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# Diversity in biological function and mechanism of the tRNA methyltransferase Trm10

**Isobel E. Bowles**, **Jane E. Jackman***

Department of Chemistry and Biochemistry, Center for RNA Biology and Ohio State Biochemistry Program, 484 W. 12th Avenue, Columbus, OH, 43210, USA

## Conspectus

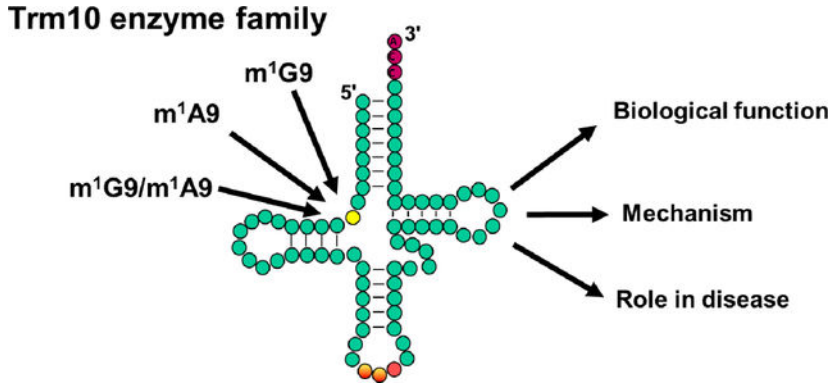
Transfer ribonucleic acid (tRNA) are the most highly modified RNA species in the cell and loss of tRNA modifications can lead to growth defects in yeast, as well as metabolic, neurological, and mitochondrial disorders in humans. Significant progress has been made toward identifying the enzymes that are responsible for installing diverse modifications in tRNA, revealing a landscape of fascinating biological and mechanistic diversity that remains to be fully explored. Most early discoveries of tRNA modification enzymes were in model systems where each enzyme was not strictly required for viability, an observation somewhat at odds with the extreme conservation of many of the same enzymes throughout multiple domains of life. Moreover, many tRNA modification enzymes act on more than one type of tRNA substrate, which is not necessarily surprising given the similar overall secondary and tertiary structure of tRNA, yet biochemical characterization has revealed interesting patterns of substrate specificity that can be challenging to rationalize on a molecular level. Questions about how many enzymes efficiently select a precise set of target tRNAs from among a structurally similar pool of molecules persist.

The tRNA methyltransferase Trm10 provides an exciting paradigm to study biological and mechanistic questions surrounding tRNA modifications. Even though the enzyme was originally characterized in *Saccharomyces cerevisiae* where its deletion causes no detectable phenotype under standard lab conditions, several more recently identified phenotypes provide insight into the requirement for this modification in the overall quality control of the tRNA pool. Studies of Trm10 in yeast also revealed another characteristic feature that has turned out to be a conserved feature of enzymes throughout the Trm10 family tree. We were initially surprised to see that purified *S. cerevisiae* Trm10 was capable of modifying tRNA substrates that were not detectably modified by the enzyme *in vivo* in yeast. This pattern has continued to emerge as we and others have studied Trm10 orthologs from Archaea and Eukarya, with enzymes exhibiting *in vitro* substrate specificities that can differ significantly from *in vivo* patterns of modification. While this feature complicates efforts to predict substrate specificities of Trm10 enzymes in the absence of appropriate genetic systems, it also provides an exciting new system for studying how enzyme activities can be regulated to achieve dynamic patterns of biological tRNA modification, which have been shown to be increasingly important for stress responses and human disease. Finally, the intriguing diversity in target nucleotide modification that has been revealed among Trm10 orthologs is distinctive among known tRNA modifying enzymes and necessitates unusual

*To whom correspondence should be addressed jackman.14@osu.edu.

and likely novel catalytic strategies for methylation that are being revealed by biochemical and structural studies directed toward various family members. These efforts will no doubt yield more surprising discoveries in terms of tRNA modification enzymology.

## Graphical Abstract




## Introduction

tRNA modifications are generally categorized into two regions of tRNA structure: body modifications and anticodon stem-loop (ACL) modifications[4, 5]. Modifications to the ACL frequently aid in the efficiency or fidelity of translation, while body modifications have been more challenging to understand due to their distant location from the site of codon-anticodon interaction. Body modifications generally exhibit a more indirect yet important role in tRNA folding and stability, while also playing a part in tRNA quality control to ensure a functional pool of tRNA molecules is maintained[4, 5]. In *S. cerevisiae*, there are 10 chemically unique modifications found in the tRNA body region while the ACL region contains the remaining 15 different tRNA modifications[5, 6]. The chemical diversity of tRNA nucleotide base and ribose modifications is vast, ranging from addition of relatively “simple” chemical groups via acetylation or methylation, to more profound chemical and structural changes with complex modifications such as queuosine or wybutosine[5, 7]. Base methylation, the focus of this Account, is comprised of 8 chemically unique modifications of cytoplasmic tRNAs in *S. cerevisiae*: 1-methylguanosine ($m^1G$), 2-methylguanosine ($m^2G$), N2,N2-dimethylguanosine ($m^{2,2}G$), 7-methylguanosine ($m^7G$), 1-methyladenine ($m^1A$), 3-methylcytidine ($m^3C$), 5-methylcytidine ($m^5C$), and 1-methylinosine ($m^1I$) (Figure 1). Many of these modifications and their corresponding enzymes are conserved throughout Eukarya, Bacteria, and Archaea[5, 8–10].

The tRNA methyltransferases that perform these distinct methylations are correspondingly diverse, representing different structural classes and catalytic strategies for transferring the methyl group from the methyl donor S-adenosyl methionine (SAM) to the target atom of the tRNA nucleotide[11]. tRNA methyltransferases have been categorized into classes based on the presence of conserved structural features, and include the Rossmann fold methyltransferases (also known as Class I; e.g. Trm8), SPOUT methyltransferases (also known as Class IV; e.g. Trm10), and a more recently identified class consisting of radical

SAM methyltransferases (e.g. MiaB)[12, 13]. In all three structural classes, however, many tRNA methyltransferases share a common theme of multi-tRNA substrate specificity, resulting in modification of specific subsets of substrate tRNAs. For some enzymes, the molecular basis for recognition of specific substrates has been attributed to specific sequence motifs, such as for the bacterial $m^1G37$ methyltransferase TrmD, which methylates the G37 nucleotide when it is found in the specific purine36G37 sequence context of the ACL[14, 15]. In other cases, a specific structural feature is recognized, such as the position 34 2'-O methyltransferase TrmL, which depends on the structure of the ACL[16, 17]. However, for many tRNA methyltransferases, the mechanism of recognizing unique subsets of substrate tRNAs is only partially, if at all, understood. A particularly striking example of this kind of enzyme is the tRNA $m^1G9$ methyltransferase Trm10, which is a member of the SPOUT methyltransferases characterized by the presence of an unusual protein knot near the active site[18, 19]. Among tRNA methyltransferases, Trm10 exhibits a distinct pattern of substrate specificity, with only about half of the candidate tRNA species that have the appropriate target nucleotide at the $9^{th}$ position modified by the enzyme in each of the eukaryotic systems studied to date[6, 8]. This Account will highlight recent insights into the function of Trm10 and its modification at position 9 throughout Archaea and Eukarya, where it is conserved, as well as its diverse paralogs within higher eukaryotes.

## Biological function of the Trm10 modification in *S. cerevisiae*

Interestingly, despite the widely-conserved nature of the Trm10 enzyme, and therefore its modification, among Eukarya and Archaea, deletion of *TRM10* in *S. cerevisiae* where it was first discovered elicited no obvious phenotype from *trm10Δ* cells grown under a variety of conditions[18]. The apparently non-essential nature of Trm10 is a relatively common feature among the majority of conserved tRNA modification enzymes in model systems such as *E. coli* and *S. cerevisiae*, where many tRNA modification enzymes have been found to be similarly dispensable for viability[9]. Recent discoveries of roles for these modifications in tRNA quality control pathways (described in more detail below) combined with the growing number of human diseases associated with deficiency of tRNA modification enzymes has led to a more complex understanding of the biological function of individual tRNA modifications[20, 21].

