Two-subunit enzymes involved in eukaryotic post-transcriptional tRNA modification

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tRNA modifications are crucial for efficient and accurate protein translation, with defects often linked to disease. There are 7 cytoplasmic tRNA modifications in the yeast *Saccharomyces cerevisiae* that are formed by an enzyme consisting of a catalytic subunit and an auxiliary protein, 5 of which require only a single subunit in bacteria, and 2 of which are not found in bacteria. These enzymes include the deaminase Tad2-Tad3, and the methyltransferases Trm6-Trm61, Trm8-Trm82, Trm7-Trm732, and Trm7-Trm734, Trm9-Trm112, and Trm11-Trm112. We describe the occurrence and biological role of each modification, evidence for a required partner protein in *S. cerevisiae* and other eukaryotes, evidence for a single subunit in bacteria, and evidence for the role of the non-catalytic binding partner. Although it is unclear why these eukaryotic enzymes require partner proteins, studies of some 2-subunit modification enzymes suggest that the partner proteins help expand substrate range or allow integration of cellular activities.

Introduction

The complexity of the post-transcriptional tRNA modification machinery is remarkable, with 63 genes known to be required for synthesis of the 25 chemically distinct modifications found in the cytosolic tRNA of the yeast *Saccharomyces cerevisiae*.[1] Over the past decade it has become apparent that formation of a significant number of these modifications requires a complex comprised of 2 different subunits in eukaryotes, but where known, only one protein subunit in bacteria.[1,2] Remarkably, these complexes include 2 tRNA methyltransferases that share the same scaffold (along with 2 other methyltransferases), each catalyzing formation of different modifications,[3-8] as well as one methyltransferase that uses 2 different partner proteins for the same modification on different residues.[9-11] Here we discuss these modifications and the corresponding complexes, to shed light on the origin and conservation of the complexes and the functions of the subunits.

Post-transcriptional tRNA modifications occur in all domains of life, including the simplest organisms.[12,13] Modifications in and around the anticodon loop are often critical for translational fidelity and efficiency,[14] whereas modifications in the body of the tRNA often contribute to tRNA folding and stability.[15-17] The importance of tRNA modifications is underscored by their high conservation in different organisms,[18] by the frequent occurrence of a defined growth defect due to deletion of genes required for modifications,[2,19] and by the increasingly frequent association of human diseases with defects in modification.[20]

Several modifications on specific tRNA residues are known to be formed by a conserved enzyme family within all 3 domains of life.[13,18] Some examples include formation of Ψ_{38} and Ψ_{39} (pseudouridine, standard tRNA numbering system) by the *Escherichia coli* TrmA/*S. cerevisiae* Pus3 family of pseudouridylases,[22-24] formation of Ψ_{13} by the *Ec* TruD/*Sc* Pus7 family of pseudouridylases,[25-27] and formation of t^6A_{37} (N^6-threonylcarbamoyladenosine) by the core *Sc* Sua5/*Ec* YrdC and *Sc* KaeI/*Ec* YgjD families of proteins, along with other components depending on the organism.[28-32] By contrast, the highly conserved m^1G_{37} (1-methylguanosine) modification is catalyzed by the *Sc* Trm5 family of Rossman fold methyltransferases in eukaryotes and archaea, and by the unrelated *Ec* TrmD family of SPOUT methyltransferases in prokaryotes.[33,34]

Of particular interest are the modifications that are catalyzed by enzymes comprised of a catalytic subunit and a partner subunit in eukaryotes, but not, apparently, in prokaryotes. In the yeast *S. cerevisiae*, there are 5 tRNA modification enzymes that are composed of 2 distinct subunits (**Fig. 1**, **Table 1**), whereas available evidence suggests only a single gene product is required in bacteria. Moreover, there are 2 additional modifications found in *S. cerevisiae* and other eukaryotes but not in bacteria that are catalyzed by 2-subunit enzymes.[1,2] Here we discuss the 7 *S. cerevisiae* tRNA modification enzymes that modify cytoplasmic tRNAs and are comprised of 2 distinct protein subunits. These two-subunit modification enzymes fall into 2 classes, one of which can be further subdivided into 3 subclasses based on common themes, as described below. For each enzyme, we will discuss the occurrence and biological role of the corresponding modification, evidence for the requirement of a partner protein for catalytic activity of the *S. cerevisiae* protein, evidence for the conservation of this requirement in other eukaryotes, evidence for a single catalytic component in bacteria, and what is known about the function of the non-catalytic binding partner.
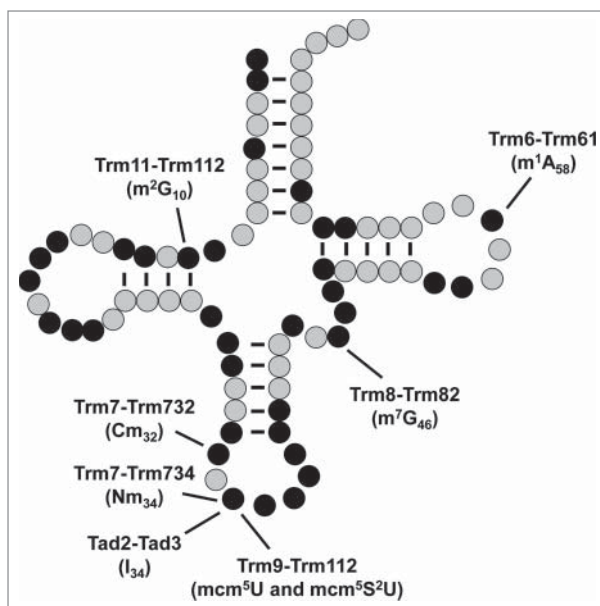
Figure 1. *S. cerevisiae* **tRNA modifications formed by 2-subunit enzymes are located throughout the tRNA.** Cloverleaf schematic depicting tRNA residues known to be modified (black) in *S. cerevisiae,* and those that are not modified (gray). Modifications (in brackets) formed by 2-subunit enzymes are labeled.

Heterodimers by Duplication and Divergence

As detailed below, there are 2 examples of eukaryotic 2-subunit enzymes that seem to have arisen by gene duplication events. For both of these complexes, it appears that the eukaryotic non-catalytic subunit has taken the place of one of the catalytic subunits of the bacterial homodimer,[35–37] perhaps to increase the substrate repertoire of the enzyme.[38,39]

Tad2-Tad3, the A_{34} deaminase

Conversion of the wobble residue A_{34} to I_{34} (inosine) is thought to occur on the majority of tRNA species that encode an A_{34} residue in bacteria and eukaryotes, but is not known to occur in archaea, which lack tRNA genes encoding A_{34}.[13,40] In

prokaryotes, I_{34} is only known to occur on tRNA$^{Arg(ACG)}$, and all 3 bacterial tRNA$^{Arg(ACG)}$ species that have been examined contain I_{34}. All 6 (of 7) *S. cerevisiae* tRNA species with an encoded A_{34} residue that have been examined have I_{34}, and all eukaryotic cytoplasmic tRNAs with an encoded A_{34} that have been examined have the I_{34} modification[13], with the exception of wheat tRNA$^{Arg(ACG)}$.[41] I_{34} increases the reading capacity of tRNAs, allowing decoding of codons ending in U and C, and often A,[42,43] and lack of the modification gene is lethal in *S. cerevisiae*, *S. pombe*, and *E. coli*.[44-46]

In *S. cerevisiae*, I_{34} is formed by the *Sc* Tad2-Tad3 protein pair.[44] *Sc TAD2* was identified as being homologous to adenosine deaminases acting on RNA, the gene was determined to be essential, a temperature sensitive mutant was generated, extracts from the temperature sensitive mutant were shown to lack A_{34} deaminase activity, and the activity was complemented by additional recombinant *Sc* Tad2 purified from *E. coli*.[44] However, purified recombinant *Sc* Tad2 itself lacked deaminase activity, and purification of *Sc* Tad2 from yeast cells resulted in co-purification of an additional polypeptide, called *Sc* Tad3. A_{34} deaminase was concluded to be comprised of an *Sc* Tad2-Tad3 heterodimer, since *Sc* Tad2 and *Sc* Tad3 co-purified in stoichiometric amounts, and biochemical fractions containing both *Sc* Tad2 and *Sc* Tad3, but neither alone, could convert A_{34} to I on a synthetic tRNA^{Ala} construct.[44]

It is likely that *TAD2* and *TAD3* arose by a gene duplication event, followed by subsequent sequence divergence, since the 120 amino acid C-terminus of *Sc* Tad3 is 26% identical and 45% similar to *Sc* Tad2, and since both *Sc* Tad2 and *Sc* Tad3 have a conserved Zn^{2+} coordination motif, as well as a conserved proline that is generally required for ammonium group binding.[44] Although both proteins are required for binding to tRNA, mutational analysis showed that *Sc* Tad2 is almost certainly the catalytic subunit of the deaminase. An *Sc* Tad2 variant with an alanine substitution in the predicted catalytic residue E56 was not active, whereas *Sc* Tad3 has a valine (V218) in this position and is presumably inactive; moreover, a complex of the *Sc* Tad3-V218E variant and *Sc* Tad2 had wild type activity, but the *Sc* Tad3-V218E variant did not restore activity to the *Sc* Tad2-E56A variant.[44]

Table 1. Eukaryotic tRNA modifications that require 2-subunit enzymes.

modification	*S. cerevisiae* enzyme[a,b]	closest *E. coli* homolog[c]	*E. coli* enzyme	references
I_{34}	Tad2-Tad3	TadA	TadA	44,45
m^1A_{58}	Trm61-Trm6 (Gcd14-Gcd10)	TrmI	TrmI	44,45,58,64,68
Nm_{32}	Trm7-Trm732	FtsJ	TrmJ	9,10,88
Nm_{34}	Trm7-Trm734 (-Rtt10, Ere2)	FtsJ	TrmL	9,10,90
m^7G_{46}	Trm8-Trm82	TrmB	TrmB	78,83
mcm^5U_{34}	Trm9-Trm112	RlmA(I)	n/a[d]	5,6,107,110
m^2G_{10}	Trm11-Trm112	YhdJ	n/a[d]	3

[a]First protein listed is the catalytic subunit.
[b]Names in brackets were original names prior to discovery of involvement in tRNA modification.
[c]Homolog of the catalytic subunit of the *S. cerevisiae* enzyme.
[d]Modification is not known to occur in bacteria.

