The open reading frame TTC1157 of *Thermus thermophilus* HB27 encodes the methyltransferase forming N^2 -methylguanosine at position 6 in tRNA

MARTINE ROOVERS,¹ YAMINA OUDJAMA,¹ MARCUS FISLAGE,^{2,3} JANUSZ M. BUJNICKI,^{4,5} WIM VERSÉES,^{2,3} and LOUIS DROOGMANS^{6,7}

¹Institut de Recherches Microbiologiques Jean-Marie Wiame, B-1070 Bruxelles, Belgium

²Structural Biology Brussels, Vrije Universiteit Brussel, 1050 Brussels, Belgium

³VIB Department of Structural Biology, 1050 Brussels, Belgium

⁴International Institute of Molecular and Cell Biology in Warsaw, PL-02-109 Warsaw, Poland

⁵Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, PL-61-614 Poznan, Poland

⁶Laboratoire de Microbiologie, Université Libre de Bruxelles (ULB), B-1070 Bruxelles, Belgium

ABSTRACT

N^2 -methylguanosine (m^2G) is found at position 6 in the acceptor stem of *Thermus thermophilus* tRNA^{Phe}. In this article, we describe the cloning, expression, and characterization of the *T. thermophilus* HB27 methyltransferase (MTase) encoded by the TTC1157 open reading frame that catalyzes the formation of this modified nucleoside. S-adenosyl-L-methionine is used as donor of the methyl group. The enzyme behaves as a monomer in solution. It contains an N-terminal THUMP domain predicted to bind RNA and contains a C-terminal Rossmann-fold methyltransferase (RFM) domain predicted to be responsible for catalysis. We propose to rename the TTC1157 gene *trmN* and the corresponding protein TrmN, according to the bacterial nomenclature of tRNA methyltransferases. Inactivation of the *trmN* gene in the *T. thermophilus* HB27 chromosome led to a total absence of m^2G in tRNA but did not affect cell growth or the formation of other modified nucleosides in tRNA^{Phe}. Archaeal homologs of TrmN were identified and characterized. These proteins catalyze the same reaction as TrmN from *T. thermophilus*. Individual THUMP and RFM domains of PF1002 from *Pyrococcus furiosus* were produced. These separate domains were inactive and did not bind tRNA, reinforcing the idea that the THUMP domain acts in concert with the catalytic domain to target a particular position of the tRNA molecule.

Keywords: tRNA; modified nucleosides; *Thermus thermophilus*; methyltransferase

INTRODUCTION

Methylation is a common RNA modification present in organisms belonging to the three domains of life (Bacteria, Eukaryota, and Archaea). In particular, methylation of the exocyclic amine of guanosine, resulting in N^2 -methylguanosine (m^2G), is widespread in rRNA of all organisms. In *Escherichia coli*, three m^2G residues are found in the 16S rRNA (at positions 966, 1207, and 1516) and two in the 23S rRNA (at positions 1835 and 2445) (Andersen and Douthwaite 2006). Genes encoding four out of the five rRNA (m^2G) methyltransferases (MTases) have been identified in the *E. coli* chromosome (Tscherne et al. 1999;

Lesnyak et al. 2006, 2007; Sergiev et al. 2006). For the RsmC and RsmD enzymes, crystal structures have been solved (Lesnyak et al. 2007; Sunita et al. 2007). RsmC and RsmD MTases (m^2G at, respectively, positions 1207 and 966 of 16S rRNA of *E. coli*) utilize assembled or assembly intermediates of the 30S subunit as substrate, while RlmG and RlmL enzymes (m^2G at, respectively, positions 1835 and 2445 of 23S RNA of *E. coli*) act on naked ribosomal RNA (for review, see Sergiev et al. 2007). The mechanism of substrate recognition by these enzymes is not known, especially since very closely related enzymes such as RsmC and RlmG exhibit very different substrate preferences (Sunita et al. 2007).

In tRNA, the m^2G modification is mostly found in Eukaryota and Archaea at positions 6, 7, 9, 10, 18, 26, 27, and 67 (Auffinger and Westhof 1998; Sprinzl and Vassilenko 2005). The tRNA MTases responsible for the formation of m^2G at positions 26 and 10 have been studied extensively in Eukaryota and Archaea. In Eukaryota two thirds of the

⁷Corresponding author.

E-mail ldroogma@ulb.ac.be.