Even in *S. cerevisiae* where Trm10 is not required for viability, evidence for a biologically significant role for the modification has emerged. In a survey of the genome-wide yeast deletion collection grown in the presence of the antitumor drug 5-fluorouracil (5FU), tRNA modification enzyme genes comprised the largest group of deletion strains that exhibited hypersensitivity to 5FU compared to the wild-type strain[22]. Since 5FU is known to be incorporated into RNA molecules, including tRNA, the hypersensitive phenotype was suggested to be due to a negative impact on overall tRNA structure due to the absence of a given tRNA modification in the deletion mutant that is exacerbated when 5FU is incorporated into the tRNA in place of the normal uracil residues. Interestingly, the *trm10*Δ strain exhibited the most severe growth defect when grown in 5FU compared to single deletion strains of other tRNA and rRNA modification genes[22] (Figure 2). Although a general negative effect on tRNA structure was implied by these original observations, subsequent studies in our lab have revealed that a single tRNA ($tRNA^{Trp}$) is responsible

for the observed hypersensitivity to 5FU in the absence of Trm10 modification due to the extremely depleted levels of $tRNA^{Trp}$, but not other Trm10 substrate tRNAs, in *trm10Δ* cells[23]. We also showed that $tRNA^{Trp}$ abundance is already significantly decreased in *trm10Δ* cells even in the absence of 5FU, where viability is not impacted[23]. Thus, the impact of the Trm10 modification even in yeast is biologically significant despite the lack of an obvious growth defect in *trm10Δ* cells grown under standard laboratory conditions.

Importantly, the antimetabolite 5FU is a common chemotherapeutic drug used to treat several solid cancer tumors including breast, colorectal, and neck cancers in humans, raising questions about similar synergies with tRNA modifications, including Trm10, in vertebrates that will be important to study in the future. Along these lines, 5FU treatment perturbs several translation-associated events in a human colorectal cancer cell line including decreased ability to perform +/−1 programmed frameshifting and cap independent translation, and decreased recognition of STOP codons that might suggest such similarities[24, 25]. The antimetabolite also directly affects RNA modification by inhibiting essential pseudouridine synthase and depleting the pseudouridine modification which is normally abundant in tRNA and other RNA molecules and is also important to pre-mRNA splicing and translational recoding[25, 26].

A 2010 study reported the genetic interaction profiles for 75% of genes in *S. cerevisiae* and revealed a strong synthetic interaction between *PUS3* and *TRM10* genes[27]. Pus3 performs the pseudouridine (Ψ) modification at position 38 and 39 in *S. cerevisiae* and its deletion and loss of Ψ at position 38 and/or 39 causes slow growth, temperature sensitivity, and reduced −1 frameshifting[28, 29]. When deleted together, a *trm10Δ pus3Δ* strain is not viable, but growth is fully rescued by complementation of either a *PUS3* or *TRM10* plasmid[30] (Figure 2). Of the tRNAs in *S. cerevisiae* that contain both the Trm10 $m^1G9$ and Pus3 Ψ38 or Ψ39 modifications, only $tRNA^{Trp}$ overexpression (naturally containing Ψ39, not Ψ38) was able to partially suppress the double deletion growth defect, although it did not restore growth to the level observed in the presence of either modification[30]. According to structure prediction, $tRNA^{Trp}$ contains a relatively unstable anticodon stem compared with other tRNA species, with a free energy prediction of −2.4 kcal/mol without modifications[31]. This compares to an average free energy prediction of −4.78 kcal/mol calculated for the other 42 tRNAs[30].

Pseudouridine modifications are generally thought to contribute positively to the overall stability of tRNA structures, thus the Pus3 modification may be necessary to stabilize the anticodon stem, as the Ψ39 modification was shown to stabilize the ACL of $tRNA^{Lys}$ and increase its Tm by 5°C[32]. In combination with the absence of the $m^1G9$ modification in the double *trm10Δ pus3Δ* mutant, $tRNA^{Trp}$ stability could further decrease leading to the observed results. Since 5FU inhibits formation of pseudouridine which are introduced at many positions of cellular tRNAs by other Pus enzymes in *S. cerevisiae*, the global loss of pseudouridine may contribute to the observed 5FU sensitivities of some modification enzyme deletions[22]. However, 5FU hypersensitivity of *trm10Δ* may also be due to other reasons besides simple destabilizing loss of pseudouridine. We note that the deletion of *pus3* caused slow growth at high temperatures also due to poor function of $tRNA^{Gln}_{UUG}$ with the loss of Ψ38 and $s^2U$, as the tRNA overexpression rescued growth, but the gene related

to formation of $s^2U$ in the cell, *UBA4*, when individually deleted and grown under 5FU conditions had minimal sensitivity to the drug[22, 30]. Thus, the loss of pseudouridine affects tRNA species differently, also depending on the landscape of other modifications on the tRNA, and its overall inherent stability with and without modifications.

In humans, a homozygous mutation leading to a truncated PUS3 variant caused disease states similar to TRMT10A variants in humans presenting as intellectual disability[33]. A similar phenomenon was observed upon homozygous mutations leading to missense and frameshift deletions of PUS7, also impairing neurological development leading to microcephaly, intellectual disability, and behavioral defects[34, 35]. While the connection between Trm10 and Pus enzymes has not been further studied in humans, the similarity in the disease states caused by variants of both enzymes reveal intriguing questions as to whether there remains a similar connection in humans between the biological impact of $m^1G9$ and Ψ modifications as there is in *S. cerevisiae.* Overall, this underscores the importance of understanding the biological roles for tRNA modification and tRNA stability in the context of human disease.

Due to the importance of modifications like methylation to tRNA structure and function for fidelity of translation, and ultimately cell growth, multiple pathways have been shown to degrade aberrant tRNAs to avoid downstream problems that these lingering tRNAs could cause[5]. Much of this work has so far centered on *S. cerevisiae*, where known tRNA degradation pathways include the rapid tRNA decay pathway (RTD) acting on hypomodified mature tRNA, the Met22 dependent decay pathway (MPD) acting on pre-tRNA with an aberrant intron-exon junction, and the nuclear TRAMP complex acting on aberrant initiator tRNA/pre-tRNA[36–38]. When any of these tRNA species does not undergo normal processing and/or modification, it becomes significantly more susceptible to recognition by these various tRNA quality control pathways, leading to its degradation in the yeast cell.

Interestingly, even though many tRNA modifications can be found in multiple different tRNAs, tRNA quality control has been observed to be much more selective, often targeting only one of the tRNA species that is impacted by loss of the modification and causing it to be degraded, while the loss of modification on other tRNAs has little to no effect on their overall stability. Just one example of how defects in tRNA methylation can selectively impact one tRNA is $tRNA^{Val}_{AAC}$, which undergoes RTD upon the loss of $m^7G46$ and $m^5C49$ that occurs upon deletion of the methyltransferases Trm8 and Trm4 respectively, and leads to temperature sensitivity of the double mutant strain[36]. Although Trm8 and Trm4 enzymes each modify multiple tRNA substrates (10 tRNAs have $m^7G46$ and 12 tRNAs have $m^5C49$), only four tRNAs share both modifications, $tRNA^{iMet}$, $tRNA^{Phe}_{GAA}$, $tRNA^{Val}_{AAC}$, and $tRNA^{Val}_{CAC}$. Yet, even among these 4 species, overexpression of only one of these, $tRNA^{Val}_{AAC}$, is able to rescue the temperature sensitivity of the double mutant strain, even though the three other tRNAs are also missing $m^7G46$ and $m^5C49$ modifications in the deletion strains[36]. Similarly, of the 12 elongator tRNA species Trm10 modifies in *S. cerevisiae*, only mature $tRNA^{Trp}$ lacking $m^1G9$ modification undergoes quality control, providing another example of a tRNA modification that is present on multiple substrates in the cell but is seemingly only important for one tRNA[23]. Interestingly, Trm10-dependent surveillance of $tRNA^{Trp}$ appears to be mediated by a new pathway that shares some, but not

all, features with previously described tRNA quality control mechanisms[23]. The identify of specific exonucleases that degrade tRNA lacking the $m^1G9$ modification to cause the 5FU hypersensitive and synthetic temperature sensitive phenotypes associated with the gene in *S. cerevisiae* is an ongoing area of investigation, but our observations and those of the Phizicky lab should help to answer these questions about the diverse biological impacts of Trm10 in *S. cerevisiae*.