Tad2 and Tad3 homologs are widely found in eukaryotes,[47] and evidence from eukaryotes other than *S. cerevisiae* further suggests that the Tad2-Tad3 complex is required for A_{34} deaminase activity. Thus, a screen for temperature sensitive *S. pombe* mutants identified a mutant encoding an *Sp* Tad3 variant with greatly reduced binding of *Sp* Tad2 and associated with reduced levels of inosine in tRNA.[48] Moreover, the temperature sensitive phenotype of the *Sp tad3* mutant could be suppressed by additional copies of *Sp tad2*$^+$, suggesting that overexpression drove formation of catalytically active complexes.[48] Similarly, analysis of *Trypanosoma brucei* A_{34} deaminase activity suggests the requirement of a Tad2-Tad3 complex, since a *Tb* Tad2-Tad3 complex has activity, but a *Tb* Tad2 homodimer lacks catalytic activity.[39]

By contrast, A_{34} deamination of tRNA$^{Arg(ACG)}$ is catalyzed only by TadA (the homolog of Tad2 and Tad3) in prokaryotes,[13,40] based on the occurrence of only this homolog in bacteria,[47] and the activity of the purified proteins from *E. coli* and *Agrobacterium tumefaciens*.[38,45]

TadA was shown to be a homodimer based on its crystal structure from *Staphylococcus aureus, A. aeolicus,* and *A. tumefaciens*.[36,38,49] The co-crystal structure of the *S. aureus* TadA homodimer bound to the 15-mer tRNA$^{Arg(AGC)}$ anticodon stem-loop suggests that substrate binding occurs via an induced fit of the anticodon to the rigid interface between the homodimer via specific contacts predominantly with the 5 nucleotides of the anticodon loop and the C_{32}-A_{38} pair at the top of the loop.[36]

Many residues shown to be important for tRNA binding in bacterial TadA are not conserved in *Tb* Tad2, suggesting a role for *Tb* Tad3 in substrate binding.[50] Indeed, some findings suggest that the eukaryotic heterodimer is important for recognition of a larger region on substrate tRNAs to catalyze modification of the multiple different tRNA targets, as opposed to local structural elements of the single tRNA recognized by homodimeric TadA. Thus, although bacterial TadA has activity toward only a stem-loop RNA construct, *Sc* Tad2-Tad3 requires a full tRNA construct and a proper 3-dimensional structure for activity.[38,44,51] Consistent with these findings, TadA can deaminate eukaryotic tRNA$^{Arg(ACG)}$, but cannot deaminate other eukaryotic Tad2-Tad3 tRNA substrates.[45] Remarkably, the *Tb* Tad2-Tad3 enzyme has C to U ssDNA deaminase activity both in vivo and in vitro, further demonstrating the increased substrate repertoire of eukaryotic Tad2-Tad3 as compared to bacterial TadA.[52] Alfonzo and colleagues have proposed a model wherein binding of the Zn^{2+} ion occurs intermolecularly, possibly granting increased ability to diversify substrates (as needed for the 7 substrate tRNAs in *S. cerevisae*) while still maintaining specificity for A_{34}.[39]

Trm6-Trm61, the m^1A_{58} methyltransferase

The m^1A_{58} (1-methyladenosine) modification is commonly found in eukaryotic tRNAs, including 21 cytoplasmic *S. cerevisiae* tRNAs. This modification is also found less frequently in bacterial and archaeal tRNAs, with a greater frequency of occurrence in tRNA from thermophilic organisms[13]. m^1A_{58} likely contributes to tRNA stability,[53] and *S. cerevisiae* mutants lacking this

modification are inviable due to degradation of hypomodified tRNA$_i^{Met}$ by the TRAMP complex (Trf4/Air2/Mtr4p polyadenylation complex) and the nuclear exosome.[16,54-57] This specific degradation of only tRNA$_i^{Met}$ is likely due to loss of stability in an important substructure unique to eukaryotic initiator tRNAs, wherein m^1A_{58} is involved in hydrogen bonding interactions with residues A_{20}, A_{54}, and A_{60}.[53,58] The requirement of m^1A_{58} for viability does not extend to all eukaryotes, since *S. pombe* mutants lacking this modification are viable,[46] albeit with a slow growth defect.[59]

In *S. cerevisiae*, m^1A_{58} is formed by Trm6/Trm61.[58] *Sc TRM6* (also named *GCD10*) and *Sc TRM61* (*GCD14*) were first identified in screens selecting for mutations that increased *GCN4* expression in an *Sc gcn2–101 gcn3–101* mutant.[60-62] *GCN4* expression is higher when there is less functional eIF2-GTP-tRNA$_i^{Met}$ (eIF2: eukaryotic initiation factor 2) initiation ternary complex, and it was found that high copy tRNA$_i^{Met}$ suppressed the temperature sensitive phenotype of *Sc gcd10–504* (*trm6*) mutants and the lethality of *Sc trm6Δ* and *Sc trm61Δ* mutants by restoring levels of the initiator tRNA.[58,63] It was also shown that tRNA from *Sc trm6Δ* and *Sc trm61* mutants lacked m^1A_{58} modification,[58,64] that *Sc* Trm6 and *Sc* Trm61 form a complex with m^1A_{58} catalytic activity, and that activity was dependent on the S-adenosyl methionine (AdoMet) binding domain of *Sc* Trm61, which is the catalytic subunit.[58,64] The *Sc* Trm6-Trm61complex appears to be a dimer of heterodimers based on size exclusion chromatography.[65]

The m^1A_{58} modification enzyme also appears to consist of a Trm6-Trm61 complex in other eukaryotes. Trm6 and Trm61 family proteins are found in yeast, plants, and animals,[66,67] and the human methyltransferase appears to require both Trm6 and Trm61. Thus, co-expression of human *TRM6* and human *TRM61* suppressed the temperature sensitive growth of *Sc trm6–504* and *Sc trm61–2* mutants, restored levels of m^1A_{58} on tRNA in these mutants, and led to m^1A_{58} formation on human tRNA^{Lys} introduced on a plasmid, as measured by an altered electrophoretic mobility of the tRNA.[67] Furthermore, expression of only human *TRM6* or human *TRM61* did not lead to a substantial increase in m^1A_{58} modification in mutants, and a complex of human Trm6-Trm61 purified from yeast was able to specifically methylate yeast tRNA$_i^{Met}$.[67]

It is likely that *TRM6* and *TRM61* arose from a gene duplication event followed by sequence divergence, based on the sequence similarity between predicted bacterial Trm61 homologs and eukaryotic Trm6, as well as the conservation of predicted secondary structural elements in eukaryotic Trm6, eukaryotic Trm61, and predicted bacterial Trm61 homologs.[66] This argument is further strengthened by the finding that many of the residues involved in *Mycobacterium tuberculosis* TrmI (a bacterial Trm61 homolog) homotetramer formation are also involved in the interaction between *Sc* Trm6 and *Sc* Trm61.[35,65]

By contrast, m^1A_{58} modification of bacterial and archaeal tRNA is formed by only TrmI (the homolog of Trm61), based on the occurrence of only one Trm61 homolog and no obvious Trm6 homolog in bacterial and archaeal species,[66,68,69] and the activity of the purified *Thermus thermophilus, M. tuberculosis,* and

Pyrococcus abyssi proteins.[68–70] Based on electrospray ionization mass spectrometry of the native complex, *Tt* TrmI is a homotetramer,[37] and it is also likely that the *M. tuberculosis* protein is homotetrameric, based on gel filtration analysis.[70]

One of the major functions of Trm6 in the Trm6-Trm61 complex appears to be tRNA binding. Thus, Trm6 contains an RNA recognition motif, and wild type *Sc* Trm6-Trm61 binds tRNA, as does a complex containing *Sc* Trm6 and an *Sc* Trm61 variant with mutations in the AdoMet binding domain, whereas *Sc* Trm61 by itself does not.[64] Moreover, mutations of conserved residues predicted to be involved in the interface between *Sc* Trm6 and *Sc* Trm61 abrogate tRNA binding and m^1A_{58} activity (based on the TrmI crystal structure[35]), but do not appear to disrupt the heterotetrameric complex or AdoMet binding.[65] These results have led to the speculation that these conserved residues are required to form a Trm6/Trm61 interface that is required for tRNA binding, rather than for complex formation of the proteins themselves.[65]

Acquisition of an Unrelated Partner Protein

As detailed below, there are 5 eukaryotic methyltransferases that have acquired partner proteins unrelated to the catalytic subunit. We further divide these methyltransferases into 3 distinct subclasses.

Acquisition of an unrelated subunit by eukaryotes for the same bacterial modification

This class of modification enzymes catalyzes conversion of G_{46} to m^7G_{46} (7-methylguanosine). The m^7G_{46} modification occurs in bacterial and eukaryotic tRNAs,[13] but to date has not been found in tRNA from archaea, although m^7G_{49} is found on tRNA$^{Leu(UAG)}$ from *Thermoplasma acidophilum*.[71] m^7G_{46} plays a role in stabilizing the tertiary fold of the tRNA, and is part of a commonly occurring base triple with N_{13} and N_{22}.[72,73] *S. cerevisiae* strains lacking m^7G_{46} have a mild growth defect when grown at 38°C on synthetic media containing glycerol,[74] and mutants lacking m^7G_{46} and m^5C (5-methylcytidine) are temperature-sensitive due to degradation of tRNA$^{Val(AAC)}$ by the rapid tRNA decay pathway, which also affects strains lacking m^7G_{46} in combination with lack of any of several other modifications in the body of the tRNA.[17,75,76] Additionally, m^7G_{46} was shown to be required for growth of *T. thermophilus* at high temperature, and to be required for subsequent Gm_{18} (2′-*O*-methylguanosine) and m^1G_{37} modification on tRNA^{Phe} as part of a tRNA modification network.[77]

In *S. cerevisiae*, m^7G_{46} is formed by the *Sc* Trm8-Trm82 protein pair.[78] *Sc* Trm8-Trm82 was discovered using a biochemical genomics approach[79] when it was found that protein purified from *S. cerevisiae* strains expressing tagged open reading frames for either *Sc TRM8* or *Sc TRM82* yielded m^7G formation activity on pre-tRNA^{Phe}.[78] Evidence indicating that the enzyme is composed of the *Sc* Trm8-Trm82 complex includes the observation that deletion of either gene results in lack of m^7G modification on tRNA, that the 2 proteins form a stoichiometric complex,

that recombinant *Sc* Trm8 purified from *E. coli* has low in vitro m^7G formation activity that is increased 250-fold when co-expressed with *Sc* Trm82, and that co-translation is required for an active complex.[78] *Sc* Trm8 contains a methyltransferase domain and is the catalytic subunit of the enzyme, whereas *Sc* Trm82 is a WD-repeat protein.