Article published online ahead of print. Article and publication date are at <http://www.rnajournal.org/cgi/doi/10.1261/rna.030411.111>.

tRNAs sequenced so far have N^2,N^2 -dimethylguanosine (m^2_2G) at position 26 (Grosjean et al. 1995), in the bend between the D-stem and the anticodon stem, with m^2G being the intermediate of this dimethylation. The dimethylated guanosine m^2_2G26 prevents pairing with a cytidine (contrary to the monomethylated m^2G) in the tRNA D-stem and controls the pairing with adenine by limiting the pairing mode to the imino-hydrogen bonded form, thereby stabilizing the structure (Pallan et al. 2008).

The gene encoding this tRNA (m^2_2G26) MTase was identified in lower as well as in higher Eukaryota (Ellis et al. 1986; Reinhart et al. 1986; Edqvist et al. 1992, 1994; Liu et al. 1999; Niederberger et al. 1999; Liu and Straby 2000). In yeast, the TRM1 gene is responsible for m^2_2G formation in both the cytoplasmic and mitochondrial tRNAs (Hopper et al. 1982; Ellis et al. 1986).

In Archaea, the m^2_2G modification is present at position 26 as well as position 10 of half of the tRNAs sequenced so far (Grosjean et al. 1995). These dimethylations are catalyzed by two distinct MTases. Biochemical (Constantinesco et al. 1998) as well as structural (Ihsanawati et al. 2008) studies were performed with the Trm1 enzyme of the hyperthermophilic euryarchaeon *Pyrococcus furiosus*. The p_f Trm1 enzyme needs specific base-pairing in the D-loop, limited size of the variable loop, and a correct overall tRNA folding for N^2,N^2 -dimethylation of G26 (Constantinesco et al. 1999). Although initially thought as being excluded from Bacteria, a Trm1 ortholog has been found in the hyperthermophilic bacterium *Aquifex aeolicus* (Takeda et al. 2002; Awai et al. 2009). Besides formation of m^2_2G26 , the bacterial Trm1 can also catalyze m^2G27 formation. The enzyme transfers the methyl group of S-adenosylmethionine (AdoMet) only in the presence of an intact T-loop (Awai et al. 2009). Further, unlike the archaeal Trm1, its bacterial ortholog requires neither specific base-pairing in the D-loop nor a restricted size of the variable loop (Awai et al. 2005).

Exocyclic amine methylation of guanosine is also detected at position 10 of the tRNAs of Eukaryota and Archaea. In Archaea, dimethylation of this guanosine can occur, while in yeast, the eukaryotic representative, only monomethylation is observed (Armengaud et al. 2004). In yeast, two different polypeptides are required to fulfill the G10 monomethylation (Purushothaman et al. 2005; Okada et al. 2009): Trm11p, which is the catalytic MTase subunit, and Trm112p, a small auxiliary protein that is required for the activity of at least three other MTases and is probably involved in various cellular functions (Mazauric et al. 2010). Deletion of the TRM11 gene did not lead to difference in cell growth, while a double deletion of TRM11 and TRM112 affected growth (Purushothaman et al. 2005). In Archaea, the enzyme responsible for m^2G10 formation is a monomer, called TrmG10 (Armengaud et al. 2004). The elements that determine the m^2G10 formation in tRNA of the archaeon *Pyrococcus abyssi*, such as variable loop length and base-pairing in the D-loop, are totally opposed to those required

for the m^2_2G26 formation (Urbonavicius et al. 2006), underlying the fact that TrmG10 and Trm1 are only remotely related. The Trm1 enzyme bears a putative helix–turn–helix motif at its C terminus, while the TrmG10 protein contains an N-terminal THUMP domain (Armengaud et al. 2004). The THUMP domain, present in thiouridine synthases, RNA MTases, and pseudouridine synthases (Aravind and Koonin 2001), was proposed to interact with a specific region of the tRNA, thereby targeting the catalytic domain of the enzyme toward a particular region of the tRNA structure (Gabant et al. 2006).

No tRNA (m^2G10) MTase nor potential homologs were detected so far in Bacteria. In this article, we report the identification of a TrmG10 homolog in the extreme thermophilic bacterium *Thermus thermophilus*. As TrmG10, this enzyme contains a catalytic domain and a THUMP domain. However, in contrast with TrmG10, the bacterial enzyme methylates the N^2 position of guanosine at position 6 of tRNA. The corresponding gene was inactivated in the *T. thermophilus* chromosome, and the biochemical characteristics of the enzyme were compared to those of its archaeal ortholog.