## Diversity in tRNA substrate recognition by Trm10

Trm10 was first discovered as the $m^1G9$ methyltransferase in *S. cerevisiae* through a genome-wide approach directed toward identifying previously unknown tRNA modification enzymes[18]. The enzyme was demonstrated to be sufficient for tRNA modification in *S. cerevisiae* based on the complete absence of detectable $m^1G9$ in *trm10Δ* cells upon analysis of 8 different tRNA species that were known at the time to carry the $m^1G9$ modification[18]. Even from the limited set of tRNAs studied at the time, it was clear that the presence of the target G9 nucleotide was not sufficient to specify activity of Trm10 since only around half of the 24 sequenced G9-containing tRNAs in *S. cerevisiae* were modified by Trm10 *in vivo*[6, 8]. This observation prompted us to carry out a deeper investigation to define the complete set of tRNA substrates for Trm10 modification in *S. cerevisiae*, including 8 G9-containing tRNA whose tRNA modification status had not yet been defined. We identified 2 additional tRNA substrates that are quantitatively modified with $m^1G9$ by Trm10, as well as 2 additional tRNA that are modified only partially by Trm10 *in vivo*, resulting in a total of 13 different tRNA that are Trm10 substrates in *S. cerevisiae* grown under wild-type conditions[1] (Table 1). The observation of these “partial” Trm10 substrates was a particularly important observation because it suggested some additional tRNA-specific features that may dictate the levels of Trm10 activity in individual tRNAs, although these features remain to be fully identified. To date, the full spectrum of tRNA that are modified by Trm10 has only been demonstrated in *S. cerevisiae* and human cells, which exhibit overlapping but not identical sets of tRNAs that carry $m^1G9$ modification[1, 39, 40] (Table 1). An evolutionary understanding of this divergence, along with all the factors that determine tRNA substrate specificity within the Trm10 family in diverse species will require further study to ascertain.

*In vitro* characterization of *S. cerevisiae* Trm10 activity added even more complexity to the picture of tRNA substrate recognition. Despite there being no detectable $m^1G9$ modification of some tRNAs *in vivo* in wild-type yeast, it was surprising to see robust methylation of several of these same tRNAs when tested *in vitro* using purified S. cerevisiae Trm10[1] (Table 1). A striking example is $tRNA^{Val}_{UAC}$, which was readily methylated by the recombinant purified Trm10 enzyme *in vitro*, despite this tRNA having no detectable $m^1G9$ methylation in yeast cells. Kinetic analysis showed a minimal difference in catalytic efficiency between modification of $tRNA^{Val}_{UAC}$ transcripts compared to modification of a bona fide *in vivo* substrate $tRNA^{Gly}_{GCC}$[1]. Moreover, the direct human ortholog of Trm10 (TRMT10A) was also able to robustly methylate both S. cerevisiae $tRNA^{Val}_{UAC}$ and $tRNA^{Gly}_{GCC}$[2, 3], despite the fact that the human G9-containing $tRNA^{Gly}_{GCC}$ is not modified by Trm10 *in vivo* in any human cell line tested to date[6, 39, 40] (Table 1). Using tRNA isolated from *trm10Δ* cells that contains all other modifications except for $m^1G9$, we demonstrated that this discrepancy between the observed *in vitro* and *in vivo* substrate specificity cannot be explained by the

use of *in vitro* transcripts in our assays, since the otherwise fully modified $tRNA^{Val}_{UAC}$ substrate was also modified to some extent by purified Trm10 *in vitro*[1]. This observation that a given Trm10 ortholog's *in vitro* methylation activity does not necessarily correlate directly with its *in vivo* modification pattern has important implications for the entire Trm10 family. First, caution is warranted when trying to assign specific tRNA modification activities *in vivo* based solely on the results of *in vitro* activity assays of enzymes from other species, as is starkly clear from the analysis of the yeast and human enzymes described above. Second, the molecular basis for Trm10 substrate selection *in vivo* must rely on factors that are yet to be understood and likely will require determination of patterns of position 9 modification in many additional species before sufficient evidence can be obtained to truly rationalize the substrate recognition parameters for this enzyme.

Recently, we have obtained some insight into at least one of the factors that seems to be associated with tRNA selection by Trm10 *in vitro*. The selective 2'OH acylation analyzed by primer extension (SHAPE) reactivity of five *in vitro* transcribed tRNAs was performed, comparing the observed reactivities of the free tRNA to the tRNA bound by Trm10[41]. The studied transcripts included two *in vivo* substrates of *S. cerevisiae* Trm10, $tRNA^{Gly}_{GCC}$ and $tRNA^{Trp}_{CCA}$, a tRNA only modified *in vitro*, $tRNA^{Val}_{UAC}$, and two tRNAs that are not modified by Trm10 *in vitro* or *in vivo*, $tRNA^{Leu}_{CAA}$ and $tRNA^{Ser}_{UGA}$[1]. SHAPE data reveals that when Trm10 binds to a catalytically competent substrate tRNA, the D-stem becomes more flexible, potentially to allow the enzyme access to the core G9 position for methylation. The non-substrates exhibit significantly less flexibility of the D-stem upon Trm10 binding, indicating that the tRNA may not have efficient access to methylate G9, which is likely due to the presence of the extended variable loop in these type II tRNAs. Surprisingly, Trm10 substrate tRNAs also exhibit decreased flexibility in the ACL region, distant from the G9 site of methylation, whereas the flexibility of the ACL region in non-substrate tRNAs is mostly unchanged. These data suggest a possible mechanism whereby rigidifying the ACL region may aid Trm10 in methylating the G9 position and indicates that Trm10 interaction with substrate causes a more global change to tRNA flexibility. Following up *in vitro* characterization with *in vivo* approaches to determine how other modifications affect tRNA flexibility upon interaction with Trm10 would provide interesting insight into Trm10 substrate specificity.

While crystal structures have been resolved for examples of archaeal and eukaryotic Trm10 enzymes in complex with cofactor SAM (or its analogs) that reveal intricacies of this interaction, molecular features associated with the enzyme's interaction with tRNA have not yet been directly observed in most available structures[42–44]. The only exception is the structure of human mitochondrial TRMT10C, which is a part of an unusual protein-only RNaseP complex (further discussed below), recently resolved by cryo-EM with substrate tRNA bound[45]. While this structure provides important details about tRNA recognition in the context of 5'-end processing, the presence of TRMT10C's required cofactor protein SDR5C1 and the 5'-end nuclease enzyme PRORP in this RNase P complex, features that are not a shared by any other archaeal or eukaryotic enzyme, limits the insight that can be transferred from this important structure to those of the majority of other Trm10 family members.

## Diversity in Trm10 function revealed through characterization of human orthologs

In our initial phylogeny of Trm10 after its discovery in yeast, we were puzzled by the distinct patterns of Trm10 gene distribution we observed[18]. Similar to fungi, archaeal species, appear to encode a single readily identifiable Trm10 ortholog in their genomes, which was consistent with our demonstration that a single enzyme was capable of modifying all $m^1G9$-containing tRNA in *S. cerevisiae* (and presumably these orthologs could function similarly in Archaea to modify all substrate tRNAs). However, metazoa appeared to have undergone duplication in the Trm10 family, with up to 3 different Trm10 paralogs encoded in vertebrates[18]. This observation raised the question of whether there was redundancy of function among the different Trm10 enzymes, or if specialization of the enzymes occurred in the context of multicellular eukaryotes. The first answer to this question was suggested by the demonstration that one of the 3 human paralogs (TRMT10C) functioned in human mitochondria, where it is responsible for not only $m^1G9$ methylation of mitochondrial tRNAs but also acts on other mitochondrial tRNA as an $m^1A9$ methyltransferase[46, 47]. Interestingly, this Trm10 paralog is part of an unusual protein-only version of the 5'-end tRNA maturation enzyme Ribonuclease P (RNase P) where it is one of three essential subunits required for 5'-maturation activity (and thus is also known as RNase P-protein 1 (RPP1))[46].