In other eukaryotes, the m^7G_{46} modification enzyme also appears to consist of the Trm8-Trm82 protein pair. Trm8 and Trm82 homologs are found in yeast, plants, and animals,[78] and co-expression of human *METTL1* (human *TRM8*) and *WDR4* (human *TRM82*) complemented the m^7G_{46} modification defect in *trm8Δ* or *trm82Δ* mutant cells, whereas expression of only human *METTL1* or human *WDR4* did not.[78]

In humans, it was also recently reported that HeLa cells with reduced levels of human *METTL1* and human *NSUN2* (required for m^5C) are sensitive to 5-fluorouracil, resulting in a decrease in tRNA$^{Val(AAC)}$ levels.[80] Interestingly, human *METTL1* was found to be inactivated by phosphorylation at Ser27 by protein kinase B, suggesting a possible mechanism to regulate m^7G modification levels in the cell.[81]

By contrast, in bacteria m^7G_{46} is formed by the Trm8 homolog TrmB alone, based on the apparent absence of Trm82 homologs,[82] the activity of purified *E. coli* TrmB,[83] and the ability of *E. coli* TrmB to complement the lack of *Sc* Trm8-Trm82 in *S. cerevisiae trm8Δ trm82Δ* double mutants.[74] $_{Ec}$ TrmB is monomeric,[83] whereas *Bacillus subtilis* TrmB is homodimeric in solution and in its crystal structure, which has been proposed as a first evolutionary step in the requirement for a dimeric enzyme.[84]

It is clear that part of the role of *Sc* Trm82 is to maintain levels of *Sc* Trm8 in yeast, and that *Sc* Trm82 is also required for other reasons, since *Sc* Trm8 levels are greatly reduced in *Sc trm82Δ* mutants, but restoration of levels in *S. cerevisiae* through *Sc* Trm8 overexpression only marginally restores m^7G_{46} activity.[74] In the crystal structures of members of the $_{Ec}$ TrmB/*Sc* Trm8 family, the B4-αD loop region of unbound *Sc* Trm8 is in a much different conformation than that of the *Sc* Trm8-Trm82 complex and that of the *B. subtilis* TrmB, which have a similar conformation to one another.[84,85] In *Sc* Trm8, the distinct conformation of the unbound form is stabilized by a salt bridge between R195 and E204, which is unable to form in the *Sc* Trm8-Trm82 dimer due to steric constraints imposed by *Sc* Trm82.[85] The equivalent residue to *Sc* Trm8 R195 (R129) in *B. subtilis* TrmB points in the opposite direction compared to unbound R195 in *Sc* Trm8,[84] and alanine substitution of the equivalent arginine residue in *E. coli* TrmB results in loss of more than 90% of its methyltransferase activity,[85,86] suggesting that this residue is important for Trm8/TrmB activity.

Sc Trm82 does not appear to be involved in tRNA binding since tRNA^{Phe} cross-links only to *Sc* Trm8, and not to *Sc* Trm82,[74] and since the best fit small-angle X-ray scattering (SAXS) model of the *Sc* Trm8-Trm82 complex bound to tRNA suggests that only *Sc* Trm8 is involved in tRNA binding.[85] This SAXS model also suggests that *Sc* Trm8 binds the tRNA through the local structure around the variable region, especially the D-stem and T-stem,[85] which is consistent with the finding that deletion of these stems leads to complete loss of methylation

activity by *Sc* Trm8-Trm82, whereas deletion of the acceptor or anticodon stems does not.[87]

Acquisition of 2 different unrelated subunits by eukaryotes for the same modification at different locations

The Trm7 methyltransferase is an example of this subclass, wherein a catalytic subunit engages 2 distinct partner proteins to direct the same 2'-*O*-methylation activity to different residues: to C_{32} and N_{34} to form Cm_{32} and Nm_{34}. Nm_{32} and Nm_{34} modifications are found in tRNAs from eukaryotes, bacteria, and archaea.[13] Cm_{32} and Nm_{34} occur in tandem on 3 tRNA species from *S. cerevisiae*, and Cm_{32} and Gm_{34} modification of tRNA^{Phe} appears to be highly conserved in eukaryotes, occurring in 16 of 17 eukaryotic tRNA^{Phe} species that have been examined.[13] Although Cm_{32} and Gm_{34} are found in tandem on tRNA^{Phe}, they are in chemically distinct environments from each other.[72] Other Nm_{32} and Nm_{34} modifications are found in mammalian, insect, plant, bacterial, and archaeal tRNAs, although not always in tandem,[13] and Nm_{32} and Nm_{34} modifications appear to be important in eukaryotes, although their roles are not well understood. Thus, *S. cerevisiae* and *S. pombe* mutants lacking Cm_{32} and Gm_{34}, and *S. pombe* mutants lacking Gm_{34}, have a severe growth defect due to reduced function of tRNA^{Phe}, whereas *S. cerevisiae* or *S. pombe* mutants lacking only Cm_{32} are healthy.[9-11] Furthermore, Cm_{32} and Gm_{34} on tRNA^{Phe} are also important for the formation of wybutosine (yW_{37}) from m^1G_{37} in *S. cerevisiae* and *S. pombe*[10,11] (**Fig. 2**). In bacteria and archaea, there are no reported deleterious phenotypes associated with lack of Nm_{32},[88,89] but lack of Nm_{34} in *E. coli* causes a defect in amber (UAG) suppression by tRNA$^{Leu(CUA)}$, which was suggested to implicate Nm_{34} in wobble codon:anticodon pairing.[90]

In *S. cerevisiae*, Cm_{32} is formed by the *Sc* Trm7-Trm732 protein pair, and Nm_{34} is formed by the *Sc* Trm7-Trm734 pair.[9,10] *Sc TRM7* was identified by searching for *S. cerevisiae* homologs of the *E. coli* 2'-*O*-methyltransferase *FtsJ* (*RrmJ*),[9] which 2'-*O*-methylates U_{2552} residue of the 23S rRNA subunit.[91,92] It was found that tRNA^{Phe}, tRNA$^{Leu(UAA)}$, and tRNA^{Trp} from *S. cerevisiae trm7Δ* mutants lacked Cm_{32} and Nm_{34}, and that tagged *Sc* Trm7 purified from yeast cells was able to form Cm_{32}, and to a

lesser extent, Gm_{34} on tRNA^{Phe}.[9] It was later shown that *Sc* Trm732 is required for Cm_{32} formation and that *Sc* Trm734 is required for Nm_{34} formation by showing that all 3 *Sc* Trm7 tRNA substrates from *Sc trm732Δ* or *Sc trm734Δ* mutants completely lacked their respective Cm_{32} or Nm_{34} modifications, that extracts from *Sc trm732Δ* or *Sc trm734Δ* mutants were unable to form their respective Cm_{32} or Nm_{34} modifications on synthetic substrates, and that *Sc* Trm7 forms a distinct complex with *Sc* Trm732, and a distinct separate complex with *Sc* Trm734.[10] The requirement of *Sc* Trm732 for Cm_{32} modification on tRNA^{Phe} and of *Sc* Trm734 for Gm_{34} modification was further demonstrated by the failure of overexpressed *Sc* Trm7 to suppress the slow growth phenotype of *Sc trm732Δ trm734Δ* mutants.[10] *Sc* Trm732 is an armadillo repeat protein that contains a domain of unknown function (DUF2428), and *Sc* Trm734 is a WD-repeat protein.

The requirement for the Trm7-Trm732 protein pair for Nm_{32} formation, and of the Trm7-Trm734 protein pair for Nm_{34} formation is likely conserved throughout eukaryotes. Thus, analysis of 25 eukaryotic genomes comprising all 5 eukaryotic supergroups readily identified Trm7 homologs in all 25 organisms, Trm732 homologs in 22 organisms, and Trm734 homologs in 14 organisms.[11,93] In *S. pombe*, *Sp trm7Δ* mutants lack Cm_{32}, Gm_{34}, and yW_{37} on their tRNA^{Phe}, *Sp trm732Δ* mutants lack Cm_{32}, and *Sp trm734Δ* mutants lack Gm_{34}.[11] Furthermore, expression of human *FTSJ1* (the predicted *TRM7* human homolog) suppressed the growth defect of *Sc trm7Δ* mutants by forming Cm_{32} on tRNA^{Phe}, and suppression and modification by human FTSJ1 required either *Sc TRM732* or human *THADA* (the predicted *TRM732* human homolog).[11] These findings implicate defective Nm_{32} and Nm_{34} modifications in non-syndromic X-linked intellectual disability, since mutations in *FTSJ1* are strongly linked to this disease.[94–97]

The formation of Nm_{32} in *E. coli* and in the archaeon *Sulfolobus acidocaldarius* requires members of the homodimeric TrmJ SPOUT methyltransferase family,[88,89] which are not obviously related to Trm7, Trm732, or Trm734. *Ec* TrmJ appears to require elements in the D-stem and loop for modification activity, whereas *Sa* TrmJ appears to require elements solely in the anticodon loop.[89]

The formation of Nm_{34} requires distinct genes in bacteria and archaea, neither of which are related to components of the *TRM7* modification machinery. Thus, in *E. coli*, Cm_{34} and Um_{34} on certain tRNA^{Leu} species are formed by the SPOUT methyltransferase TrmL, which recognizes its substrates by interactions with specific residues, including the N^6-(isopentenyl)-2-methylthioadenosine modification formed from A_{37} in the anticodon loop of substrate tRNAs.[90,98] By contrast, in the archaeon *Haloferax volcanii*, Cm_{34} formation on tRNA^{Trp} and on elongator tRNA^{Met} require box C/D snoRNPs (small nucleolar ribonucleoprotein) specific to each corresponding pre-tRNA.[99,100]

Comparison of Trm7 to other FtsJ family proteins suggests a possible reason for the requirement of additional proteins for Trm7 activity, since the stem-loop tRNA substrates modified by Trm7 are slightly different than the rRNA stem-loop substrates modified by other proteins in this family (**Fig. 2**). Thus, *E. coli*
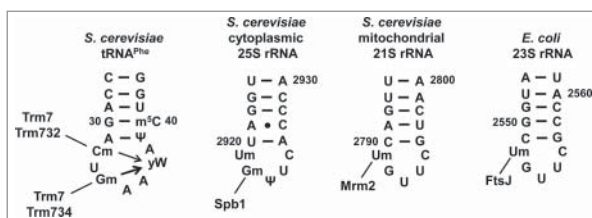

Figure 2. Schematic of FtsJ methyltransferase stem-loop substrates. *S. cerevisiae* Trm7 requires Trm732 for Cm_{32} modification and Trm734 for Gm_{34} modification of the anticodon loop of tRNA^{Phe} which, as for all tRNAs, has 7 bases. These modifications then drive formation of yW_{37} from its m^1G precursor. The thicker arrow from Gm_{34} indicates that yW formation is more dependent on this modification. Other FtsJ family members modify the 5-base A-loop in the rRNA large subunit in different organisms and organelles, as indicated.

