Met22 [45]. Two lines of evidence suggest that this degradation is specific for $tRNA^{Val(AAC)}$, and not other species lacking $m^7G_{46}$ and $m^5C$. First, the temperature sensitive phenotype of *trm8*-Δ *trm4*-Δ mutants can be restored by introduction of multicopy plasmids expressing $tRNA^{Val(AAC)}$ [44], strongly suggesting that this tRNA is the only species that is adversely affected in the mutant. Second, whereas the levels of $tRNA^{Val(AAC)}$ are reduced to less than 20% of wild type levels in *trm8*-Δ *trm4*-Δ mutants at high temperature, the levels of a number of control tRNAs remain almost constant (Fig. 1), including all three tRNA species that, like $tRNA^{Val(AAC)}$, have both $m^7G_{46}$ and $m^5C_{49}$ ($tRNA^{Phe}$ [45], $tRNA_i^{Met}$ [44] and $tRNA^{Val(CAC)}$ (J. Whipple and E. M. P., unpbulished), and the three other known tRNAs with $m^7G_{46}$ and $m^5C$ at other positions ($tRNA^{Cys}$ I.S. Chernyakov and E. M. P., unpublished), $tRNA^{Lys(UUU)}$ [44] and $tRNA^{Met}$ [44]).

These results demonstrate clearly that the combined loss of $m^7G$ and $m^5C$ has only a minor effect on targeting other tRNAs for degradation by the RTD pathway, and suggest that perhaps these modifications have only minor stabilizing effects on the function of the resistant tRNAs. Presumably the modest but distinct role of $m^5C$ of $tRNA^{Phe}$ in stabilizing binding between the two halves of the tRNA [35] is not crucial in vivo under these conditions. Presumably also, the known tertiary interactions between $m^7G_{46}$ and $G_{22}$ of the $C_{13}$:$G_{22}$ base pair of $tRNA^{Phe}$ are not grossly perturbed by loss of methylation at $m^7G$, since these interactions involve donation of hydrogen bonds from the N1 and exocyclic N2 position of the $G_{46}$ residue [46], which are available in the presence or absence of the methyl group.

### 2.2. Lack of $ac^4C_{12}$ and $Um_{44}$ leads to specific degradation of mature $tRNA^{Ser(CGA)}$ and $tRNA^{Ser(UGA)}$

We have also previously shown that yeast cells lacking $ac^4C_{12}$ and $Um_{44}$ due to lack of Tan1 and Trm44 are temperature sensitive due to degradation of mature $tRNA^{Ser(CGA)}$ and $tRNA^{Ser(UGA)}$ by the RTD pathway [47]. Two lines of evidence suggest that this degradation is specific for these two tRNA species, and not the two other $tRNA^{Ser}$ species. First, overproduction of $tRNA^{Ser(CGA)}$ and $tRNA^{Ser(UGA)}$ restores healthy growth at high temperature. Second, there is no observed reduction in levels of $tRNA^{Ser(IGA)}$, the only other tRNA with both of these modifications, or of $tRNA^{Ser(GCA)}$, the only other tRNA likely to have these modifications [47]. In addition, *tan1*-Δ *trm44*-Δ strains have mildly reduced levels of $tRNA^{Leu(GAG)}$ at high temperature, presumably due to the lack of $ac^4C_{12}$ since this tRNA does not have $Um_{44}$; however, none of the other three $tRNA^{Leu}$ species (which are the only other tRNA species with $ac^4C_{12}$) has reduced levels under these conditions.

### 2.3. Lack of $m^1A_{58}$ in tRNA appears to lead to specific degradation of pre-$tRNA_i^{Met}$ in yeast

Anderson and co-workers have shown that pre-$tRNA_i^{Met}$ lacking $m^1A_{58}$ is recognized by a nuclear surveillance system in a *trm6*$^{ts}$ mutant at non-permissive temperature, polyadenylated by Trf4 of the TRAMP complex, and degraded by Rrp6 and the nuclear exosome [6,48,49].