We sought to further clarify the role of the other two non-mitochondrial human enzymes (TRMT10A and TRMT10B), revealing that these two enzymes do exhibit distinct non-redundant functions in humans[2]. Human TRMT10A acts similarly to *S. cerevisiae* Trm10, modifying a diverse set of multiple tRNA species with $m^1G9$[2, 3] (Table 1). This result is consistent with the observation that human and other vertebrate TRMT10A paralogs cluster more closely with the fungal $m^1G9$ methyltransferase than do any of the other human paralogs. However, human TRMT10B exhibited a new activity in the context of eukaryotic enzymes, catalyzing $m^1A9$ methylation of a single tRNA, human $tRNA^{Asp}$ [2]. A subsequent experiment using knockout human cell lines confirmed this observation *in vivo*, demonstrating complete loss of $m^1G9$ modification on all its substrate tRNAs upon loss of TRMT10A expression, while only $m^1A9$ modification of $tRNA^{Asp}$ was impacted by silencing of TRMT10B[39].

Having identified these distinct biochemical functions for the human orthologs of Trm10, questions about the biological role for this conserved modification have become more important to address. Interestingly, familial mutations in the human TRMT10A homolog have been identified in patients who exhibit a disease syndrome characterized largely by neurological and metabolic defects[48–55]. While TRMT10A is ubiquitously expressed in humans, it is enriched in the brain and pancreas, consistent with the observed biological impact of mutation or loss of TRMT10A[49]. Intriguingly, there are subtle differences between the phenotypic effects observed with different TRMT10A mutations suggesting a complex impact of the $m^1G9$ modification on human tRNA that are yet to be explained. For example, the first identified TRMT10A mutation led to formation of a premature termination codon (PTC), and the affected patients exhibited early onset diabetes[49]. Studies

of β-cell mass in primary rat β-cells and dispersed human islets indicate that β-cells undergo apoptosis upon silencing of TRMT10A, but that TRMT10A silencing did not alter insulin secretion[49]. We participated in a subsequent characterization of a different missense mutation in TRMT10A and showed that it completely inactivated enzyme methyltransferase activity. However, this missense mutation was associated with a different pattern of disturbance of glucose metabolism characterized largely by hypolycemia[48]. Additional distinct mutations in TRMT10A have been described, with similar diversity in their apparent impact on affected patients. These data suggest that our understanding of the molecular role of Trm10 and its $m^1G9$ modification is not currently sufficient to explain these distinct biological effects in humans. Interestingly, in the earlier PTC mutant patients, $tRNA^{Gln}$ lacking $m^1G9$ was shown to become susceptible to tRNA fragmentation, leading to accumulation of 5'-tRFs derived from this tRNA, while 3'-tRF levels remained largely the same in patient and control cells, thus suggesting a role for these fragments in the human disease that will be important to explore further[56]. Excitingly, we recently established the first loss of function mutant strains for the zebrafish orthologs of the TRMT10A and TRMT10B enzymes and demonstrated that each enzyme is a true functional ortholog of the human enzymes in terms of its modification activities in the fish (Jepson and Jackman, unpublished) and we are excited about the possibility of using this system to address these questions moving forward.

## Unique catalytic mechanisms of Trm10 SPOUT methyltransferases

Even though two other enzymes capable of catalyzing $m^1G$ modification have also been discovered and extensively characterized (Trm5, the $m^1G37$ modification enzyme in eukaryotes and TrmD, the $m^1G37$ modification enzyme in Bacteria)[14], subsequent structural and mechanistic characterization of various Trm10 orthologs by us and others has confirmed its many unique catalytic properties. For example, although Trm10 enzymes share a structurally similar overall SPOUT methyltransferase fold with TrmD, Trm10 enzymes from several tested organisms do not utilize any of the same catalytic features that have been associated with TrmD[57]. In particular, despite initial suggestions of a requirement for a conserved general base residue in catalysis to abstract the N1 proton from the target G9 atom, we demonstrated that Trm10 does not use an obligate general base residue to fulfill this role, a result that was also observed for the bifunctional Trm10 methyltransferase from *Thermococcus kodakarensis*[3, 44] (Figure 3). In fact, mutation of any of the highly conserved carboxylate residues in *S. cerevisiae* Trm10, human TRMT10A or *T. kodakarensis* Trm10 only caused minimal to moderate decreases in overall reaction rates[3, 44]. Moreover, pH rate profiles for the mutant *S. cerevisiae* enzymes demonstrated that each retained a single basic ionization that was required for efficient catalysis[3]. Intriguing further insight into the molecular basis for this ionization was revealed by our work with the archaeal Trm10 homolog from *T. kodakarensis*[58]. This enzyme, like several archaeal orthologs described in more detail below, is like the human TRMT10C in that it is capable of catalyzing both $m^1G9$ and $m^1A9$ modification activity, depending on the identity of the N9 nucleotide on different tRNA substrates[59]. We used this information to also study the pH dependence of the different activities catalyzed by *T. kodakarensis* Trm10 and showed that while the $m^1G9$ activity exhibited a single basic pKa like the *S. cerevisiae* $m^1G9$ methyltransferase, the

$m^1A9$ activity occurred independent of pH across the same range[58]. Thus, we believe that this is strong evidence that the ionization observed in the $m^1G9$ activity is for the ionization of the N-1 atom on the target G9 base itself and that this represents a rate-determining step for this reaction (Figure 3). This would be consistent with the lack of pH dependence for $m^1A9$ methylation, since the N1 position of the A9 residue would be predictably already deprotonated at most physiological pH values. The molecular contributions of specific enzyme residues to overcoming this rate-determining step will be exciting to reveal as we obtain further structural and mechanistic insight into catalysis by Trm10.

The observation of Trm10 enzymes with distinct abilities to modify different nucleotide bases is a particularly unusual feature of the Trm10 family. Although some tRNA methyltransferases modify multiple distinct nucleotides at the same position of a tRNA, such as Trm13 that creates 2'-O-methyl modifications at either A4 or C4 nucleotides of target RNAs[60], the actual chemistry of the reaction occurs at the 2'-hydroxyl which is chemically identical in the context of both nucleotides. In Trm10, the predictably different pKa values of a G9 vs. A9 nucleotide yield important protonation differences at the N-1 atom that is the site of catalysis, and subsequently distinct chemical strategies are likely to be employed to modify these two target purines. The identification of all possible combinations of catalytic activities, including enzymes that can act solely as an $m^1G9$ methyltransferase (e.g. *S. cerevisiae* Trm10 and human TRMT10A), enzymes that are uniquely $m^1A9$ methyltransferases (e.g. *Sulfolobus acidocaldarius* Trm10 and human TRMT10B) and enzymes that can act as bifunctional $m^1A9$ and $m^1G9$ methyltransferases (e.g. *T. kodakarensis* Trm10 and human TRMT10C) now positions us to provide critically needed insight into the molecular basis for these distinct catalytic features.

## Conclusions and future outlook

As our knowledge of the biological impact and catalytic mechanisms associated with the Trm10 tRNA methyltransferase family continues to grow, it is clear that there is much more to learn about this intriguing enzyme family. Although the study of phenotypes associated with the loss of the Trm10 modification in simpler unicellular models suggests an impact of this modification specifically on $tRNA^{Trp}$, whether this is a shared feature across all domains of life, and especially in metazoan species that contain multiple Trm10 paralogs, remains to be assessed. This is a particularly important question to answer since there is currently no explanation for the different clinical effects of patient-associated TRMT10A mutations that have been observed from many groups, including ours. The evaluation of vertebrate model systems is likely to be important in this regard. Likewise, the diversity of enzymatic function exhibited across the Trm10 family, with several new examples of distinct purine methylation specificities despite the different chemical nature of the target A vs. G nucleotide, will require additional biochemical and structural approaches to fully understand. These studies are also likely to impact our understanding of the larger SPOUT family of methyltransferases to which Trm10 belongs. Despite the presence of a common core protein fold in all family members, insight into the molecular basis for each enzyme's distinct substrate specificities is limited. Indeed, the chemical nature of the modifications catalyzed with this structurally similar core vary significantly, from pyrimidine and purine base methylation to ribose 2'O-methylation and these enzymes act on

a variety of macromolecular substrates. Understanding how the catalytic properties of each SPOUT enzyme are achieved will be an important future goal.