FtsJ (which is ~34% identical to *Sc* Trm7) methylates the first residue (Um$_{2552}$) of the 5-base A-loop in the 23S rRNA,[91,92] *S. cerevisiae* Mrm2 (~29% identical to *Sc* Trm7) methylates the first residue (Um$_{2791}$) of the 5-base A-loop in the the 21S mitochondrial rRNA,[101] and *S. cerevisiae* Spb1 (~34% identical to *Sc* Trm7) methylates the second residue (Gm$_{2992}$) of the same 5-base A-loop of cytoplasmic 27S pre-rRNA,[102] each apparently acting alone. We therefore speculate that *Sc* Trm732 may help *Sc* Trm7 to recognize and modify the first residue of the 7-base loop of tRNA (as opposed to the 5-base loop in rRNA), and that *Sc* Trm734 may help *Sc* Trm7 recognize the N$_{34}$ residue, which is the third residue in the anticodon loop, and chemically distinct from the substrate residues of the other known FtsJ family members.

S. cerevisiae Trm734 has also been implicated in endoplasmic recycling, seemingly unrelated to tRNA modification. *Sc TRM734* (*ERE2*) was identified in a genome-wide screen for deletion mutants with increased canavanine resistance due to defects in endoplasmic recycling.[103] It was suggested that *Sc* Trm734 regulates the function of *Sc* Ere1, which was identified in the same screen, since it interacts with Trm734 in membrane bound fractions on a glycerol gradient, co-immunoprecipitates with *Sc* Trm734, and since some Trm734 colocalized with *Sc* Ere1 in endosomal compartments in ESCRT (endosomal sorting complexes required for transport) mutant cells. Thus, it may be that cytosolic *Sc* Trm734 functions for tRNA modification, and that membrane-bound *Sc* Trm734 plays a role in endoplasmic recycling.[103]

Sc TRM734 (*RTT10*) was also identified in a screen for *S. cerevisiae* deletion mutants that showed increased Ty1 retrotransposition.[104] In retrospect, however, this defect is likely due to decreased expression of an important protein(s) involved in repression of Ty1 transposition caused by lack of Nm$_{34}$ modification, since *Sc TRM7* was identified in the same screen.

Acquisition of a common unrelated partner protein for different methyltransferases

The Trm9 and Trm12 methyltransferases are examples of proteins in this subclass, wherein different catalytic subunits engage the same partner protein Trm112 to direct different chemical modifications on different residues. The first tRNA methyltransferase that requires Trm112 is responsible for formation of the terminal methyl group of mcm^{5}U$_{34}$ (5-methoxycarbonylmethyluridine) and related modifications. The mcm^{5}U family of modifications is found only in eukaryotes and is implicated in efficient reading of AGA and AAG codons by tRNA^{Arg} $^{(UCU)}$ and tRNA$^{Glu(UUC)}$, respectively in *S. cerevisiae*.[105] Lack of the modification is associated with sensitivity to DNA damaging agents in yeast and in humans,[105,106] as well as sensitivity to aminoglycosides at high temperature and resistance to zymocin-mediated tRNA cleavage and cell death in yeast.[107-109]

In *S. cerevisiae*, the terminal methyl group of mcm^{5}U$_{34}$ and mcm^{5}S^{2}U$_{34}$ (5-methoxycarbonylmethyl-2-thiouridine) is formed by the *Sc* Trm9-Trm112 protein pair.[5,6,107,109,110] *Sc TRM9* was identified as a putative methyltransferase by bioinformatics, tRNA from *S. cerevisiae trm9Δ* mutants was shown to lack the

mcm^{5}U and mcm^{5}S^{2}U modifications, and tagged *Sc* Trm9 protein purified from yeast was shown to methylate a saponified tRNA extract, demonstrating that *Sc* Trm9 is required for formation of the terminal methyl group of mcm^{5}U.[107] Subsequently, several suppressors of zymocin toxicity in *S. cerevisiae* were identified as mutants encoding *Sc* Trm9 variants lacking the C-terminal domain required for interaction with *Sc* Trm112.[109,110] The requirement of *Sc* Trm112 for mcm^{5}U and mcm^{5}S^{2}U was later determined explicitly by showing that tRNA from *Sc trm112Δ* mutants lacked the terminal methyl group of the mcm^{5}U modification, and that purified *Sc* Trm9- Trm112 from *E. coli* had this methylation activity, whereas *Sc* Trm9 alone did not.[5,6] Interestingly, lack of either *Sc* Trm9 or *Sc* Trm112 gives rise to ncm^{5}U (5-carbamoylmethyluridine) instead of the expected cm^{5}U modification in yeast, raising questions about the biochemistry of mcm^{5}U formation.[6]

The requirement of Trm9-Trm112 for mcm^{5}U is likely conserved in other eukaryotes. Thus, predicted Trm9 proteins are found in yeast, worms, flies, and humans,[107] and predicted Trm112 proteins are found in fungi, animals, and plants.[4] Additionally, the *Mus musculus* Trm9 and Trm112 homologs are required for mcm^{5}U formation in mice,[111] and the Trm9 homolog is required for this modification in human cells.[106] However, the role that Trm112 plays for the methyltransferase activity of Trm9 is not yet clear.

Trm112-Trm11, the m^{2}G$_{10}$ methyltransferase

The second tRNA methyltransferase that requires Trm112 is responsible for formation of the m^{2}G$_{10}$ (N^2-methylguanosine) modification, which is found in eukaryotes and archaea, but not in bacteria.[13] Although the precise role of this modification is not clear, it is likely involved in tRNA stability since lack of this modification in combination with lack of m^{2,2}G$_{26}$ (N^2,N^2,-dimethylguanosine) in *S. cerevisiae* results in slow growth.[3]

In *S. cerevisiae*, m^{2}G$_{10}$ is formed by the *Sc* Trm11-Trm112 pair.[3] *Sc TRM11* was identified by bioinformatics approaches as a putative methyltransferase, and it was found that extracts from *S. cerevisiae* cells lacking *Sc TRM11* were unable to catalyze formation of m^{2}G on synthetic tRNA$^{Ile(UAU)}$. However, although purified *Sc* Trm11 from yeast yielded m^{2}G activity, *Sc* Trm11 purified from *E. coli* did not, and it was shown that *Sc trm112Δ* mutants lacked m^{2}G activity, that tagged *Sc* Trm112 purified from yeast exhibited m^{2}G activity, and that *Sc* Trm11 and *Sc* Trm112 formed a complex.[3] *Sc* Trm112 was later shown explicitly to be required for *Sc* Trm11 activity using a wheat germ cell free system.[112]

Since *Sc* Trm11 and *Sc* Trm112 homologs are found widely in eukaryotes and in archaea,[3] it is likely that they are required for m^{2}G$_{10}$ activity in other organisms, although to our knowledge this has not been tested experimentally. The precise role of *Sc* Trm112 for *Sc* Trm11 activity is not known, but *Sc* Trm11 stability does not appear to be affected by *Sc* Trm112 levels.[3]

Trm112 has distinct roles in other methyltransferase complexes

Trm112 is also required for the function of 2 other 2-subunit methyltransferase enzymes. We will briefly discuss these enzymes

because studies on their structure and function suggest several possible roles for Trm112 in Trm9 and Trm11 function, and because the relative amounts of each complex may affect the methylation activity of the other Trm112-containing complexes, since Trm112 appears to be limiting in *S. cerevisiae*.[8,113]

Analysis of the Mtq2-Trm112 complex suggests that Trm112 functions to solubilize Mtq2 and to place it in an active conformation.[4,114] In *S. cerevisiae*, *Sc* Mtq2-Trm112 is the enzyme responsible for N^5 methylation of the glutamine residue of the GGQ tripeptide motif of eukaryotic release factor 1 (eRF1).[4,115,116] The crystal structure of Mtq2-Trm112 from *Encephalitozoon cuniculi* consists of a single heterodimer, and suggests that *E. cuniculi* Trm112 may function to solubilize *E. cuniculi* Mtq2 by masking a hydrophobic patch involved in the dimer interface.[114] Indeed, the presence of *Sc* Trm112 increases the solubility of *Sc* Mtq2 when the proteins are expressed in *E. coli*.[4] Analysis of the crystal structure also suggests that *E. cuniculi* Trm112 may be important for the conformation of the AdoMet binding domain of *E. cuniculi* Mtq2, since a loop motif is involved in both interaction with *E. cuniculi* Trm112 and with the AdoMet cofactor.[114]

Analysis of the overall structure of the *E. cuniculi* Mtq2-Trm112 complex suggests that Trm112 may also function in substrate binding.[114] Although the structure of the methyltransferase domain of *E. cuniculi* Mtq2 is most similar to that of *E. coli* PrmC (the protein that N^5 methylates the Gln residue of the GGQ motif of bacterial release factors RF1 and RF2[117,118]), the overall structure of the *E. cuniculi* Mtq2-Trm112 complex most closely resembles that of *E. coli* RlmA(I),[119] which methylates 23S rRNA residue G_{745}.[120,121] The Zn^{2+} binding domain of *E. cuniculi* Trm112 superimposes well with the Zn^{2+} binding domain of *E. coli* RlmA(1), which has been implicated in rRNA binding,[119] suggesting that Trm112 may be important for substrate recognition or binding.[114]

In addition, analysis of RlmA(I) structure suggested a role for Trm112 in binding and activity of Trm9, since RlmA(I) is the closest *E. coli* homolog of *S. cerevisiae* Trm9, and a model of Trm112 binding to Trm9 correctly predicted residues important for both Trm9-Trm112 protein-protein interactions and activity.[114]