RESULTS

Bioinformatic identification of a putative tRNA (m^2G6) methyltransferase in *T. thermophilus* HB27

Among the six sequenced tRNAs from the extreme thermophilic bacterium *T. thermophilus*, only the tRNA^{Phe} bears an m^2G at position 6 (m^2G6). The presence of m^2G in this bacterial tRNA is intriguing because this modification is generally considered as being exclusively eukaryotic or archaeal. The *T. thermophilus* MTase responsible for m^2G6 formation, however, remained to be discovered.

In order to identify the open reading frame (ORF) encoding this enzyme, BLASTP searches were performed on the *T. thermophilus* HB27 proteome using well-characterized tRNA (m^2G) MTases as queries: Trm1 forming m^2G26 and TrmG10 forming m^2G10 . By use of this approach, no close relative of Trm1 was found, in agreement with the previous study (Bujnicki et al. 2002). However, using TrmG10 (the product of the PAB1283 ORF) as a query revealed the existence of a homolog (E value = 7×10^{-4}) in *T. thermophilus* HB27, encoded by the *TTC1157* gene. This protein is annotated as a putative MTase. Curiously, the equivalent protein in *T. thermophilus* HB8 present in the database (TTHA1521) is N-terminally truncated by 29 residues with respect to TTC1157. Similarly to TrmG10, the TTC1157 protein contains two domains: an N-terminal THUMP domain and a C-terminal AdoMet-dependent MTase domain. Since none of the sequenced tRNAs from *T. thermophilus* contain m^2G10 , TTC1157 appeared as

a potential candidate for catalyzing the formation of m²G6.

Inactivation of the *T. thermophilus* *TTC1157* gene results in the loss of an enzyme forming m²G in tRNA^{Phe}

To generate a *T. thermophilus* strain in which the *TTC1157* gene is inactivated, a kanamycin-resistance cassette was introduced into the chromosomal *TTC1157* gene by homologous recombination. The mutant strain was obtained by a double homologous recombination event between the wild-type (WT) chromosomal allele and a nonreplicating plasmid-borne allele in which the coding sequence was interrupted by a kanamycin nucleotidyltransferase (*knt*) gene (see Materials and Methods). The resulting disrupted strain was resistant to 60 µg/mL kanamycin. The presence of the kanamycin-resistance cassette within the *TTC1157* gene was checked by PCR (data not shown). The growth of the mutant strain was not affected compared with the WT HB27 strain on a temperature range from 55°C–80°C.

In order to test whether the mutant strain has lost a tRNA (m²G) MTase activity, total tRNA was extracted from the WT and the mutant strain and hydrolyzed to nucleosides for high performance liquid chromatography (HPLC) analysis. The results presented in Figure 1 show the presence of m²G in tRNA from the WT cells and its total absence in tRNA from the mutant cells. On the other hand, extracts of the WT and mutant strains were compared for their capacity to form m²G in a synthetic transcript of *T. thermophilus* tRNA^{Phe} devoid of any modification. T7 RNA polymerase was used for in vitro transcription, and the synthetic tRNA was labeled by the addition of [α-³²P] GTP in the reaction mixture (see

Materials and Methods). Equal amounts of extracts were incubated 30 min at 60°C with this transcript in the presence of AdoMet as the methyl donor. After incubation, the tRNA was recovered by phenol extraction and ethanol precipitation and completely hydrolyzed into 5'-phosphate nucleosides by nuclease P1. The resulting hydrolysate was analyzed by two-dimensional thin layer chromatography (2D TLC) followed by autoradiography. Figure 2 shows that a cell extract of WT *T. thermophilus* HB27 catalyzes the formation of m⁷G, 2'-O-methylguanosine (Gm), and m²G in the synthetic transcript. However, the same experiment with a cell extract from the mutant strain revealed only the formation of m⁷G and Gm but no m²G, indicating that the product of the *TTC1157* gene is a tRNA (m²G) MTase.

The purified recombinant *TTC1157* protein catalyzes the formation of m²G in tRNA

The *T. thermophilus* *TTC1157* gene was PCR-amplified and cloned into the pET28b expression vector, allowing the production of an N-terminal His-tagged protein in *E. coli* (see Materials and Methods). The expression of the recombinant protein was induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 h at 15°C in the *E. coli* strain Rosetta (DE3). The His-tagged protein was purified by immobilized metal-ion affinity chromatography. Figure 3A shows that the recombinant protein is at least 90% pure, the apparent molecular mass (43 kDa) being close to the expected value from the amino acid sequence (39.143 kDa). Gel filtration chromatography of the purified *TTC1157* protein showed that it behaves as a monomer. To test the activity of the purified recombinant protein, an [α-³²P] GTP-labeled transcript of tRNA^{Phe} was incubated for 30 min at 60°C

FIGURE 1. Inactivation of the *TTC1157* gene of *T. thermophilus* results in the loss of m²G in tRNA. Total tRNA from the *T. thermophilus* wild-type strain (A) and mutant strain (B) was extracted and hydrolyzed by nuclease P1 and alkaline phosphatase and then analyzed by HPLC. The peak corresponding to m²G is indicated by an arrow. A standard curve with the reference nucleosides C, U, G, A, and m²G is shown in C.