## Biographies

Isobel E. Bowles earned a BS in ACS Chemistry from Butler University. She is a current PhD candidate at the Ohio State University where she studies tRNA modification enzyme Trm10 and its biological significance in *S. cerevisiae.*

Jane E. Jackman earned a BS in Biochemistry from the University of Rochester and a PhD in Biochemistry from Duke University. She has been on the faculty at Ohio State University since 2007, where she is currently Professor of Chemistry and Biochemistry and Vice Chair for Undergraduate Studies in Chemistry and Biochemistry. Dr. Jackman has been studying tRNA modification and processing enzymes for over 20 years, participating in discoveries of unusual enzymatic mechanisms and biological functions associated with many of these enzymes in diverse organisms from all domains of life.

## REFERENCES

(1). Swinehart WE; Henderson JC; Jackman JE Unexpected expansion of tRNA substrate recognition by the yeast m1G9 methyltransferase Trm10. RNA 2013, 19, 1137–1146. DOI: 10.1261/rna.039651.113. [PubMed: 23793893] The comprehensive characterization of Saccharomyces cerevisiae Trm10 m1G9 modification activity in vitro and in vivo led to the discovery of the difference between substrate specificity exhibited by the purified enzyme in vitro and the enzyme in its cellular context.

(2). Howell NW; Jora M; Jepson BF; Limbach PA; Jackman JE Distinct substrate specificities of the human tRNA methyltransferases TRMT10A and TRMT10B. RNA 2019, 25, 1366–1376. DOI: 10.1261/rna.072090.119. [PubMed: 31292261] The first bona fide biologically-relevant activity of the human TRMT10B paralog (as a tRNA-specific m1A9 methyltransferase) was demonstrated, demonstrating the presence of multiple paralogs that each exhibit unique functions in the context of multicellular eukaryotes.

(3). Krishnamohan A; Jackman JE Mechanistic features of the atypical tRNA m1G9 SPOUT methyltransferase, Trm10. Nucleic Acids Res 2017, 45, 9019–9029. DOI: 10.1093/nar/gkx620. [PubMed: 28911116] This paper was the first to rule out a proposed mechanism involving an obligate general base residue for Trm10 catalysis, as well as other catalytic features exhibited by other m1G methyltransferases, supporting the likely novel mechanism associated with this enzyme.

(4). Howell NW; Jackman JE Impact of Chemical Modification on tRNA Function. eLS 2019. DOI: 10.1002/9780470015902.a0028527.

(5). Phizicky EM; Hopper AK The life and times of a tRNA. RNA 2023, 29, 898–957. DOI: 10.1261/rna.079620.123. [PubMed: 37055150]

(6). Boccaletto P; Stefaniak F; Ray A; Cappannini A; Mukherjee S; Purta E; Kurkowska M; Shirvanizadeh N; Destefanis E; Groza P; Avsar G; Romitelli A; Pir P; Dassi E; Conticello SG; Aguilo F; Bujnicki JM MODOMICS: a database of RNA modification pathways. 2021 update. Nucleic Acids Res 2022, 50, D231–D235. DOI: 10.1093/nar/gkab1083. [PubMed: 34893873]

(7). Jackman JE; Alfonzo JD Transfer RNA modifications: nature's combinatorial chemistry playground. Wiley interdisciplinary reviews. RNA 2013, 4, 35–48. DOI: 10.1002/wrna.1144. [PubMed: 23139145]

(8). Juhling F; Morl M; Hartmann RK; Sprinzl M; Stadler PF; Putz J tRNAdb 2009: compilation of tRNA sequences and tRNA genes. Nucleic Acids Res 2009, 37, D159–162. DOI: gkn772 [pii] 10.1093/nar/gkn772. [PubMed: 18957446]

(9). Machnicka MA; Milanowska K; Osman Oglou O; Purta E; Kurkowska M; Olchowik A; Januszewski W; Kalinowski S; Dunin-Horkawicz S; Rother KM; Helm M; Bujnicki JM; Grosjean H MODOMICS: a database of RNA modification pathways−-2013 update. Nucleic Acids Res 2013, 41, D262–267. DOI: 10.1093/nar/gks1007. [PubMed: 23118484]

(10). de Crecy-Lagard V; Boccaletto P; Mangleburg CG; Sharma P; Lowe TM; Leidel SA; Bujnicki JM Matching tRNA modifications in humans to their known and predicted enzymes. Nucleic Acids Res 2019, 47, 2143–2159. DOI: 10.1093/nar/gkz011. [PubMed: 30698754]

(11). Hori H Methylated nucleosides in tRNA and tRNA methyltransferases. Frontiers in genetics 2014, 5, 144. DOI: 10.3389/fgene.2014.00144. [PubMed: 24904644]

(12). Esakova OA; Grove TL; Yennawar NH; Arcinas AJ; Wang B; Krebs C; Almo SC; Booker SJ Structural basis for tRNA methylthiolation by the radical SAM enzyme MiaB. Nature 2021, 597, 566–570. DOI: 10.1038/s41586-021-03904-6. [PubMed: 34526715]

(13). Schubert HL; Blumenthal RM; Cheng X Many paths to methyltransfer: a chronicle of convergence. Trends in Biochemical Sciences 2003, 28, 329–335. [PubMed: 12826405]

(14). Christian T; Hou YM Distinct determinants of tRNA recognition by the TrmD and Trm5 methyl transferases. J Mol Biol 2007, 373, 623–632. DOI: S0022–2836(07)01081–9 [pii] 10.1016/j.jmb.2007.08.010. [PubMed: 17868690]

(15). Takeda H; Toyooka T; Ikeuchi Y; Yokobori S; Okadome K; Takano F; Oshima T; Suzuki T; Endo Y; Hori H The substrate specificity of tRNA (m1G37) methyltransferase (TrmD) from Aquifex aeolicus. Genes Cells 2006, 11, 1353–1365. DOI: GTC1022 [pii] 10.1111/j.1365-2443.2006.01022.x. [PubMed: 17121543]

(16). Zhou M; Long T; Fang ZP; Zhou XL; Liu RJ; Wang ED Identification of determinants for tRNA substrate recognition by Escherichia coli C/U34 2'-O-methyltransferase. RNA Biol 2015, 12, 900–911. DOI: 10.1080/15476286.2015.1050576. [PubMed: 26106808]

(17). Grosjean H; Edqvist J; Straby KB; Giege R Enzymatic formation of modified nucleosides in tRNA: dependence on tRNA architecture. J Mol Biol 1996, 255, 67–85. DOI: 10.1006/jmbi.1996.0007. [PubMed: 8568876]

(18). Jackman JE; Montange RK; Malik HS; Phizicky EM Identification of the yeast gene encoding the tRNA m1G methyltransferase responsible for modification at position 9. RNA 2003, 9, 574–585. [PubMed: 12702816]

(19). Strassler SE; Bowles IE; Dey D; Jackman JE; Conn GL Tied up in knots: Untangling substrate recognition by the SPOUT methyltransferases. J Biol Chem 2022, 298, 102393. DOI: 10.1016/j.jbc.2022.102393. [PubMed: 35988649]

(20). Ramos J; Fu D The emerging impact of tRNA modifications in the brain and nervous system. Biochim Biophys Acta Gene Regul Mech 2019, 1862, 412–428. DOI: 10.1016/j.bbagrm.2018.11.007. [PubMed: 30529455]

(21). Suzuki T The expanding world of tRNA modifications and their disease relevance. Nat Rev Mol Cell Biol 2021, 22, 375–392. DOI: 10.1038/s41580-021-00342-0. [PubMed: 33658722]

(22). Gustavsson M; Ronne H Evidence that tRNA modifying enzymes are important in vivo targets for 5-fluorouracil in yeast. RNA 2008, 14, 666–674. DOI: rna.966208 [pii] 10.1261/rna.966208. [PubMed: 18314501]

(23). Bowles IE; Jackman JE A tRNA-specific function for tRNA methyltransferase Trm10 is associated with a new tRNA quality control mechanism in Saccharomyces cerevisiae. bioRxiv 2023, 2023.2010.2006.561306. DOI: 10.1101/2023.10.06.561306.