Analysis of the Bud23-Trm112 complex indicates that Trm112 maintains Bud23 protein levels.[7] In *S. cerevisiae*, the *Sc* Bud23-Trm112 complex is required for the m^{7}G modification of G_{1575} on the 18S subunit of rRNA in yeast.[7,8,122] *Sc trm112Δ* mutants have severely reduced levels of *Sc* Bud23,[7] and surprisingly, the *Sc bud23Δ* slow growth phenotype can be rescued by expression of a catalytic null *Sc* Bud23 variant,[122] further indicating that the role of *Sc* Trm112 in this complex is not for catalysis. Moreover, the slow growth phenotype of the *Sc trm112Δ* mutant is almost completely due to Bud23 defects, since the *Sc bud23Δ trm112Δ* double mutant grew as poorly as the *Sc bud23Δ* or *Sc trm112Δ* single mutants.[5,8]

It appears that *Sc* Trm112 may also act to integrate methylation signals between tRNA, the ribosome, and release factors (**Fig. 3**).[114] Because *Sc* Trm112 is likely present in non-stoichiometric amounts compared to its methyltransferase partners,[8,113]
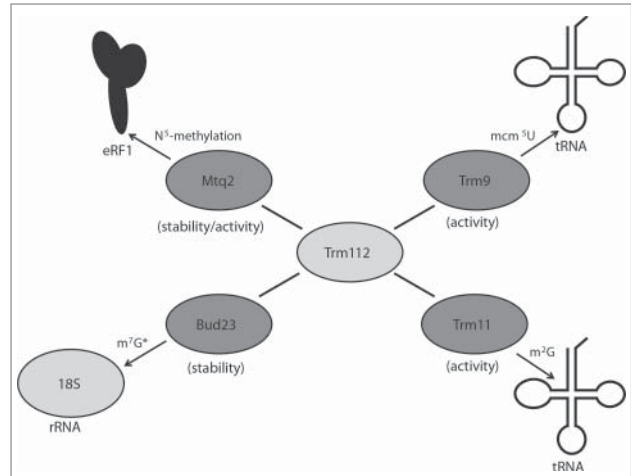

Figure 3. Trm112 partners with several methyltransferases involved in diverse translational processes. Schematic of the *S. cerevisiae* Trm112 physical interactions known to affect the activity or stability (in brackets) of its partner methyltransferases. (*) The *Sc* Bud23 protein, but not its methyltransferase activity, is required for m^{7}G$_{1575}$ formation on the cytoplasmic 18S rRNA subunit.

the activity of each methyltransferase partner is sensitive to the relative amounts of the other *Sc* Trm112 methyltransferase partners. For instance, overexpression of *Sc* Trm11 or *Sc* Mtq2 results in resistance to zymocin toxicity and reduced binding between *Sc* Trm112 and *Sc* Trm9. Furthermore, overexpression of *Sc* Mtq2 leads to cell growth defects, presumably by titrating *Sc* Trm112 away from *Sc* Bud23, and this defect is partially rescued by overexpression of *Sc* Bud23.[7]

Concluding Remarks

Several themes emerge upon analysis of the protein pairs involved in eukaryotic tRNA modification. As described above, tRNA modification enzymes have acquired a second subunit either by duplication of a gene or by acquisition of an unrelated partner protein, but with multiple different properties. One functional theme that has emerged is that the addition of a partner protein may allow for an expansion of the substrate repertoire for a given enzyme, as for the eukaryotic Tad2-Tad3 enzyme, which modifies an additional 6 tRNA species (in *S. cerevisiae*) in contrast to its homodimeric bacterial TadA counterpart, which appears to only modify tRNA$^{Arg(ACG)}$. A second theme is the expanded location of modifications facilitated by the acquisition of different partners for eukaryotic Trm7 activity, allowing modification of 2 different residues in chemically distinct environments in the same tRNA species. A third theme that emerges is the distinct possibility of cross-pathway regulation by modification. This is most clear for the different methyltransferases that each require association with limiting amounts of Trm112 for crucial methylations involved in translation termination, tRNA modifications, and ribosome biogenesis. The modulation of several methyltransferase activities by one protein in theory allows the integration of multiple signals to fine-tune translation.

Similarly, it is possible that Trm734 could also act to integrate different cellular functions because of the involvement of Trm734 in endoplasmic recycling and retrotransposition.

While we have pointed out some of the common themes in these 2-subunit eukaryotic tRNA modification enzymes, it remains unclear precisely how the non-catalytic subunits function in the modification complexes. Although we have cited evidence describing roles of these proteins in maintaining stability, altering conformations of proteins, expanding substrate repertoire, and promoting substrate binding, in many cases the evidence is indirect and limited. Furthermore, the mechanism by which unrelated partners were acquired is not clear in most cases. Further research will undoubtedly lead to insight into these and other questions regarding the roles of partner proteins in these important and conserved modification reactions.

References

1. Hopper AK. Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast Saccharomyces cerevisiae. Genetics 2013; 194:43-67; PMID:23633143; http://dx.doi.org/10.1534/genetics.112.147470

2. El Yacoubi B, Bailly M, de Crecy-Lagard V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Ann Rev Genet 2012; 46:69-95; PMID:22905870; http://dx.doi.org/10.1146/annurev-genet-110711-155641

3. Purushothaman SK, Bujnicki JM, Grosjean H, Lapeyre B. Trm11p and Trm112p are both required for the formation of 2-methylguanosine at position 10 in yeast tRNA. Mol Cell Biol 2005; 25:4359-70; PMID:15899842; http://dx.doi.org/10.1128/MCB.25.11.4359-4370.2005

4. Heurgue-Hamard V, Graille M, Scrima N, Ulryck N, Champ S, van Tilbeurgh H, Buckingham RH. The zinc finger protein Ynr046w is plurifunctional and a component of the eRF1 methyltransferase in yeast. J Biol Chem 2006; 281:36140-8; PMID:17008308; http://dx.doi.org/10.1074/jbc.M608571200

5. Mazauric MH, Dirick L, Purushothaman SK, Bjork GR, Lapeyre B. Trm112p is a 15-kDa zinc finger protein essential for the activity of two tRNA and one protein methyltransferases in yeast. J Biol Chem 2010; 285:18505-15; PMID:20400505; http://dx.doi.org/10.1074/jbc.M110.113100

6. Chen C, Huang B, Anderson JT, Bystrom AS. Unexpected accumulation of ncm(5)U and ncm(5)S(2) (U) in a trm9 mutant suggests an additional step in the synthesis of mcm(5)U and mcm(5)S(2)U. PloS one 2011; 6:e20783; PMID:21687733; http://dx.doi.org/10.1371/journal.pone.0020783

7. Figaro S, Wacheul L, Schillewaert S, Graille M, Huvelle E, Mongeard R, Zorbas C, Lafontaine DL, Heurgue-Hamard V. Trm112 is required for Bud23-mediated methylation of the 18S rRNA at position G1575. Mol Cell Biol 2012; 32:2254-67; PMID:22493060; http://dx.doi.org/10.1128/MCB.06623-11

8. Sardana R, Johnson AW. The methyltransferase adaptor protein Trm112 is involved in biogenesis of both ribosomal subunits. Mol Biol Cell 2012; 23:4313-22; PMID:22956767; http://dx.doi.org/10.1091/mbc.E12-05-0370

9. Pintard L, Lecointe F, Bujnicki JM, Bonnerot C, Grosjean H, Lapeyre B. Trm7p catalyses the formation of two 2'-O-methylriboses in yeast tRNA anticodon loop. EMBO J 2002; 21:1811-20; PMID:11927565; http://dx.doi.org/10.1093/emboj/21.7.1811

10. Guy MP, Podyma BM, Preston MA, Shaheen HH, Krivos KL, Limbach PA, Hopper AK, Phizicky EM. Yeast Trm7 interacts with distinct proteins for critical modifications of the tRNAPhe anticodon loop. RNA 2012; 18:1921-33; PMID:22912484; http://dx.doi.org/10.1261/rna.035287.112

11. Guy MP, Phizicky EM. Conservation of an intricate circuit for crucial modifications of the tRNAPhe anticodon loop in eukaryotes. RNA 2015; 21:61-74; PMID:25404562; http://dx.doi.org/10.1261/rna.047639.114

12. Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Fleischmann RD, Bult CJ, Kerlavage AR, Sutton G, Kelley JM, et al. The minimal gene complement of Mycoplasma genitalium. Science 1995; 270:397-403; PMID:7569993; http://dx.doi.org/10.1126/science.270.5235.397

13. Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, et al. MODOMICS: a database of RNA modification pathways–2013 update. Nucleic Acids Res 2013; 41: D262-7; PMID:23118484; http://dx.doi.org/10.1093/nar/gks1007

14. Agris PF, Vendeix FA, Graham WD. tRNA's wobble decoding of the genome: 40 years of modification. J Mol Biol 2007; 366:1-13; PMID:17187822; http://dx.doi.org/10.1016/j.jmb.2006.11.046

15. Helm M, Giege R, Florentz C. A Watson-Crick base-pair-disrupting methyl group (m1A9) is sufficient for cloverleaf folding of human mitochondrial tRNALys. Biochemistry 1999; 38:13338-46; PMID:10529209; http://dx.doi.org/10.1021/bi991061g

16. 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; 18:1227-40; PMID:15145828; http://dx.doi.org/10.1101/gad.1183804

17. 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; PMID:16387656; http://dx.doi.org/10.1016/j.molcel.2005.10.036

18. Grosjean H. Nucleic acids are not boring long polymers of only four types of nucleotides: a guided tour. In: Grosjean H, ed. DNA and RNA Modification Enzymes: Structure Mechanism, Function and Evolution. Austin, TX: Landes Bioscience, 2009:1-12.