Three lines of evidence suggest that the turnover of pre-$tRNA_i^{Met}$ lacking $m^1A_{58}$ by this nuclear surveillance system is specific for $tRNA_i^{Met}$ rather than the other species with $m^1A_{58}$. First, the normally essential Trm6/Trm61 $m^1A_{58}$ methyltransferase can be bypassed by overproduction of initiator tRNA ($tRNA_i^{Met}$) from a multicopy plasmid, demonstrating unequivocally that despite the occurrence of $m^1A$ in 23 characterized yeast tRNAs, the only essential $m^1A$ modification is that found on initiator tRNA [6]. Second, degradation of pre-$tRNA_i^{Met}$ in a *trm6*$^{ts}$ mutant is specific, since steady state levels of several other tRNAs are unaffected under these conditions, including $tRNA^{Met}$, which has $m^1A$, $tRNA^{His}$, which does not have $m^1A$, and $tRNA^{Ser(CGA)}$ and $tRNA^{Ile(UAU)}$, which are not characterized [6]. Second, treatment of RNA from a *trm6* mutant with the TRAMP complex and Rrp44 nuclease of the exosome, results in polyadenylation and partial degradation of $tRNA_i^{Met}$, but not of $tRNA^{Trp}$, $tRNA^{Pro(UGG)}$, and $tRNA^{Tyr}$, which have $m^1A$, or $tRNA^{Leu(CAA)}$ and $tRNA^{Gly(GCC)}$, which lack $m^1A$ [50]. Thus, these results suggest that lack of $m^1A$ primarily affects $tRNA_i^{Met}$.

Anderson and co-workers [49] have speculated that one plausible explanation for the specificity of the nuclear surveillance system for pre-$tRNA_i^{Met}$ lacking $m^1A_{58}$ is the unique T-loop structure of $tRNA_i^{Met}$, which is not found in elongator tRNAs, and involves hydrogen bonds between N6 and N7 of $m^1A_{58}$ with $A_{54}$, and O2′ of $m^1A_{58}$ and $A_{60}$ [51]. Lack of the methyl group of $m^1A_{58}$ may have only minor consequences on elongator tRNA species, which have a $T_{54}$:$A_{58}$ or a $T_{54}$:$m^1A_{58}$ pair instead of the $A_{54}$:$m^1A_{58}$ pair [46,52]. Nonetheless, lack of Trm6 and $m^1A_{58}$ in yeast is only partially overcome by overproduction of $tRNA_i^{Met}$, since the cells still show a growth phenotype and are temperature sensitive [6]. This suggests either that $tRNA_i^{Met}$ lacking $m^1A_{58}$ is still poorly functional, or that one or more other species of tRNA is affected by lack of the $m^1A$ modification.

All three cases examined above document clear evidence that lack of modifications leads to degradation of specific tRNA species, through the action of quality control pathways that degrade hypomodified pre-tRNA by the nuclear surveillance system, or mature tRNA by the rapid tRNA decay pathway, with no reported observable effects on the levels of other tRNAs. These results imply that despite the substantial body of evidence that modifications can stabilize tRNA in vitro, the stabilizing effect of some of the modifications may be either too minor to measure in vivo for many tRNAs under conditions that have been tested, or redundant due to other stabilizing structural features of the tRNA.

Evidence in vitro also suggests that certain tRNA modification enzymes can lack specificity. Thus, yeast Pus1 protein presumably has low specificity because it is responsible for modification of tRNA substrates at a large number of positions ($U_1$, $U_{26}$, $U_{27}$, $U_{28}$, $U_{32}$ $U_{34}$, $U_{35}$, $U_{36}$, $U_{65}$ and $U_{67}$) [20] as well as for modification of U1RNA [53]. Similarly, Trm4 is responsible for $m^5C$ modification at $C_{34}$, $C_{40}$, $C_{48}$ and $C_{49}$ in numerous tRNAs [11], as well as for modification of $C_{50}$ of $tRNA^{His}$ when Thg1 is depleted [54]. These examples emphasize the rather low substrate specificity of some modification enzymes.