(24). Bash-Imam Z; Therizols G; Vincent A; Laforets F; Polay Espinoza M; Pion N; Macari F; Pannequin J; David A; Saurin JC; Mertani HC; Textoris J; Auboeuf D; Catez F; Dalla Venezia N; Dutertre M; Marcel V; Diaz JJ Translational reprogramming of colorectal cancer cells induced by 5-fluorouracil through a miRNA-dependent mechanism. Oncotarget 2017, 8, 46219–46233. DOI: 10.18632/oncotarget.17597. [PubMed: 28515355]

(25). Ge J; Karijolich J; Zhai Y; Zheng J; Yu YT 5-Fluorouracil Treatment Alters the Efficiency of Translational Recoding. Genes 2017, 8. DOI: 10.3390/genes8110295.

(26). Hoskins J; Butler JS RNA-based 5-fluorouracil toxicity requires the pseudouridylation activity of Cbf5p. Genetics 2008, 179, 323–330. DOI: 10.1534/genetics.107.082727. [PubMed: 18493057]

(27). Costanzo M; Baryshnikova A; Bellay J; Kim Y; Spear ED; Sevier CS; Ding H; Koh JL; Toufighi K; Mostafavi S; Prinz J; St Onge RP; VanderSluis B; Makhnevych T; Vizeacoumar FJ; Alizadeh S; Bahr S; Brost RL; Chen Y; Cokol M; Deshpande R; Li Z; Lin ZY; Liang W; Marback M; Paw J; San Luis BJ; Shuteriqi E; Tong AH; van Dyk N; Wallace IM; Whitney JA; Weirauch MT; Zhong G; Zhu H; Houry WA; Brudno M; Ragibizadeh S; Papp B; Pal C; Roth FP; Giaever G; Nislow C; Troyanskaya OG; Bussey H; Bader GD; Gingras AC; Morris QD; Kim PM; Kaiser CA; Myers CL; Andrews BJ; Boone C The genetic landscape of a cell. Science 2010, 327, 425–431. DOI: 10.1126/science.1180823. [PubMed: 20093466]

(28). Bekaert M; Rousset JP An extended signal involved in eukaryotic −1 frameshifting operates through modification of the E site tRNA. Mol Cell 2005, 17, 61–68. DOI: 10.1016/j.molcel.2004.12.009. [PubMed: 15629717]

(29). Lecointe F; Simos G; Sauer A; Hurt EC; Motorin Y; Grosjean H Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of psi 38 and psi 39 in tRNA anticodon loop. J Biol Chem 1998, 273, 1316–1323. [PubMed: 9430663]

(30). Han L; Phizicky EM A rationale for tRNA modification circuits in the anticodon loop. RNA 2018, 24, 1277–1284. DOI: 10.1261/rna.067736.118. [PubMed: 30026310]

(31). Han L; Kon Y; Phizicky EM Functional importance of Psi38 and Psi39 in distinct tRNAs, amplified for tRNAGln(UUG) by unexpected temperature sensitivity of the s2U modification in yeast. RNA 2015, 21, 188–201. DOI: 10.1261/rna.048173.114. [PubMed: 25505024]

(32). Durant PC; Davis DR Stabilization of the anticodon stem-loop of tRNALys,3 by an A+-C base-pair and by pseudouridine. J Mol Biol 1999, 285, 115–131. DOI: 10.1006/jmbi.1998.2297. [PubMed: 9878393]

(33). Shaheen R; Han L; Faqeih E; Ewida N; Alobeid E; Phizicky EM; Alkuraya FS A homozygous truncating mutation in PUS3 expands the role of tRNA modification in normal cognition. Human genetics 2016, 135, 707–713. DOI: 10.1007/s00439-016-1665-7. [PubMed: 27055666]

(34). de Brouwer APM; Abou Jamra R; Kortel N; Soyris C; Polla DL; Safra M; Zisso A; Powell CA; Rebelo-Guiomar P; Dinges N; Morin V; Stock M; Hussain M; Shahzad M; Riazuddin S; Ahmed ZM; Pfundt R; Schwarz F; de Boer L; Reis A; Grozeva D; Raymond FL; Riazuddin S; Koolen DA; Minczuk M; Roignant JY; van Bokhoven H; Schwartz S Variants in PUS7 Cause Intellectual Disability with Speech Delay, Microcephaly, Short Stature, and Aggressive Behavior. American journal of human genetics 2018, 103, 1045–1052. DOI: 10.1016/j.ajhg.2018.10.026. [PubMed: 30526862]

(35). Shaheen R; Tasak M; Maddirevula S; Abdel-Salam GMH; Sayed ISM; Alazami AM; Al-Sheddi T; Alobeid E; Phizicky EM; Alkuraya FS PUS7 mutations impair pseudouridylation in humans and cause intellectual disability and microcephaly. Human genetics 2019, 138, 231–239. DOI: 10.1007/s00439-019-01980-3. [PubMed: 30778726]

(36). Alexandrov A; Chernyakov I; Gu W; Hiley SL; Hughes TR; Grayhack EJ; Phizicky EM Rapid tRNA decay can result from lack of nonessential modifications. Mol Cell 2006, 21, 87–96. DOI: 10.1016/j.molcel.2005.10.036. [PubMed: 16387656]

(37). Payea MJ; Hauke AC; De Zoysa T; Phizicky EM Mutations in the anticodon stem of tRNA cause accumulation and Met22-dependent decay of pre-tRNA in yeast. RNA 2020, 26, 29–43. DOI: 10.1261/rna.073155.119. [PubMed: 31619505]

(38). Kadaba S; Krueger A; Trice T; Krecic AM; Hinnebusch AG; Anderson J Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev 2004, 18, 1227–1240. [PubMed: 15145828]

(39). Vilardo E; Amman F; Toth U; Kotter A; Helm M; Rossmanith W Functional characterization of the human tRNA methyltransferases TRMT10A and TRMT10B. Nucleic Acids Res 2020, 48, 6157–6169. DOI: 10.1093/nar/gkaa353. [PubMed: 32392304]

(40). Clark WC; Evans ME; Dominissini D; Zheng G; Pan T tRNA base methylation identification and quantification via high-throughput sequencing. RNA 2016, 22, 1771–1784. DOI: 10.1261/rna.056531.116. [PubMed: 27613580]

(41). Strassler SE; Bowles IE; Krishnamohan A; Kim H; Kuiper EG; Hancock CJ; Comstock LR; Jackman JE; Conn GL tRNA m1G9 modification depends on substrate-specific RNA conformational changes induced by the methyltransferase Trm10. bioRxiv 2023, 2023.2002.2001.526536. DOI: 10.1101/2023.02.01.526536.