19. Phizicky EM, Hopper AK. tRNA biology charges to the front. Genes Dev 2010; 24:1832-60; PMID:20810645; http://dx.doi.org/10.1101/gad.1956510

20. Torres AG, Batlle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. Trends in Mol Med 2014; 20:306-14; http://dx.doi.org/10.1016/j.molmed.2014.01.008

21. Sprinzl M, Grueter F, Spelzhaus A, Gauss DH. Compilation of tRNA sequences. Nucleic Acids Res 1980; 8:r1-r22; PMID:6986608; http://dx.doi.org/10.1093/nar/8.1.1

22. Kammen HO, Marvel CC, Hardy L, Penhoet EE. Purification, structure, and properties of Escherichia coli tRNA pseudouridine synthase I. J Biol Chem 1988; 263:2255-63; PMID:3276686

23. 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; 273:1316-23; PMID:9430663; http://dx.doi.org/10.1074/jbc.273.3.1316

24. Blaby IK, Majumder M, Chatterjee K, Jana S, Grosjean H, de Crecy-Lagard V, Gupta R. Pseudouridine formation in archaeal RNAs: The case of Haloferax volcanii. RNA 2011; 17:1367-80; PMID:21628430; http://dx.doi.org/10.1261/rna.2712811

25. Kaya Y, Ofengand J. A novel unanticipated type of pseudouridine synthase with homologs in bacteria, archaea, and eukarya. RNA 2003; 9:711-21; PMID:12756329; http://dx.doi.org/10.1261/rna.5230603

26. Behm-Ansmant I, Urban A, Ma X, Yu YT, Motorin Y, Branlant C. The Saccharomyces cerevisiae U2 snRNA:pseudouridine-synthase Pus7p is a novel multisite-multisubstrate RNA:Psi-synthase also acting on tRNAs. RNA 2003; 9:1371-82; PMID:14561887; http://dx.doi.org/10.1261/rna.5520403

27. Muller S, Urban A, Hecker A, Leclerc F, Branlant C, Motorin Y. Deficiency of the tRNATyr:Psi 35-synthase aPus7 in Archaea of the Sulfolobales order might be rescued by the H/ACA sRNA-guided machinery. Nucleic Acids Res 2009; 37:1308-22; PMID:19139072; http://dx.doi.org/10.1093/nar/gkn1037

28. El Yacoubi B, Lyons B, Cruz Y, Reddy R, Nordin B, Agnelli F, Williamson JR, Schimmel P, Swairjo MA, de Crecy-Lagard V. The universal YrdC/Sua5 family is required for the formation of threonylcarbamoyladenosine in tRNA. Nucleic Acids Res 2009; 37:2894-909; PMID:19287007; http://dx.doi.org/10.1093/nar/gkp152

29. El Yacoubi B, Hatin I, Deutsch C, Kahveci T, Rousset JP, Iwata-Reuyl D, Murzin AG, de Crecy-Lagard V. A role for the universal Kae1/Qri7/YgjD (COG0533) family in tRNA modification. EMBO J 2011; 30:882-93; PMID:21285948; http://dx.doi.org/10.1038/emboj.2010.363

30. Srinivasan M, Mehta P, Yu Y, Prugar E, Koonin EV, Karzai AW, Sternglanz R. The highly conserved KEOPS/EKC complex is essential for a universal tRNA modification, t6A. EMBO J 2011; 30:873-81; PMID:21183954; http://dx.doi.org/10.1038/emboj.2010.343

31. Deutsch C, El Yacoubi B, de Crecy-Lagard V, Iwata-Reuyl D. Biosynthesis of threonylcarbamoyl adenosine (t6A), a universal tRNA nucleoside. J Biol Chem 2012; 287:13666-73; PMID:22378793; http://dx.doi.org/10.1074/jbc.M112.344028

32. Perrochia L, Crozat E, Hecker A, Zhang W, Bareille J, Collinet B, van Tilbeurgh H, Forterre P, Basta T. In vitro biosynthesis of a universal t6A tRNA modification in Archaea and Eukarya. Nucleic Acids Res 2013; 41:1953-64; PMID:23258706; http://dx.doi.org/10.1093/nar/gks1287

33. Bystrom AS, Hjalmarsson KJ, Wikstrom PM, Bjork GR. The nucleotide sequence of an Escherichia coli operon containing genes for the tRNA(m1G)methyltransferase, the ribosomal proteins S16 and L19 and a 21-K polypeptide. EMBO J 1983; 2:899-905; PMID:6357787

34. Bjork GR, Jacobsson K, Nilsson K, Johansson MJ, Bystrom AS, Persson OP. A primordial tRNA modification required for the evolution of life? EMBO J 2001; 20:231-9; PMID:11226173; http://dx.doi.org/10.1093/emboj/20.1.231

35. Gupta A, Kumar PH, Dineshkumar TK, Varshney U, Subramanya HS. Crystal structure of Rv2118c: an AdoMet-dependent methyltransferase from Mycobacterium tuberculosis H37Rv. J Mol Biol 2001; 312:381-91; PMID:11554794; http://dx.doi.org/10.1006/jmbi.2001.4935

36. Losey HC, Ruthenburg AJ, Verdine GL. Crystal structure of Staphylococcus aureus tRNA adenosine deaminase TadA in complex with RNA. Nat Struct Mol Biol 2006; 13:153-9; PMID:16415880; http://dx.doi.org/10.1038/nsmb1047

37. Barraud P, Golinelli-Pimpaneau B, Atmanene C, Sanglier S, Van Dorsselaer A, Droogmans L, Dardel F, Tisne C. Crystal structure of Thermus thermophilus tRNA m1A58 methyltransferase and biophysical characterization of its interaction with tRNA. J Mol Biol 2008; 377:535-50; PMID:18262540; http://dx.doi.org/10.1016/j.jmb.2008.01.041

38. Elias Y, Huang RH. Biochemical and structural studies of A-to-I editing by tRNA:A34 deaminases at the wobble position of transfer RNA. Biochemistry 2005; 44:12057-65; PMID:16142903; http://dx.doi.org/10.1021/bi050499f

39. Spears JL, Rubio MA, Gaston KW, Wywial E, Strikoudis A, Bujnicki JM, Papavasiliou FN, Alfonzo JD. A single zinc ion is sufficient for an active Trypanosoma brucei tRNA editing deaminase. J Biol Chem 2011; 286:20366-74; PMID:21507956; http://dx.doi.org/10.1074/jbc.M111.243568

40. Chan PP, Lowe TM. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. Nucleic Acids Res 2009; 37:D93-7; PMID:18984615; http://dx.doi.org/10.1093/nar/gkn787

41. Aldinger CA, Leisinger AK, Gaston KW, Limbach PA, Igloi GL. The absence of A-to-I editing in the anticodon of plant cytoplasmic tRNA (Arg) ACG demands a relaxation of the wobble decoding rules. RNA Biol 2012; 9:1239-46; PMID:22922796; http://dx.doi.org/10.4161/rna.21839

42. Crick FH. Codon–anticodon pairing: the wobble hypothesis. J Mol Biol 1966; 19:548-55; PMID:5969078; http://dx.doi.org/10.1016/S0022-2836(66)80022-0

43. Murphy FVt, Ramakrishnan V. Structure of a purine-purine wobble base pair in the decoding center of the ribosome. Nat Struct Mol Biol 2004; 11:1251-2; PMID:15558050; http://dx.doi.org/10.1038/nsmb866

44. Gerber AP, Keller W. An adenosine deaminase that generates inosine at the wobble position of tRNAs. Science 1999; 286:1146-9; PMID:10550050; http://dx.doi.org/10.1126/science.286.5442.1146

45. Wolf J, Gerber AP, Keller W. tadA, an essential tRNA-specific adenosine deaminase from Escherichia coli. EMBO J 2002; 21:3841-51; PMID:12110595; http://dx.doi.org/10.1093/emboj/cdf362

46. Kim DU, Hayles J, Kim D, Wood V, Park HO, Won M, Yoo HS, Duhig T, Nam M, Palmer G, et al. Analysis of a genome-wide set of gene deletions in the fission yeast Schizosaccharomyces pombe. Nat Biotech 2010; 28:617-23; http://dx.doi.org/10.1038/nbt.1628

47. Torres AG, Piñeyro D, Filonava L, Stracker TH, Batlle E, Ribas de Pouplana L. A-to-I editing on tRNAs: Biochemical, biological and evolutionary implications. FEBS Lett 2014; 588:4279-86; PMID:25263703; http://dx.doi.org/10.1016/j.febslet.2014.09.025

48. Tsutsumi S, Sugiura R, Ma Y, Tokuoka H, Ohta K, Ohte R, Noma A, Suzuki T, Kuno T. Wobble inosine tRNA modification is essential to cell cycle progression in G(1)/S and G(2)/M transitions in fission yeast. J Biol Chem 2007; 282:33459-65; PMID:17875641; http://dx.doi.org/10.1074/jbc.M706869200

49. Kuratani M, Ishii R, Bessho Y, Fukunaga R, Sengoku T, Shirouzu M, Sekine S, Yokoyama S. Crystal structure of tRNA adenosine deaminase (TadA) from Aquifex aeolicus. J Biol Chem 2005; 280:16002-8; PMID:15677468; http://dx.doi.org/10.1074/jbc.M414541200

50. Ragone FL, Spears JL, Wohlgamuth-Benedum JM, Kreel N, Papavasiliou FN, Alfonzo JD. The C-terminal end of the Trypanosoma brucei editing deaminase plays a critical role in tRNA binding. RNA 2011; 17:1296-306; PMID:21602302; http://dx.doi.org/10.1261/rna.2748211

51. Auxilien S, Crain PF, Trewyn RW, Grosjean H. Mechanism, specificity and general properties of the yeast enzyme catalysing the formation of inosine 34 in the anticodon of transfer RNA. J Mol Biol 1996; 262:437-58; PMID:8893855; http://dx.doi.org/10.1006/jmbi.1996.0527

52. Rubio MA, Pastar I, Gaston KW, Ragone FL, Janzen CJ, Cross GA, Papavasiliou FN, Alfonzo JD. An adenosine-to-inosine tRNA-editing enzyme that can perform C-to-U deamination of DNA. Proc Natl Acad Sci U S A 2007; 104:7821-6; PMID:17483465; http://dx.doi.org/10.1073/pnas.0702394104

53. Basavappa R, Sigler PB. The 3 A crystal structure of yeast initiator tRNA: functional implications in initiator/elongator discrimination. EMBO J 1991; 10:3105-11; PMID:1915284

54. LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell 2005; 121:713-24; PMID:15935758; http://dx.doi.org/10.1016/j.cell.2005.04.029

55. Vanacova S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W. A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol 2005; 3:e189; PMID:15828860; http://dx.doi.org/10.1371/journal.pbio.0030189

56. Kadaba S, Wang X, Anderson JT. Nuclear RNA surveillance in Saccharomyces cerevisiae: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. RNA 2006; 12:508-21; PMID:16431988; http://dx.doi.org/10.1261/rna.2305406

57. Schneider C, Anderson JT, Tollervey D. The exosome subunit Rrp44 plays a direct role in RNA substrate recognition. Mol Cell 2007; 27:324-31; PMID:17643380; http://dx.doi.org/10.1016/j.molcel.2007.06.006

58. Anderson J, Phan L, Cuesta R, Carlson BA, Pak M, Asano K, Bjork GR, Tamame M, Hinnebusch AG. The essential Gcd10p-Gcd14p nuclear complex is required for 1- methyladenosine modification and maturation of initiator methionyl-tRNA. Genes Dev 1998; 12:3650-62; PMID:9851972; http://dx.doi.org/10.1101/gad.12.23.3650