FIGURE 2. Insertion of a Km^R cassette into the *TTC1157* gene of *T. thermophilus* results in the loss of m^2G tRNA-MTase activity. Autoradiography of two-dimensional chromatograms of P1 hydrolysates of $[\alpha\text{-}^{32}\text{P}]$ GTP-labeled *T. thermophilus* $tRNA^{Phe}$ transcripts incubated for 30 min at 60°C in the presence of AdoMet and 140 μg of crude extract of *T. thermophilus* wild-type strain (WT) and mutant strain ($TTC1157::Km^R$). The number of moles of modified nucleotides per mole of tRNA is indicated between brackets.

with 0.5 μg of the purified protein and AdoMet. Figure 3 shows that the purified enzyme catalyzes the formation of a modified nucleotide with migration characteristics identical to those of m^2G 5'-phosphate (pm^2G). Therefore we propose to rename *TTC1157* as TrmN, according to the current nomenclature of bacterial tRNA MTases. In parallel, an N-terminally truncated form of *TTC1157* lacking the first 29-amino-acid residues was produced based on the proposed sequence of TTHA1521. This shortened form of the protein was found to be inactive (data not shown). Since other tRNA (m^2G) MTases (Trm1 and TrmG10) are known to form m^2G in addition to m^2G , we tested the capacity of *TTC1157* to dimethylate the target guanosine. As shown in Figure 4, the use of high amounts of enzyme (≥ 1 μg), leads to the production of 0.4–0.5 mol m^2G /mol tRNA, as well as a small amount of m^2G (up to 0.03 mol m^2G /mol tRNA).

m^2G formation by *TTC1157* occurs at position 6 of $tRNA^{Phe}$

Among the different sequenced tRNAs from *T. thermophilus*, only $tRNA^{Phe}$ contains the m^2G6 modification. To test the specificity of *TTC1157* toward $tRNA^{Phe}$, we used this tRNA and $tRNA^{Asp}$ (which should not be a substrate) for their capacity to be modified by the purified enzyme. Figure 5 shows that among the two tRNAs, only $tRNA^{Phe}$ is methylated by *TTC1157*. Moreover,

a mutant of $tRNA^{Phe}$, in which the base pair G6:C61 is replaced by C6:G61, is no longer a substrate for the enzyme (Fig. 5). The same result was obtained for mutants in which the G6:C61 base pair was substituted by A6:U61 or U6:A61 (data not shown). The mutant $tRNA^{Phe}$ C6:G61 was also incubated in a cell extract of the WT *T. thermophilus* in the presence of AdoMet. Again, no m^2G modification was observed. Taken together, these results strongly suggest that *TTC1157* catalyzes m^2G6 formation in $tRNA^{Phe}$.

To further confirm the target nucleoside of *TTC1157*, an experiment was performed in which $[\alpha\text{-}^{32}\text{P}]$ GTP-labeled transcripts of $tRNA^{Phe}$, modified by *TTC1157* or left unmodified, were digested by RNase A that cleaves RNA after pyrimidines and leaves a 3'-phosphate end. This digestion leads to several radioactive frag-

FIGURE 3. Affinity-purified *TTC1157* catalyzes the formation of m^2G in $tRNA^{Phe}$ in vitro. (A) SDS-PAGE analysis of the purified *TTC1157* protein. (Lane 1) Purified protein; (lane 2) molecular weight (in kiloDaltons) marker (Fermentas). (B) Autoradiography of two-dimensional chromatograms of P1 hydrolysates of $[\alpha\text{-}^{32}\text{P}]$ GTP-labeled *T. thermophilus* $tRNA^{Phe}$ transcripts incubated for 30 min at 60°C in the presence of purified *TTC1157* (for details, see Materials and Methods). Circles of dotted lines show the migration of the pA, pC, and pU nucleotides used as ultraviolet markers. (C) As for B but with one-dimensional chromatography and varying amounts of enzyme. The number of moles of modified nucleotides per mole of tRNA is indicated between brackets.