(42). Shao Z; Yan W; Peng J; Zuo X; Zou Y; Li F; Gong D; Ma R; Wu J; Shi Y; Zhang Z; Teng M; Li X; Gong Q Crystal structure of tRNA m1G9 methyltransferase Trm10: insight into the catalytic mechanism and recognition of tRNA substrate. Nucleic Acids Res 2014, 42, 509–525. DOI: 10.1093/nar/gkt869. [PubMed: 24081582]

(43). Van Laer B; Roovers M; Wauters L; Kasprzak JM; Dyzma M; Deyaert E; Kumar Singh R; Feller A; Bujnicki JM; Droogmans L; Versees W Structural and functional insights into tRNA binding and adenosine N1-methylation by an archaeal Trm10 homologue. Nucleic Acids Res 2016, 44, 940–953. DOI: 10.1093/nar/gkv1369. [PubMed: 26673726]

(44). Singh RK; Feller A; Roovers M; Van Elder D; Wauters L; Droogmans L; Versees W Structural and biochemical analysis of the dual-specificity Trm10 enzyme from Thermococcus kodakaraensis prompts reconsideration of its catalytic mechanism. RNA 2018, 24, 1080–1092. DOI: 10.1261/rna.064345.117. [PubMed: 29848639]

(45). Bhatta A; Dienemann C; Cramer P; Hillen HS Structural basis of RNA processing by human mitochondrial RNase P. Nat Struct Mol Biol 2021, 28, 713–723. DOI: 10.1038/s41594-021-00637-y. [PubMed: 34489609]

(46). Holzmann J; Frank P; Loffler E; Bennett KL; Gerner C; Rossmanith W RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. Cell 2008, 135, 462–474. DOI: 10.1016/j.cell.2008.09.013. [PubMed: 18984158]

(47). Vilardo E; Nachbagauer C; Buzet A; Taschner A; Holzmann J; Rossmanith W A subcomplex of human mitochondrial RNase P is a bifunctional methyltransferase--extensive moonlighting in mitochondrial tRNA biogenesis. Nucleic Acids Res 2012, 40, 11583–11593. DOI: 10.1093/nar/gks910. [PubMed: 23042678]

(48). Gillis D; Krishnamohan A; Yaacov B; Shaag A; Jackman JE; Elpeleg O TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. Journal of medical genetics 2014, 51, 581–586. DOI: 10.1136/jmedgenet-2014-102282. [PubMed: 25053765]

(49). Igoillo-Esteve M; Genin A; Lambert N; Desir J; Pirson I; Abdulkarim B; Simonis N; Drielsma A; Marselli L; Marchetti P; Vanderhaeghen P; Eizirik DL; Wuyts W; Julier C; Chakera AJ; Ellard S; Hattersley AT; Abramowicz M; Cnop M tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. PLoS genetics 2013, 9, e1003888. DOI: 10.1371/journal.pgen.1003888. [PubMed: 24204302]

(50). Lin H; Zhou X; Chen X; Huang K; Wu W; Fu J; Li Y; Polychronakos C; Dong GP tRNA methyltransferase 10 homologue A (TRMT10A) mutation in a Chinese patient with diabetes, insulin resistance, intellectual deficiency and microcephaly. BMJ Open Diabetes Res Care 2020, 8. DOI: 10.1136/bmjdrc-2020-001601.

(51). Narayanan M; Ramsey K; Grebe T; Schrauwen I; Szelinger S; Huentelman M; Craig D; Narayanan V; Group CRR Case Report: Compound heterozygous nonsense mutations in TRMT10A are associated with microcephaly, delayed development, and periventricular white matter hyperintensities. F1000Res 2015, 4, 912. DOI: 10.12688/f1000research.7106.1. [PubMed: 26535115]

(52). Siklar Z; Kontbay T; Colclough K; Patel KA; Berberoglu M Expanding the Phenotype of TRMT10A Mutations: Case Report and a Review of the Existing Cases. J Clin Res Pediatr Endocrinol 2021. DOI: 10.4274/jcrpe.galenos.2021.2021.0110.

(53). Stern E; Vivante A; Barel O; Levy-Shraga Y TRMT10A Mutation in a Child with Diabetes, Short Stature, Microcephaly and Hypoplastic Kidneys. J Clin Res Pediatr Endocrinol 2022, 14, 227–232. DOI: 10.4274/jcrpe.galenos.2020.2020.0265. [PubMed: 33448213]

(54). Yew TW; McCreight L; Colclough K; Ellard S; Pearson ER tRNA methyltransferase homologue gene TRMT10A mutation in young adult-onset diabetes with intellectual disability, microcephaly and epilepsy. Diabetic medicine : a journal of the British Diabetic Association 2016, 33, e21–25. DOI: 10.1111/dme.13024. [PubMed: 26526202]

(55). Zung A; Kori M; Burundukov E; Ben-Yosef T; Tatoor Y; Granot E Homozygous deletion of TRMT10A as part of a contiguous gene deletion in a syndrome of failure to thrive, delayed puberty, intellectual disability and diabetes mellitus. Am J Med Genet A 2015, 167A, 3167–3173. DOI: 10.1002/ajmg.a.37341. [PubMed: 26297882]

(56). Cosentino C; Toivonen S; Diaz Villamil E; Atta M; Ravanat JL; Demine S; Schiavo AA; Pachera N; Deglasse JP; Jonas JC; Balboa D; Otonkoski T; Pearson ER; Marchetti P; Eizirik DL; Cnop M; Igoillo-Esteve M Pancreatic beta-cell tRNA hypomethylation and fragmentation link TRMT10A deficiency with diabetes. Nucleic Acids Res 2018, 46, 10302–10318. DOI: 10.1093/nar/gky839. [PubMed: 30247717]

(57). Krishnamohan A; Jackman JE A Family Divided: Distinct Structural and Mechanistic Features of the SpoU-TrmD (SPOUT) Methyltransferase Superfamily. Biochemistry 2019, 58, 336–345. DOI: 10.1021/acs.biochem.8b01047. [PubMed: 30457841]

(58). Krishnamohan A; Dodbele S; Jackman JE Insights into Catalytic and tRNA Recognition Mechanism of the Dual-Specific tRNA Methyltransferase from Thermococcus kodakarensis. Genes 2019, 10. DOI: 10.3390/genes10020100.

(59). Kempenaers M; Roovers M; Oudjama Y; Tkaczuk KL; Bujnicki JM; Droogmans L New archaeal methyltransferases forming 1-methyladenosine or 1-methyladenosine and 1-methylguanosine at position 9 of tRNA. Nucleic Acids Res 2010, 38, 6533–6543. DOI: 10.1093/nar/gkq451. [PubMed: 20525789]

(60). Wilkinson ML; Crary SM; Jackman JE; Grayhack EJ; Phizicky EM The 2'-O-methyltransferase responsible for modification of yeast tRNA at position 4. RNA 2007, 13, 404–413. DOI: 10.1261/rna.399607. [PubMed: 17242307]

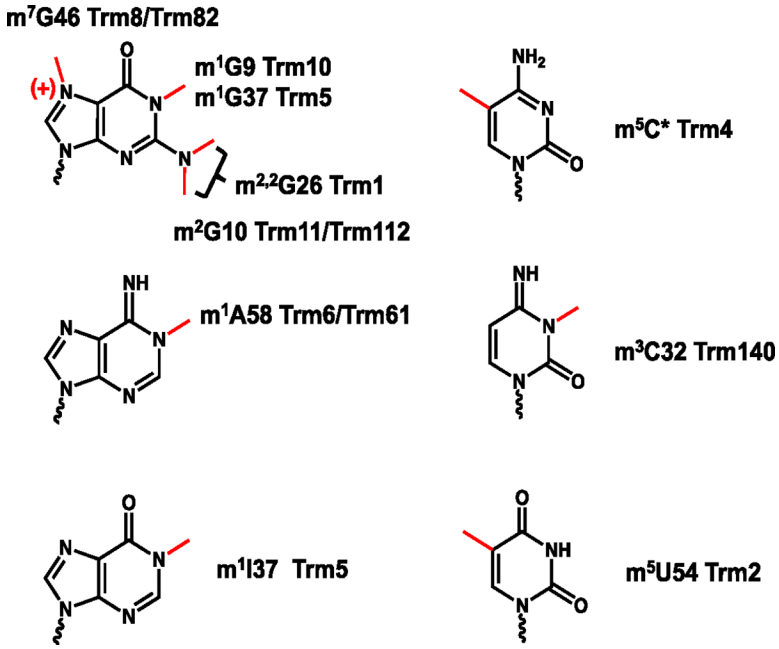



**Figure 1. tRNA nucleotide base methylations in *S. cerevisiae*.**

tRNA nucleotides are shown with the indicated methyl group base modification in red. For each base methylation, the name of the corresponding modification, its position in the tRNA and the identity of the *S. cerevisiae* methyltransferase enzyme that performs the modification are indicated. The $m^7$G46 modification results in a positive charge on the nucleotide base (indicated in parentheses). For Trm4, the asterisk indicates that this enzyme can introduce $m^5$C at 4 different positions (34, 40, 48 and 49) in *S. cerevisiae* tRNA. Both $m^1$A58 and $m^3$C32 modifications likely exist as the positively charged species at physiological pH but are indicated here as the uncharged species.