59. Zuin A, Gabrielli N, Calvo IA, Garcia-Santamarina S, Hoe KL, Kim DU, Park HO, Hayles J, Ayte J, Hidalgo E. Mitochondrial dysfunction increases oxidative stress and decreases chronological life span in fission yeast. PloS One 2008; 3:e2842; PMID:18665268; http://dx.doi.org/10.1371/journal.pone.0002842

60. Harashima S, Hinnebusch AG. Multiple GCD genes required for repression of GCN4, a transcriptional activator of amino acid biosynthetic genes in Saccharomyces cerevisiae. Mol Cell Biol 1986; 6:3990-8; PMID:3540603

61. Garcia-Barrio MT, Naranda T, Vazquez de Aldana CR, Cuesta R, Hinnebusch AG, Hershey JW, Tamame M. GCD10, a translational repressor of GCN4,

is the RNA-binding subunit of eukaryotic translation initiation factor-3. Genes Dev 1995; 9:1781-96; PMID:7542616; http://dx.doi.org/10.1101/gad.9.14.1781

62. Cuesta R, Hinnebusch AG, Tamame M. Identification of GCD14 and GCD15, novel genes required for translational repression of GCN4 mRNA in Saccharomyces cerevisiae. Genetics 1998; 148:1007-20; PMID:9539420

63. Calvo O, Cuesta R, Anderson J, Gutierrez N, Garcia-Barrio MT, Hinnebusch AG, Tamame M. GCD14p, a repressor of GCN4 translation, cooperates with Gcd10p and Lhp1p in the maturation of initiator methionyl-tRNA in Saccharomyces cerevisiae. Mol Cell Biol 1999; 19:4167-81; PMID:10330157

64. Anderson J, Phan L, Hinnebusch AG. The Gcd10p/Gcd14p complex is the essential two-subunit tRNA (1- methyladenosine) methyltransferase of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 2000; 97:5173-8; PMID:10779558; http://dx.doi.org/10.1073/pnas.090102597

65. Ozanick SG, Bujnicki JM, Sem DS, Anderson JT. Conserved amino acids in each subunit of the heteroligomeric tRNA m1A58 Mtase from Saccharomyces cerevisiae contribute to tRNA binding. Nucleic Acids Res 2007; 35:6808-19; PMID:17932071; http://dx.doi.org/10.1093/nar/gkm574

66. Bujnicki JM. In silico analysis of the tRNA:m1A58 methyltransferase family: homology-based fold prediction and identification of new members from Eubacteria and Archaea. FEBS Lett 2001; 507:123-7; PMID:11684083; http://dx.doi.org/10.1016/S0014-5793(01)02962-3

67. Ozanick S, Krecic A, Andersland J, Anderson JT. The bipartite structure of the tRNA m1A58 methyltransferase from S. cerevisiae is conserved in humans. RNA 2005; 11:1281-90; PMID:16043508; http://dx.doi.org/10.1261/rna.5040605

68. Droogmans L, Roovers M, Bujnicki JM, Tricot C, Hartsch T, Stalon V, Grosjean H. Cloning and characterization of tRNA (m1A58) methyltransferase (TrmI) from Thermus thermophilus HB27, a protein required for cell growth at extreme temperatures. Nucleic Acids Res 2003; 31:2148-56; PMID:12682365; http://dx.doi.org/10.1093/nar/gkg314

69. Roovers M, Wouters J, Bujnicki JM, Tricot C, Stalon V, Grosjean H, Droogmans L. A primordial RNA modification enzyme: the case of tRNA (m1A) methyltransferase. Nucleic Acids Res 2004; 32:465-76; PMID:14739239; http://dx.doi.org/10.1093/nar/gkh191

70. Varshney U, Ramesh V, Madabushi A, Gaur R, Subramanya HS, RajBhandary UL. Mycobacterium tuberculosis Rv2118c codes for a single-component homotetrameric m1A58 tRNA methyltransferase. Nucleic Acids Res 2004; 32:1018-27; PMID:14960715; http://dx.doi.org/10.1093/nar/gkh207

71. Tomikawa C, Ohira T, Inoue Y, Kawamura T, Yamagishi A, Suzuki T, Hori H. Distinct tRNA modifications in the thermo-acidophilic archaeon, Thermoplasma acidophilum. FEBS Lett 2013; 587:3575-80; PMID:24076028; http://dx.doi.org/10.1016/j.febslet.2013.09.021

72. Kim SH, Suddath FL, Quigley GJ, McPherson A, Sussman JL, Wang AH, Seeman NC, Rich A. Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. Science 1974; 185:435-40; PMID:4601792; http://dx.doi.org/10.1126/science.185.4149.435

73. Gautheret D, Damberger SH, Gutell RR. Identification of base-triples in RNA using comparative sequence analysis. J Mol Biol 1995; 248:27-43; PMID:7537339; http://dx.doi.org/10.1006/jmbi.1995.0200

74. Alexandrov A, Grayhack EJ, Phizicky EM. tRNA m7G methyltransferase Trm8p/Trm82p: evidence

linking activity to a growth phenotype and implicating Trm82p in maintaining levels of active Trm8p. RNA 2005; 11:821-30; PMID:15811913; http://dx.doi.org/10.1261/rna.2030705

75. Chernyakov I, Whipple JM, Kotelawala L, Grayhack EJ, Phizicky EM. Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. Genes Dev 2008; 22:1369-80; PMID:18443146; http://dx.doi.org/10.1101/gad.1654308

76. Dewe JM, Whipple JM, Chernyakov I, Jaramillo LN, Phizicky EM. The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs lacking one or more of several modifications. RNA 2012; 18:1886-96; PMID:22895820; http://dx.doi.org/10.1261/rna.033654.112

77. Tomikawa C, Yokogawa T, Kanai T, Hori H. N7-Methylguanine at position 46 (m7G46) in tRNA from Thermus thermophilus is required for cell viability at high temperatures through a tRNA modification network. Nucleic Acids Res 2010; 38:942-57; PMID:19934251; http://dx.doi.org/10.1093/nar/gkp1059

78. Alexandrov A, Martzen MR, Phizicky EM. Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA. RNA 2002; 8:1253-66; PMID:12403464; http://dx.doi.org/10.1017/S1355838202024019

79. Martzen MR, McCraith SM, Spinelli SL, Torres FM, Fields S, Grayhack EJ, Phizicky EM. A biochemical genomics approach for identifying genes by the activity of their products. Science 1999; 286:1153-5; PMID:10550052; http://dx.doi.org/10.1126/science.286.5442.1153

80. Okamoto M, Fujiwara M, Hori M, Okada K, Yazama F, Konishi H, Xiao Y, Qi G, Shimamoto F, Ota T, et al. tRNA Modifying Enzymes, NSUN2 and METTL1, Determine Sensitivity to 5-Fluorouracil in HeLa Cells. PLoS Genet 2014; 10:e1004639; PMID:25233213; http://dx.doi.org/10.1371/journal.pgen.1004639

81. Cartlidge RA, Knebel A, Peggie M, Alexandrov A, Phizicky EM, Cohen P. The tRNA methylase METTL1 is phosphorylated and inactivated by PKB and RSK in vitro and in cells. EMBO J 2005; 24:1696-705; PMID:15861136; http://dx.doi.org/10.1038/sj.emboj.7600648

82. Michaud J, Kudoh J, Berry A, Bonne-Tamir B, Lalioti MD, Rossier C, Shibuya K, Kawasaki K, Asakawa S, Minoshima S, et al. Isolation and characterization of a human chromosome 21q22.3 gene (WDR4) and its mouse homologue that code for a WD-repeat protein. Genomics 2000; 68:71-9; PMID:10950928; http://dx.doi.org/10.1006/geno.2000.6258

83. De Bie LG, Roovers M, Oudjama Y, Wattiez R, Tricot C, Stalon V, Droogmans L, Bujnicki JM. The yggH gene of Escherichia coli encodes a tRNA (m7G46) methyltransferase. J Bact 2003; 185:3238-43; PMID:12730187; http://dx.doi.org/10.1128/JB.185.10.3238-3243.2003

84. Zegers I, Gigot D, van Vliet F, Tricot C, Aymerich S, Bujnicki JM, Kosinski J, Droogmans L. Crystal structure of Bacillus subtilis TrmB, the tRNA (m7G46) methyltransferase. Nucleic Acids Res 2006; 34:1925-34; PMID:16600901; http://dx.doi.org/10.1093/nar/gkl116

85. Leulliot N, Chaillet M, Durand D, Ulryck N, Blondeau K, van Tilbeurgh H. Structure of the yeast tRNA m7G methylation complex. Structure 2008; 16:52-61; PMID:18184583; http://dx.doi.org/10.1016/j.str.2007.10.025

86. Purta E, van Vliet F, Tricot C, De Bie LG, Feder M, Skowronek K, Droogmans L, Bujnicki JM. Sequence-structure-function relationships of a tRNA (m7G46) methyltransferase studied by homology modeling and site-directed mutagenesis. Proteins 2005; 59:482-8; PMID:15789416; http://dx.doi.org/10.1002/prot.20454

87. Matsumoto K, Toyooka T, Tomikawa C, Ochi A, Takano Y, Takayanagi N, Endo Y, Hori H. RNA recognition mechanism of eukaryote tRNA (m7G46) methyltransferase (Trm8-Trm82 complex). FEBS Lett 2007; 581:1599-604; PMID:17382321; http://dx.doi.org/10.1016/j.febslet.2007.03.023

88. Purta E, van Vliet F, Tkaczuk KL, Dunin-Horkawicz S, Mori H, Droogmans L, Bujnicki JM. The yfhQ gene of Escherichia coli encodes a tRNA:Cm32/Um32 methyltransferase. BMC Mol Biol 2006; 7:23; PMID:16848900; http://dx.doi.org/10.1186/1471-2199-7-23

89. Somme J, Van Laer B, Roovers M, Steyaert J, Versees W, Droogmans L. Characterization of two homologous 2'-O-methyltransferases showing different specificities for their tRNA substrates. RNA 2014; 20:1257-71; PMID:24951554; http://dx.doi.org/10.1261/rna.044503.114

90. Benitez-Paez A, Villarroya M, Douthwaite S, Gabaldon T, Armengod ME. YibK is the 2'-O-methyltransferase TrmL that modifies the wobble nucleotide in Escherichia coli tRNA(Leu) isoacceptors. RNA 2010; 16:2131-43; PMID:20855540; http://dx.doi.org/10.1261/rna.2245910