The in vivo and in vitro results described above suggest further that there may be some modifications of tRNAs that occur as an indirect consequence of the need to modify one or a few particular tRNAs for which the modification is crucial. According to this model, the modification of other tRNA species that occurs as a consequence of shared recognition features

with the crucial tRNA substrates, may confer little or no beneficial (or deleterious) function to these other accidentally modified tRNAs.

## 3. Nonessential body modifications may have unappreciated roles

Despite the evidence described above suggesting the presence of some possibly ancillary or redundant modifications in tRNAs, it is important to recall that only a limited number of conditions have been tested for the function of modifications in vivo. Thus, although only specific tRNAs lacking particular modifications are targeted for degradation, that is not to say that modifications of the tRNA body only serve this one stabilizing role in the body and always act to prevent degradation by these two pathways. It is well known that modifications such as dihydrouridine can add to tRNA flexibility [3,55], that several modifications can affect the specificity of aminoacyl tRNA synthetases [56-58], that modifications can affect the folding of tRNA [43], and that modifications can affect the activity and targeting of endotoxins [59]. Thus, it seems plausible that other seemingly redundant modifications may exert their effects in one of these ways or in different ways on different tRNAs. It is also certainly possible that modifications might act by affecting other functions of tRNA, such as their interaction with cellular trafficking proteins, with translation elongation factors or with other components of the ribosome, or that modifications play different or unanticipated roles in response to different growth conditions, developmental states or stresses. These roles of modifications will undoubtedly emerge as increasingly sophisticated assays are used to probe function.

## 4. Some modifications likely have important roles in all modified tRNAs and are added in a highly specific manner

Several examples demonstrate that certain modification enzymes are exquisitely specific. Thus, for example, $tRNA^{His}$ guanyltransferase is specific for its anticodon to ensure that only $tRNA^{His}$ can obtain the extra $G_{-1}$ residue that is used by HisRS to direct histidylation of the tRNA [54,60-62], $t^6A_{37}$ formation is directed specifically by $U_{36}$ and $A_{38}$ in the anticodon of tRNA in oocytes [63] and, as described above, a number of other modifications have well known specificity for residues around the anticodon of substrate tRNAs, and play important roles in decoding mRNAs and maintaining the reading frame during translation. In these cases the driving force for specificity is clearly translation.

Specificity is also often driven by exceedingly subtle architectural factors. Formation of a 2′-O-methylated $U_{34}$ in $tRNA^{[Ser]Sec}$ in Xenopus oocytes clearly illustrates this point. There are two isoacceptors for this tRNA in vertebrates: one has the modified nucleotide $mcm^5U$ at the first position of the anticodon; and the second has the nucleotide $mcm^5Um$. Notably, replacement of $U_{55}$ (where Ψ is normally found) by $G_{55}$ prevents ribose methylation at $U_{34}$, preventing the formation of $mcm^5U_m$ without affecting the levels of $mcm^5U$. Biologically this difference is very relevant in that 2′-O-methylation of $mcm^5U$ at position 34 is enhanced in the presence of selenium and may have a role in the formation of selenoproteins. Given the role of $Ψ_{55}$ in stabilizing tRNA tertiary structure, this suggests that structural changes caused by the lack of $Ψ_{55}$ indeed affect anticodon methylation, creating connectivity between the two modified sites however distant they may be.