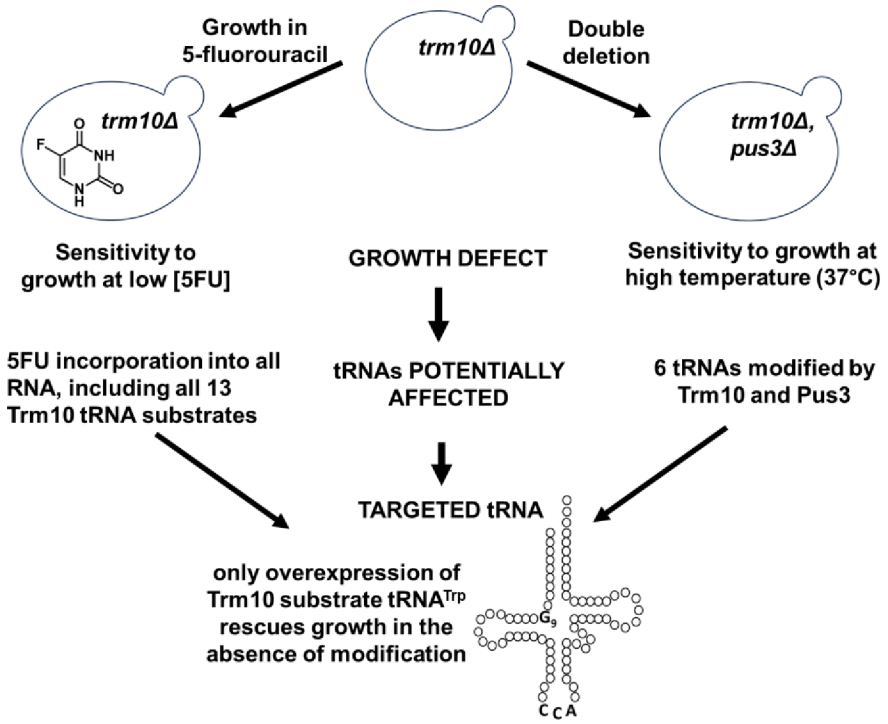



**Figure 2. Phenotypes associated with loss of the m$^1$G9 modification in *S. cerevisiae*.**

The *trm10Δ* yeast strain has been associated with two biological phenotypes that suggest a role for the m$^1$G9 modification in tRNA. In the presence of the antitumor drug 5-fluorouracil (5FU), the *trm10Δ* strain is unable to grow at concentrations that are tolerated by the *TRM10* wild-type strain. Since 5FU is incorporated into RNA molecules, including tRNA, the cause of the hypersensitivity is likely due to a combined effect of the presence of 5FU and absence of the m$^1$G9 modification on any of 13 different substrates for Trm10. Deletion of both *pus3* and *trm10* causes the strain to be hypersensitive to growth at high temperatures (37°C), and could potentially impact the fitness of any of 6 different tRNA that would lack both modifications. The overexpression of only one tRNA, modified by both enzymes, rescues growth under these stress conditions.

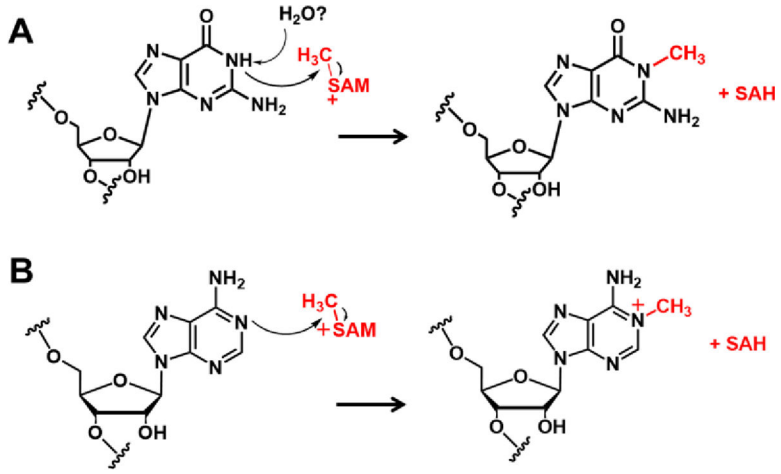



**Figure 3: Proposed mechanisms of purine methylation by Trm10 family enzymes.**

Predicted mechanisms of $m^1G9$ (A) and $m^1A9$ (B) methylation consistent with analysis of *S. cerevisiae, H. sapiens (TRMT10A)* and *T. kodakarensis* Trm10. The lack of a conserved protein residue that could act as a required general base to abstract the N1 proton from G9 is explained by a rate-determining ionization of the G9 using a collaborative active site that facilitates transfer of the N1 proton to water. This ionization is not necessary to methylate A9 nucleotides, resulting in a distinct pH dependence for this reaction.

**Table 1:**

Comparison of $m^1G9$ modification between *S. cerevisiae* and human tRNA and their $m^1G9$ methyltransferases

| *G9-containing tRNA* [a] | *S. cerevisiae* (Trm10) | | Human (TRMT10A) | |
|---|---|---|---|---|
| | *In vivo* | *In vitro* | *In vivo* | *In vitro* |
| *Ala AGC* | ✔ | NT | N/A ($A_9$) | |
| *Ala UGC* | ✔ | NT | N/A ($A_9$) | |
| *Arg ACG* | ✔ | ✔ [c] | ✔ | ✔ [e] |
| *Arg UCU* | ✔ | NT | ✔ | NT |
| *Gly CCC* | ✔ [b] | ✔ | X | ✔ |
| *Gly GCC* | ✔ | ✔ | X | ✔ |
| *Ile AAU* | ✔ | NT | N/A ($A_9$) | |
| *iMet CAU* | ✔ | ✔ [c] | ✔ | ✔ |
| *Pro AGG* | ✔ | NT | ✔ | ✔ |
| *Pro UGG* | ✔ | ✔ | ✔ | NT |
| *Thr CGU* | ✔ [b] | NT | ✔ | NT |
| *Trp CCA* | ✔ | ✔ | ✔ | ✔ |
| *Val AAC* | ✔ | NT | N/A ($A_9$) | |
| *Asn GUU* | X | ✔ | ✔ | ✔ |
| *Cys GCA* | X | ✔ | X | NT |
| *Lys CUU* | X | ✔ [d] | ✔ | NT |
| *Thr AGU* | X | ✔ | ✔ | NT |
| *Val UAC* | X | ✔ | X | ✔ [f] |
| *Leu CAA* | X | X | X | X |
| *Leu UAA* | X | NT | X | NT |
| *Leu GAG* | X | NT | X | NT |
| *Leu UAG* | X | NT | X | NT |
| *Ser AGA* | X | NT | X | NT |
| *Ser UGA* | X | NT | X | NT |

[a] The table includes the 24 tRNA isotypes for which modification status at G9 has been determined in *S. cerevisiae*. No modification data are available for tRNAs Ser(GCU), Ser(CGA) and Arg(CCU).

[b] tRNA not 100% modified in wild-type *S. cerevisiae*

[c] Activity tested using human tRNA transcript, *S. cerevisiae* transcript not available.

[d] Activity inferred based on ability of Trm10 to modify this tRNA when overexpressed in *S. cerevisiae*

[e] Tested Arg isoacceptor is not indicated in ref. 26.

[f] Activity tested using *S. cerevisiae* transcript; human transcript not available.