91. Caldas T, Binet E, Bouloc P, Costa A, Desgres J, Richarme G. The FtsJ/RrmJ heat shock protein of Escherichia coli is a 23 S ribosomal RNA methyltransferase. J Biol Chem 2000; 275:16414-9; PMID:10748051; http://dx.doi.org/10.1074/jbc.M001854200

92. Bugl H, Fauman EB, Staker BL, Zheng F, Kushner SR, Saper MA, Bardwell JC, Jakob U. RNA methylation under heat shock control. Mol Cell 2000; 6:349-60; PMID:10983982; http://dx.doi.org/10.1016/S1097-2765(00)00035-6

93. Feder M, Pas J, Wyrwicz LS, Bujnicki JM. Molecular phylogenetics of the RrmJ/fibrillarin superfamily of ribose 2'-O-methyltransferases. Gene 2003; 302:129-38; PMID:12527203; http://dx.doi.org/10.1016/S0378-1119(02)01097-1

94. Freude K, Hoffmann K, Jensen LR, Delatycki MB, des Portes V, Moser B, Hamel B, van Bokhoven H, Moraine C, Fryns JP, et al. Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. Am J Hum Genet 2004; 75:305-9; PMID:15162322; http://dx.doi.org/10.1086/422507

95. Ramser J, Winnepenninckx B, Lenski C, Errijgers V, Platzer M, Schwartz CE, Meindl A, Kooy RF. A splice site mutation in the methyltransferase gene FTSJ1 in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). J Med Genet 2004; 41:679-83; PMID:15342698; http://dx.doi.org/10.1136/jmg.2004.019000

96. Froyen G, Bauters M, Boyle J, Van Esch H, Govaerts K, van Bokhoven H, Ropers HH, Moraine C, Chelly J, Fryns JP, et al. Loss of SLC38A5 and FTSJ1 at Xp11.23 in three brothers with non-syndromic mental retardation due to a microdeletion in an unstable genomic region. Human Genet 2007; 121:539-47; http://dx.doi.org/10.1007/s00439-007-0343-1

97. Takano K, Nakagawa E, Inoue K, Kamada F, Kure S, Goto Y. A loss-of-function mutation in the FTSJ1 gene causes nonsyndromic X-linked mental retardation in a Japanese family. Am J Med Genet 2008; 147B:479-84; PMID:18081026; http://dx.doi.org/10.1002/ajmg.b.30638

98. Liu RJ, Zhou M, Fang ZP, Wang M, Zhou XL, Wang ED. The tRNA recognition mechanism of the minimalist SPOUT methyltransferase, TrmL. Nucleic Acids Res 2013; 41:7828-42; PMID:23804755; http://dx.doi.org/10.1093/nar/gkt568

99. Clouet d'Orval B, Bortolin ML, Gaspin C, Bachellerie JP. Box C/D RNA guides for the ribose methylation of archaeal tRNAs. The tRNATrp intron guides the formation of two ribose-methylated nucleosides in the mature tRNATrp. Nucleic Acids Res 2001; 29:4518-29; PMID:11713301; http://dx.doi.org/10.1093/nar/29.22.4518

100. Joardar A, Malliahgari SR, Skariah G, Gupta R. 2'-O-methylation of the wobble residue of elongator pre-tRNA(Met) in Haloferax volcanii is guided by a box C/D RNA containing unique features. RNA Biol 2011; 8:782-91; PMID:21654217; http://dx.doi.org/10.4161/rna.8.5.16015

101. Pintard L, Bujnicki JM, Lapeyre B, Bonnerot C. MRM2 encodes a novel yeast mitochondrial 21S rRNA methyltransferase. EMBO J 2002; 21:1139-47; PMID:11867542; http://dx.doi.org/10.1093/emboj/21.5.1139

102. Lapeyre B, Purushothaman SK. Spb1p-directed formation of Gm2922 in the ribosome catalytic center occurs at a late processing stage. Mol Cell 2004; 16:663-9; PMID:15546625; http://dx.doi.org/10.1016/j.molcel.2004.10.022

103. Shi Y, Stefan CJ, Rue SM, Teis D, Emr SD. Two novel WD40 domain-containing proteins, Ere1 and Ere2, function in the retromer-mediated endosomal recycling pathway. Mol Biol Cell 2011; 22:4093-107; PMID:21880895; http://dx.doi.org/10.1091/mbc.E11-05-0440

104. Nyswaner KM, Checkley MA, Yi M, Stephens RM, Garfinkel DJ. Chromatin-associated genes protect the yeast genome from Ty1 insertional mutagenesis. Genetics 2008; 178:197-214; PMID:18202368; http://dx.doi.org/10.1534/genetics.107.082602

105. Begley U, Dyavaiah M, Patil A, Rooney JP, DiRenzo D, Young CM, Conklin DS, Zitomer RS, Begley TJ. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. Mol Cell 2007; 28:860-70; PMID:18082610; http://dx.doi.org/10.1016/j.molcel.2007.09.021

106. Fu D, Brophy JA, Chan CT, Atmore KA, Begley U, Paules RS, Dedon PC, Begley TJ, Samson LD. Human AlkB homolog ABH8 Is a tRNA methyltransferase required for wobble uridine modification and DNA damage survival. Mol Cell Biol 2010; 30:2449-59; PMID:20308323; http://dx.doi.org/10.1128/MCB.01604-09

107. Kalhor HR, Clarke S. Novel methyltransferase for modified uridine residues at the wobble position of tRNA. Mol Cell Biol 2003; 23:9283-92; PMID:14655538; http://dx.doi.org/10.1128/MCB.23.24.9283-9292.2003

108. Lu J, Huang B, Esberg A, Johansson MJ, Bystrom AS. The Kluyveromyces lactis gamma-toxin targets tRNA anticodons. RNA 2005; 11:1648-54; PMID:16244131; http://dx.doi.org/10.1261/rna.2172105

109. Jablonowski D, Zink S, Mehlgarten C, Daum G, Schaffrath R. tRNAGlu wobble uridine methylation by Trm9 identifies Elongator's key role for zymocin-induced cell death in yeast. Mol Microbiol 2006; 59:677-88; PMID:16390459; http://dx.doi.org/10.1111/j.1365-2958.2005.04972.x

110. Studte P, Zink S, Jablonowski D, Bar C, von der Haar T, Tuite MF, Schaffrath R. tRNA and protein methylase complexes mediate zymocin toxicity in yeast. Mol Microbiol 2008; 69:1266-77; PMID:18657261; http://dx.doi.org/10.1111/j.1365-2958.2008.06358.x

111. Songe-Moller L, van den Born E, Leihne V, Vagbo CB, Kristoffersen T, Krokan HE, Kirpekar F, Falnes PO, Klungland A. Mammalian ALKBH8 possesses tRNA methyltransferase activity required for the biogenesis of multiple wobble uridine modifications implicated in translational decoding. Mol Cell Biol 2010; 30:1814-27; PMID:20123966; http://dx.doi.org/10.1128/MCB.01602-09

112. Okada K, Muneyoshi Y, Endo Y, Hori H. Production of yeast (m2G10) methyltransferase (Trm11 and Trm112 complex) in a wheat germ cell-free translation system. Nucleic Acids Symp Ser 2009:303-4; PMID:19749381; http://dx.doi.org/10.1093/nass/nrp152

113. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. Global analysis of protein expression in yeast. Nature

2003; 425:737-41; PMID:14562106; http://dx.doi.org/10.1038/nature02046

114. Liger D, Mora L, Lazar N, Figaro S, Henri J, Scrima N, Buckingham RH, van Tilbeurgh H, Heurgue-Hamard V, Graille M. Mechanism of activation of methyltransferases involved in translation by the Trm112 'hub' protein. Nucleic Acids Res 2011; 39:6249-59; PMID:21478168; http://dx.doi.org/10.1093/nar/gkr176

115. Heurgue-Hamard V, Champ S, Mora L, Merkulova-Rainon T, Kisselev LL, Buckingham RH. The glutamine residue of the conserved GGQ motif in Saccharomyces cerevisiae release factor eRF1 is methylated by the product of the YDR140w gene. J Biol Chem 2005; 280:2439-45; PMID:15509572; http://dx.doi.org/10.1074/jbc.M407252200

116. Polevoda B, Span L, Sherman F. The yeast translation release factors Mrf1p and Sup45p (eRF1) are methylated, respectively, by the methyltransferases Mtq1p and Mtq2p. J Biol Chem 2006; 281:2562-71;

PMID:16321977; http://dx.doi.org/10.1074/jbc.M507651200

117. Nakahigashi K, Kubo N, Narita S, Shimaoka T, Goto S, Oshima T, Mori H, Maeda M, Wada C, Inokuchi H. HemK, a class of protein methyl transferase with similarity to DNA methyl transferases, methylates polypeptide chain release factors, and hemK knockout induces defects in translational termination. Proc Natl Acad Sci U S A 2002; 99:1473-8; PMID:11805295; http://dx.doi.org/10.1073/pnas.032488499

118. Heurgue-Hamard V, Champ S, Engstrom A, Ehrenberg M, Buckingham RH. The hemK gene in Escherichia coli encodes the N(5)-glutamine methyltransferase that modifies peptide release factors. EMBO J 2002; 21:769-78; PMID:11847124; http://dx.doi.org/10.1093/emboj/21.4.769

119. Das K, Acton T, Chiang Y, Shih L, Arnold E, Montelione GT. Crystal structure of RlmAI: implications for understanding the 23S rRNA G745/G748-methylation at the macrolide antibiotic-binding site. Proc

Natl Acad Sci U S A 2004; 101:4041-6; PMID:14999102; http://dx.doi.org/10.1073/pnas.0400189101

120. Gustafsson C, Persson BC. Identification of the rrmA gene encoding the 23S rRNA m1G745 methyltransferase in Escherichia coli and characterization of an m1G745-deficient mutant. J Bact 1998; 180:359-65; PMID:9440525

121. Liu M, Douthwaite S. Resistance to the macrolide antibiotic tylosin is conferred by single methylations at 23S rRNA nucleotides G748 and A2058 acting in synergy. Proc Natl Acad Sci U S A 2002; 99:14658-63; PMID:12417742; http://dx.doi.org/10.1073/pnas.232580599

122. White J, Li Z, Sardana R, Bujnicki JM, Marcotte EM, Johnson AW. Bud23 methylates G1575 of 18S rRNA and is required for efficient nuclear export of pre-40S subunits. Mol Cell Biol 2008; 28:3151-61; PMID:18332120; http://dx.doi.org/10.1128/MCB.01674-07