Another example of a modification that depends on another modification occurs in mitochondrial $tRNA^{Trp}$ of *T. brucei*. This tRNA undergoes mitochondrial thiolation and contains 2-thiouridine ($s^2U$) at an unusual position, $U_{33}$ of the anticodon loop (Fig. 2). This modification is commonly found at $U_{34}$ (the first position of the anticodon) in $tRNA^{Glu}$, $tRNA^{Gln}$ and $tRNA^{Lys}$ in bacteria and eukarya [64,65]. $tRNA^{Trp}$ also undergoes C to U editing at the first position of the anticodon; however, $tRNA^{Trp}$ is not 100% edited and both UCA and CCA anticodon-containing isoacceptors co-exist in mitochondria. These two tRNAs are then

presumably dedicated to the decoding of the UGA and UGG codons in mitochondria. This has raised the question about the relationship between C to U editing and the unusual thiolation at position 33 (Fig. 2). A recent report showed that if $s^2U$ levels decrease, the levels of $tRNA^{Trp}$ that undergo C to U editing (a specialized form of postranscriptional modification) go up to nearly 100% [66]. In this latter case, thiolation serves as a negative determinant for C to U editing and helps keep the ratios of edited/unedited tRNAs in check, perhaps suggesting some biological role for both forms of the tRNA.

Yet another way that specificity is controlled in vivo is by compartmentalization. In principle, the modification content of a given tRNA can be affected either by the localization of modification enzymes in specific compartments, or by the localization of the tRNA. Some modification enzymes are imported into the nucleus following their synthesis in the cytoplasm [67], some are strictly cytoplasmic [68], and others are imported into the mitochondria [69] [70,71]. This trafficking creates a situation in which a particular enzyme, because of its intracellular localization, may never encounter a particular substrate. Although the discovery of retrograde tRNA nuclear import implies that tRNAs that escape to the cytoplasm without certain modifications can still in principle be imported back into the nucleus to obtain these modifications [72-74], it is still true that different nuclear-cytoplasmic trafficking patterns can preclude the encounter between tRNA and its substrate. In addition, tRNAs that are synthesized or imported into mitochondria are confined to this compartment, and are subject to modification only by enzymes that can be imported there.

One well-studied example of modification specificity apparently conferred by mitochondrial location is the C to U editing of the *T. brucei* $tRNA^{Trp}$ species discussed above. This editing is confined to mitochondrial $tRNA^{Trp}$ and as far as we know does not affect cytoplasmic $tRNA^{Trp}$ or any other tRNA species in either compartment. This finding suggests strongly that editing is driven by location of the editing enzyme, as well as by $tRNA^{Trp}$ specificity.

A second example of modification specificity apparently conferred by mitochondrial location is the formation of $s^2U$ at $U_{33}$ of this same *T. brucei* $tRNA^{Trp}$. Although both cytoplasm and mitochondria share a need for the desulfurase Nifs1 (the eukaryotic homolog of bacterial iscS) [66,75-77] to initiate the sulfur transfer reaction, the cytoplasmic thiolation pathway differs from that in mitochondria (and bacteria) [10,78,79] (Fig. 2). In the cytoplasm, Nifs1 transfers the sulfur group from cysteine to a series of ubiquitin-like proteins (UBLs) [78,80], and finally to the tRNA. In mitochondria, the route to tRNA thiolation is less clear but appears to involve Nifs1 and Mtu1 (the eukaryotic homolog to mnmA) [80]. Since there are no tRNA genes in the *T. brucei* mitochondrial genome, $tRNA^{Trp}$ is transcribed in the nucleus and transits through the cytoplasm where a portion of it is maintained in the cytoplasm for translation of nucleus-encoded mRNAs, and another portion is imported into the mitochondria. Surprisingly $tRNA^{Trp}$ is not thiolated in the cytoplasm but receives the unusual $U_{33}$ thiolation following mitochondrial import (Fig. 2). This result suggests strongly that specificity for $s^2U_{33}$ modification of $tRNA^{Trp}$ derives from the mitochondrial location of the $U_{33}$ thiolation machinery, thereby preventing modification of cytoplasmic $tRNA^{Trp}$, whereas the cytoplasmic thiolation system is specific for $U_{34}$ modification of cytoplasmic $tRNA^{Gln}$, $tRNA^{Glu}$ and $tRNA^{Lys}$ [66,75-77].