FIGURE 4. Formation of m²G in tRNA^{Phe} of *T. thermophilus* in the presence of high amounts of enzyme. Autoradiography of one-dimensional chromatograms of P1 hydrolysates of [α -³²P] GTP-labeled *T. thermophilus* tRNA^{Phe} transcripts incubated for 30 min at 60°C in the presence of increasing amounts of purified TTC1157. Circles of dotted lines show the migration of the pG, pA, pC, and pU nucleotides used as ultraviolet markers.

ments with a length of 1-, 2-, and 3-nt residues and two fragments consisting of five residues (Fig. 6). The latter two fragments correspond to nucleotides 4–8 and 21–25. They can be separated by a 30% polyacrylamide gel electrophoresis (PAGE) and distinguished by the nearest neighbor analysis using RNase T2 that generates 3'-phosphate mononucleosides. Indeed, only the fragment containing G6 has two adjacent guanosines. The G6 position is present in the slower migrating five-residue-long fragment. Our results show that the m²G formed by TTC1157 is present in the five-residue-long fragment corresponding to positions 4–8 and that the m²G is 5'-adjacent to another G, demonstrating that TTC1157 targets G6 of tRNA^{Phe} (Fig. 6).

m²G6 is not required for other modifications of tRNA^{Phe}

The existence of a functionally relevant modification network in *T. thermophilus* tRNAs has been reported recently. In this network, the absence of a given modification affects the formation of other modified nucleosides (Tomikawa et al. 2010; Ishida et al. 2011). To determine whether other modifications in tRNA^{Phe} depend on the presence of m²G6, we incubated transcripts of tRNA^{Phe} (radiolabeled with [α -³²P] GTP, [α -³²P] ATP, [α -³²P] UTP, or [α -³²P] CTP) with extracts from the WT *T. thermophilus* and from the mutant strain in which the *TTC1157* gene is inactivated, and with AdoMet. This experiment allowed us to observe the formation of m⁷G, Gm, m¹A, i⁶A, ψ , and m⁵U. The amount of these

modified nucleosides appeared independently of the presence or absence of m²G6 (data not shown).

Archaeal homologs of TTC1157

BLASTP analyses revealed homologs of TTC1157 in Euryarchaeota but not in Crenarchaeota. Several of these homologs were cloned and expressed in *E. coli*: TK1863 from *Thermococcus kodakaraensis*, MK0969 from *Methanopyrus kandleri*, AF2178 from *Archaeoglobus fulgidus*, PAB0923 from *P. abyssi*, MJ0438 from *Methanocaldococcus jannaschii*, and PF1002 from *P. furiosus*. All these proteins were shown to catalyze m²G formation in tRNA. Among these proteins, PF1002 showed the highest production level and solubility in *E. coli*. Therefore, this protein was purified and further characterized, leading to the determination of its crystal structure (Fislage et al. 2011, 2012). We observed that addition of a reducing agent (β -mercaptoethanol or dithiothreitol) was required to keep the protein fully active. Indeed, the active enzyme is a monomer, and inactive dimers were formed in the absence of a reducing agent, indicating that these dimers result from disulfide bridge formation between cysteines of two mono-

FIGURE 5. In the absence of G6 in tRNA, no m²G is formed by TTC1157. Autoradiography of two-dimensional chromatograms of P1 hydrolysates of [α -³²P] GTP-labeled *T. thermophilus* tRNA^{Phe}, tRNA^{Asp}, and tRNA^{Phe}(G6C; C67G) transcripts incubated for 30 min at 60°C in the presence of 0.5 μ g of purified TTC1157. Circles of dotted lines show the migration of the pA, pC, and pU nucleotides used as ultraviolet markers.

mers. Similar experiments as those described in the previous paragraphs for TTC1157 were performed with PF1002 and gave identical results, showing that these enzymes catalyze the same reaction (formation of m^2G6 in tRNA) in two different kingdoms of life. This is in accordance with the high similarity of the three-dimensional structure of the two proteins (Fislage et al. 2012).

Importance of the two domains of tRNA (m^2G6) MTases for the enzymatic activity

The bacterial and archaeal tRNA (m^2G6) MTases contain two domains: an N-terminal THUMP domain and a C-terminal Rossmann-fold-like MTase (RFM) domain. We have expressed these domains separately in *E. coli*. Although the two domains of PF1002 (N terminus to residue 189 for the THUMP domain; residue 189 to C terminus for the RFM domain) could successfully be produced in a soluble form, for TTC1157 only the THUMP domain was soluble. The two domains of PF1002 were purified and tested for enzymatic activity and tRNA binding. The THUMP domain is considered as an RNA binding module (Aravind and Koonin 2001). Separate domains showed no enzymatic activity, and the binding to tRNA^{Phe} was totally impaired compared with the WT protein as measured by bandshift assays (Fig. 7), demonstrating that in tRNA (m^2G6) MTases the putative RNA-binding and catalytic domains cannot function autonomously.

DISCUSSION

The modifications m^2G and m^2_2G are frequently found in archaeal and eukaryotic tRNAs, in particular at positions 10 and 26. The enzymes responsible for these methylations have been extensively studied, including the determination of the structure for an archaeal Trm1 enzyme that forms m^2_2G26 (Ihsanawati et al. 2008). In contrast, m^2G is rather scarce in bacterial tRNA. Sequencing data revealed the presence of m^2_2G26 and m^2G/m^2_2G27 in tRNA^{Cys} of the hyperthermophilic bacterium *A. aeolicus*. A single MTase of the Trm1 family is responsible for these modifications (Awai et al. 2009). Intriguingly, m^2G at position 6 has been found in only one bacterial tRNA: tRNA^{Phe} of the extreme thermophilic bacterium *T. thermophilus*. In this article, we have identified the TTC1157 ORF in the *T. thermophilus* HB27 genome as coding for the MTase that catalyzes the m^2G6 formation in tRNA^{Phe}. We have also identified archaeal orthologs catalyzing the same reaction. We propose to rename the TTC1157 ORF

FIGURE 6. Localization of m^2G in tRNA. (Left) Nucleotide sequence of *T. thermophilus* tRNA^{Phe}. The arrow points to position 6 of tRNA, and the line indicates the 5-nt fragment isolated after RNase digestion. (Center) Autoradiogram of the 30% PAGE used to separate the RNase A fragments of the modified and unmodified tRNA^{Phe} transcripts. The obtained 5-, 3-, and 2-nt-long fragments are indicated by arrows. The 5-nt-long fragment indicated by an asterisk corresponds to the lined nucleotides in A. (Right) Autoradiography of two-dimensional chromatograms of T2 hydrolysates of the labeled 5-nt (*) fragment obtained from transcripts of [α - ^{32}P] GTP-labeled *T. thermophilus* tRNA^{Phe} incubated for 30 min at 60°C in the presence or absence of purified TTC1157.

as *trmN*, according to the bacterial nomenclature of tRNA MTases, and the archaeal orthologs as *trm14*, for consistency with the article by Menezes et al. (2011) reporting the *M. jannaschii* tRNA (m^2G6) MTase, which was accepted for publication during the preparation of this manuscript. These proteins belong to COG0116, which contains bacterial, archaeal, and eukaryotic members. Interestingly, not all of these proteins are capable of catalyzing the same reaction with similar substrates, since the YpsC protein from *Bacillus subtilis* was not capable of forming m^2G in tRNA (data not shown).

The new family of MTases is evolutionary linked to TrmG10 proteins that catalyze the formation of m^2G/m^2_2G10 in archaeal and eukaryotic tRNAs. Indeed, these proteins contain a THUMP domain fused to a RFM domain. Similarly to TrmG10, TrmN and Trm14 can form m^2_2G , in addition to m^2G , at high enzyme concentrations. Our results indicate that the THUMP domain alone is not sufficient for tRNA binding. A similar observation was made by Gabant et al. (2006) on the THUMP domain of archaeal TrmG10. Also, the individual MTase domain does not show any tRNA binding. This reinforces the idea that both domains collaborate and that the THUMP domain assists the catalytic domain to target a particular position of the tRNA molecule.

The role of m^2G6 in tRNA is not known yet. A mutant of *T. thermophilus* devoid of TrmN is viable and does not show any growth defect in a large range of temperatures. Therefore, m^2G6 does not appear to be involved in an essential function. This is, however, not exceptional for mutants of tRNA modification enzymes, in which growth

FIGURE 7. Bandshift assay with an [α -³²P] GTP-labeled *T. thermophilus* tRNA^{Phe} transcript and PF1002 or the separated MTase and THUMP domains. Increasing amounts of the proteins were used in a binding experiment on [α -³²P] GTP-labeled *T. thermophilus* tRNA^{Phe} transcript. The complexes were separated on a 6% PAGE.

phenotypes appear only when several modifications are absent (Purushothaman et al. 2005; Chernyakov et al. 2008). Consistent with this finding, m²G6 does not seem to be required for the formation of other modifications in tRNA, at least in tRNA^{Phe}.