## 5. Summary and Concluding Remarks

The picture that emerges from this discussion is the intriguing possibility that modifications may not necessarily have the same beneficial effect on all tRNAs. Two lines of evidence are cited above to support this claim. First, we summarized findings supporting the view that although some modifications may stabilize specific tRNA species from degradation by either the nuclear surveillance or the rapid decay pathway, several tRNAs with the same modifications

are largely resistant to these pathways. Second, we summarized evidence that some modifications in the body of the tRNA, are catalyzed by enzymes with low specificity. We have also made three arguments that each modification might be important for each tRNA. First, we pointed out that only a limited number of growth conditions and assays have been tested, and suggested that new roles of modifications would be uncovered as more sophisticated assays are used to explore the effects of modifications on tRNA charging, folding, and flexibility, as well as on other aspects of translation and toxin defense. Second, we highlighted the high specificity of tRNA modification enzymes that act near the anticodon, or that otherwise affect translation by affecting charging fidelity. Third, we provided support for increased levels of specificity of particular modification enzymes acting around the anticodon, due to subtle and complex modes of substrate recognition. We also described how intracellular compartmentalization of tRNAs and modification enzymes may affect substrate availability and indirectly influence specificity.

We emphasize that it is not that surprising that modifications are not equally useful for all tRNAs. Since as argued above, the substrate specificity of at least some tRNA modification enzymes is necessarily relaxed to accommodate their disparate targets, it seems plausible that particular modifications on some tRNA species are not as important as the same modification on other tRNA species. It is also conceivable that some modifications serve no actual role on certain tRNAs. Thus, while the driving force for maintaining the modification enzymes is their important role on specific tRNAs, we suggest that the seemingly redundant modification of other tRNA species by these enzymes can occur because of overlapping substrate specificity, and the benign effects of these additional modifications on the tRNA species that receive them.

## Acknowledgments

We are grateful to E. Grayhack for advice on the manuscript. Research in the authors' laboratories is supported by grants GM 52347 to E.M.P and GM084065 to J.D.A. from the National Institutes of Health and by an MCB0620707 grant from the National Science Foundation to J.D.A.

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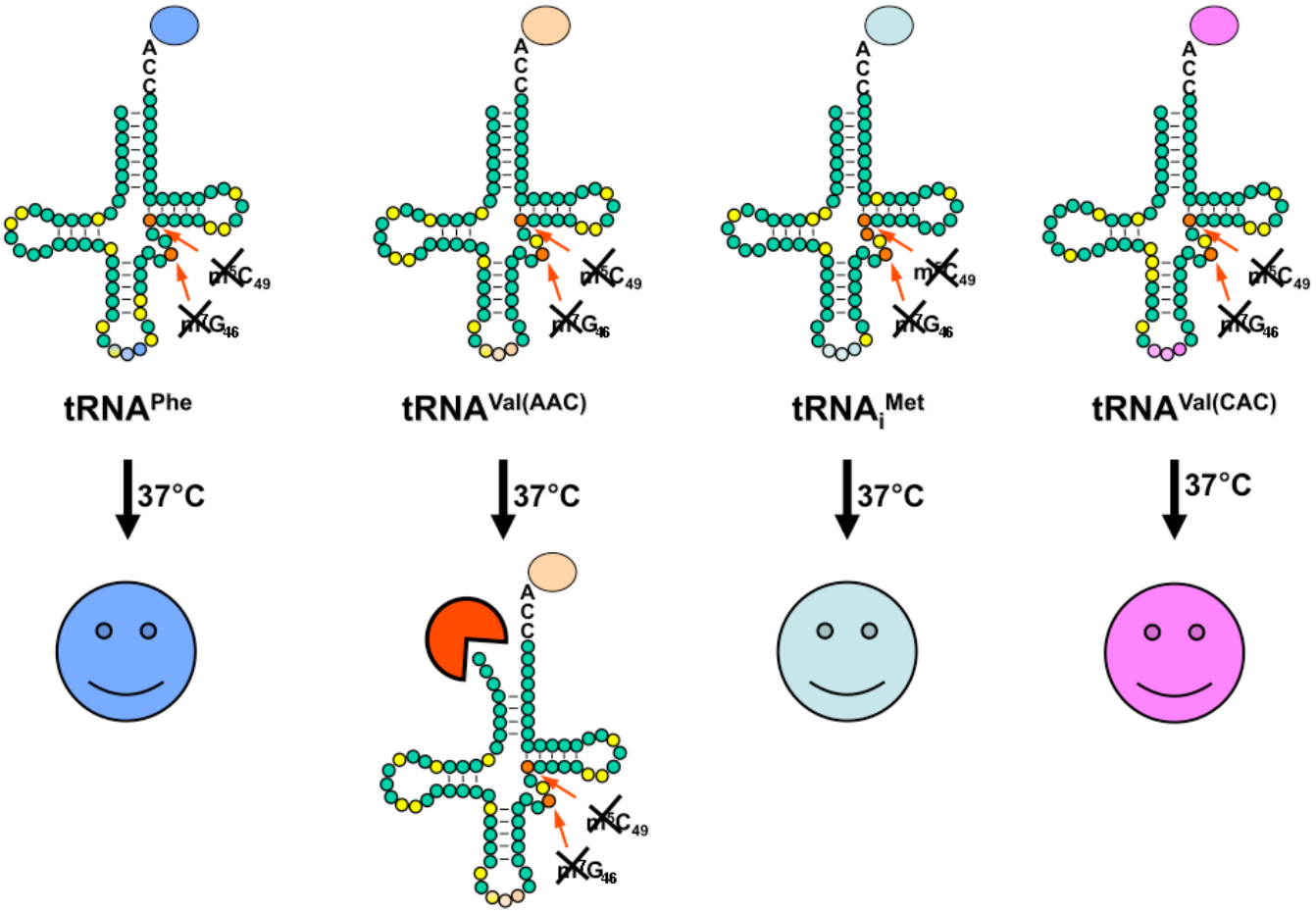