MATERIALS AND METHODS

General procedures

Ampicillin was used at a concentration of 50 μ g/mL; kanamycin, at 30 μ g/mL; and chloramphenicol, at 30 μ g/mL. Restriction

endonucleases and T4 DNA ligase were purchased from Fermentas. [α -³²P] NTP was from Perkin Elmer. Oligonucleotides were synthesized by Sigma. Nuclease P1 and RNase T2 were from Sigma, and RNase A was from Fermentas. Sequences and purposes of oligonucleotides used in this study are given in Table 1. *T. thermophilus* genomic DNA was isolated according to the method described by Ramseier et al. (1993). *P. furiosus* genomic DNA was isolated according to the method described by Roovers et al. (1997). Genomic DNA from *A. fulgidus* and *M. jannaschii* were purchased from ATCC. Genomic DNA from *M. kandleri*, *P. abyssi*, and *T. kodakaraensis* were kind gifts from H. Huber, H. Grosjean, and T.J. Santangelo.

Detection of m²G by HPLC

A sample of $\sim$ 100 μ g of totally hydrolyzed tRNA from *T. thermophilus* WT and mutant strain was obtained by nuclease P1 and alkaline phosphatase (both from Sigma) treatment. The hydrolysate was injected on a Supelco Discovery C18 (2502 $\times$ 24.6)-mm HPLC column equilibrated with 0.25 M ammonium acetate (pH8). The column was eluted with a linear gradient of acetonitrile/water (40/60) at a flow rate of 1.2 mL/min. The nucleosides were detected by ultraviolet absorption at 254 nm. The canonical nucleosides and m²G used as reference markers were from Sigma.

Plasmid constructions for TTC1157 gene inactivation in *T. thermophilus* HB27

A 3.2-kb *T. thermophilus* genomic fragment bearing the TTC1157 gene was amplified from *T. thermophilus* HB27 genomic DNA

TABLE 1. Oligonucleotides used in this study

Oligo	Sequence	Purpose
TTC1157-1	GATCCATATGTGGCTTGAGGCCACCAACCC	Amplification of ORF TTC1157
TTC1157-2	GATCGTCGACCTAGAGCTTCTCTAGGACGAAGACC	
PF1002-1	GACCATATGAAGTTTTTGCTCACAACAGCC	Amplification of ORF PF1002
PF1002-2	GACCTCGAGTTTCACTACATACAAGTGAAC	
AF2178-1	GACCCATGGGCATGAAGCGAGTTCAGAATCTC	Amplification of ORF AF2178
AF2178-2	GACGTCGACAATCATGAAAACCATAATTTTTG	
MJ0438-1	GACCATATGGATTACTATGTTACACTATCC	Amplification of ORF MJ0438
MJ0438-2	GACCTCGAGTAAAGTTAAATAAACACCC	
MK0969-1	GACCATATGACCTCGGAGTACTGTGCACG	Amplification of ORF MK0969
MK0969-2	GACGGATCCTTAGCGAAAGACTAGGAGCCTCAC	
PAB0923-1	GATCCATATGCTGAAGCTATTGCTAACGACTGCTC	Amplification of ORF PAB0923
PAB0923-2	GATCGTCGACTTACTCAATAACGTACGCGTGAACCCCTAAG	
TK1863-1	GACCCATGGGCATGAGACTCTTACTCACAACC	Amplification of ORF TK1863
TK1863-2	GACCTCGAGTTCCACAACATATGTGTGCACC	
TTC1157-1	GATCCATATGTGGCTTGAGGCCACCAACCC	Amplification of THUMP-TTC1157
TTC-Ser1	GATCGTCGACCTAGGAGAGGGGCTTTCCGTGAG	
TTC-Ser2	GATCCATATGTCCCGCCGCTTCCCAAGGCG	Amplification of MTase-TTC1157
TTC1157-2'	GATCGTCGACGAGCTTCTCTAGGACGAAGACCCG	
PF1002-1	GACCATATGAAGTTTTTGCTCACAACAGCC	Amplification of THUMP-PF1002
PF1002-His1	GATCCTCGAGTTAGTGAAGTGAGCTATCTCCAGTTG	
PF1002-His2	GATCCATATGCACAAAAGGCTTGAGAGATTATG	Amplification of MTase-PF1002
PF1002-2	GACCTCGAGTTTCACTACATACAAGTGAAC	
TTC3-1	GGAGGCGGAGGCCGAGGAGG	Amplification of 3.2 kbp <i>Thermus</i> DNA with TTC1157
TTC3-2	GGAGGAGTGCGGCATCCTCG	
KmAcl-1	CTAGGTCTACCCTCCTCCGGAACCTAGG	Amplification of Km ^R cassette
KmAcl-2	CTAGGTAGACCGTAACCAACATGATTAAC	
TTC-PHE1	TATTAATACGACTCACTATAGCCGAGGTAGCTCAGTTGGTAGAG	Amplification of tRNA ^{Phe} gene of <i>T. thermophilus</i>
TTC-PHE2	TATCCTGGTGCCGAGGGGCGGAATCGAACCG	