**Fig. 1.**

Illustration of the specificity of the rapid tRNA degradation pathway. The figure depicts four yeast tRNAs known to have $m^7G_{46}$ and $m^5C_{49}$, only one of which is a substrate for the rapid tRNA decay pathway in *trm8*-Δ *trm4*-Δ mutants, which lack these modifications. Mature $tRNA^{Val(AAC)}$ is rapidly degraded in *trm8*-Δ *trm4*-Δ mutants upon shift to 37 °C by the 5′-3′ exonucleases Rat1 or Xrn1, whereas $tRNA^{Phe}$, $tRNA^{Val(CAC)}$, and $tRNA_i^{Met}$ are resistant. Green circles represent each residue, yellow circles represent known modifications, orange circles represent sites of $m^7G$ and $m^5C$ modification, and the anticodon is colored to match its amino acid, indicated by an oval.

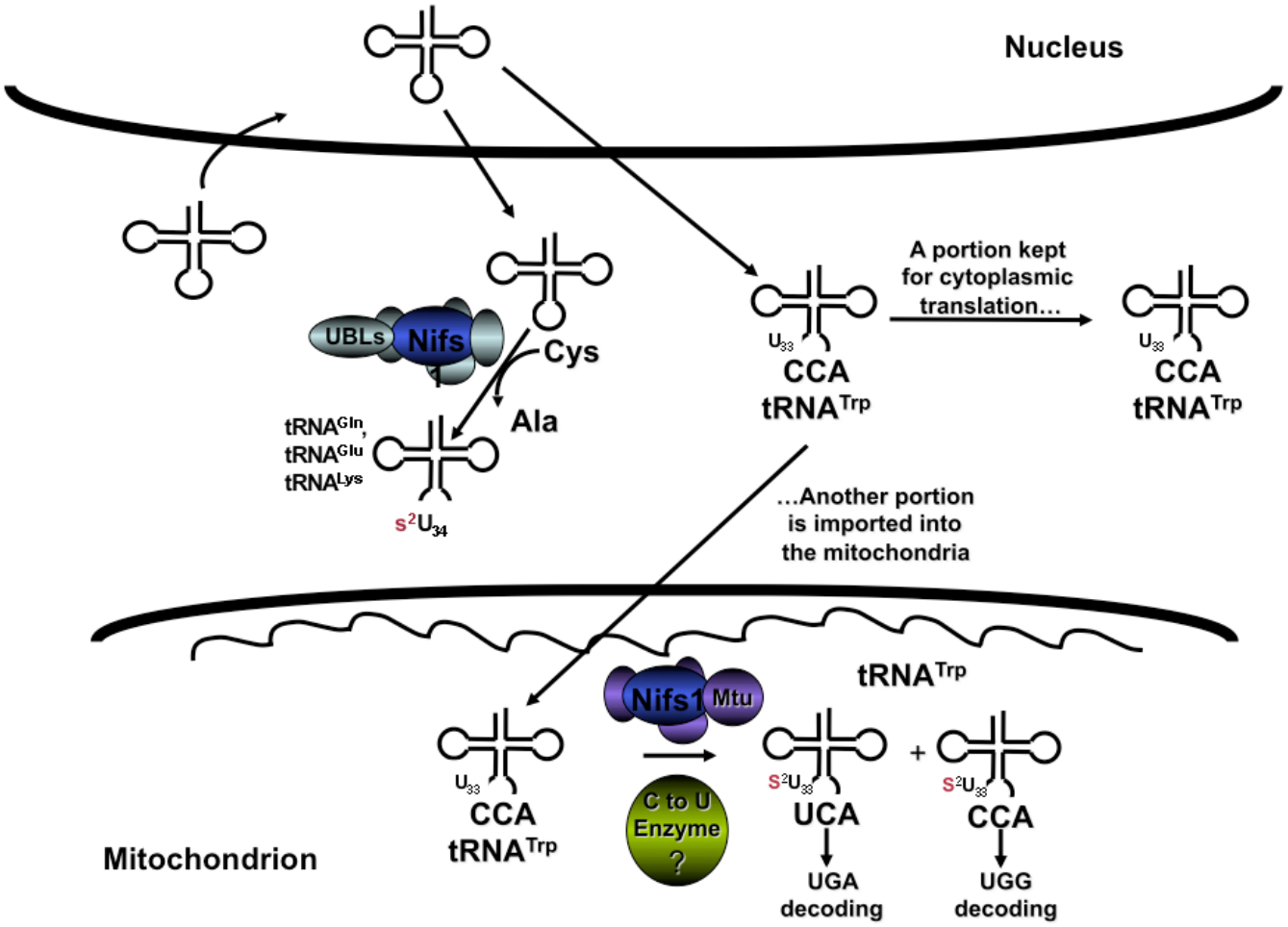


**Fig. 2.**

The role of intracellular localization on tRNA modification specificity. In eukaryotes there are three locations where tRNAs can be modified: the nucleus, cytoplasm and organelles (chloroplasts and mitochondria). Highlighted here are two examples where localization may impact tRNA modification. In the case of cytoplasmic thiolation, only $tRNA^{Gln}$, $tRNA^{Glu}$ and $tRNA^{Lys}$ with a U at position 34 are substrates for thiolation. In some cases like the example of $tRNA^{Trp}$ in trypanosomes, thiolation occurs at an unusual position ($U_{33}$) following import into the mitochondria. Thus $tRNA^{Trp}$ transits through the nucleus and is only a thiolation substrate for the mitochondrial enzymes. Likewise, this tRNA only undergoes C to U editing following mitochondrial import, suggesting the requirement for mitochondria-specific modifications for editing, and highlighting the possible interrelation among different modifications in the same substrate. Nifs refers to the universally conserved desulfurase involved in tRNA thiolation in all organisms. UBLs are the ubiquitin-like factors involved in cytoplasmic thiolation. Mtu is the mitochondrial homolog of the bacterial mnmA, responsible for transferring the sulfur to tRNAs. The question mark denotes the fact that the C to U editing enzyme still remains unknown.