using Pwo DNA polymerase, DMSO, and the oligonucleotides TTC3-1 and TTC3-2. The amplified fragment was cloned in the pJET PCR cloning vector (Fermentas), now called pJET-TTC. A thermo-resistant *knt* gene derivative (Km^R) encoding kanamycin nucleotidyltransferase (Liao et al. 1986) was PCR amplified from the *Thermus* plasmid pT8-231, using Pwo polymerase and the oligonucleotides KmAccI-1 and KmAccI-2 with AccI restriction sites to facilitate the cloning into the cognate site present in the middle of the *TTC1157* gene. The plasmid pJET-TTC containing the Km^R cassette in the unique AccI site (present in the *TTC1157* gene) was used to transform *T. thermophilus* HB27 cells. Transformation was performed according to the method described by Koyama et al. (1986).

Cloning of the *T. thermophilus* *TTC1157* gene and its archaeal homologs

The *T. thermophilus* *TTC1157* gene and the archaeal *PF1002*, *AF2178*, *MJ0438*, *MK0969*, *PAB0923*, and *TK1863* genes were amplified by PCR from their respective genomic DNAs using the respective oligonucleotide set (Table 1) and Pwo polymerase. The obtained fragments were first subcloned in the pJET PCR cloning vector. The sequence of the inserts was checked. Subsequently the inserts were cloned in a pET expression vector with the adequate restriction enzymes, generating an N- or C-terminal His-tagged protein (Table 2).

Expression and purification of recombinant proteins

The His-tagged *T. thermophilus* *TTC1157*, *P. furiosus* *PF1002*, and other archaeal proteins (*AF2178*, *MJ0438*, *MK0969*, *PAB0923*, and *TK1863*) were expressed in the *E. coli* strain Rosetta (DE3). Transformed cells were grown at 37°C in 2 L (for *TTC1157* and *PF1002*) or 100 mL (for *AF2178*, *MJ0438*, *MK0969*, *PAB0923*, and *TK1863*) of Luria broth supplemented with kanamycin and chloramphenicol to an optical density at 660 nm of 0.6. At this stage, IPTG was added to a final concentration of 0.1 mM to induce recombinant protein expression. After overnight induction at 15°C, the cells were harvested and resuspended in 50 mL buffer A (50 mM Tris-HCl at pH 8, 250 mM NaCl) for *TTC1157*, in 50 mL buffer B (50 mM Tris-HCl at pH 8, 500 mM NaCl) for *PF1002*, and in 3 mL of buffer A for the other proteins. Cells were lysed by 30 min or 5 min sonication at 4°C using a Vibracell 75041 sonicator. The lysates were cleared by centrifugation (20,000g for 30 min) and applied to, respectively, a column of Chelating Sepharose Fast Flow (1 × 30 cm; GE Healthcare) charged with Ni^{2+} for *TTC1157* and *PF1002*, and

a column from the Protino Ni-IDA 1000 kit (Macherey-Nagel) for the other proteins. The latter proteins were purified according to the manufacturers' instructions. For the *TTC1157* and *PF1002* proteins, the column was washed with buffer A and B, respectively. The adsorbed material was eluted with a linear gradient (750 mL, from 0 to 500 mM) of imidazole in the respective buffer. Eluted fractions were analyzed by SDS-PAGE. The fractions containing the recombinant proteins were pooled, glycerol was added (20% final), and aliquots of the preparations were flash-frozen in liquid nitrogen and stored at −80°C.

tRNA MTase assays

The tRNA MTase assay based on the procedure described previously (Droogmans and Grosjean 1991) involved in vitro transcribed, ^{32}P -labeled *T. thermophilus* tRNA^{Phe/Asp} as a substrate. The general procedure for generating in vitro transcripts of tRNA genes is based on the method described by Reyes and Abelson (1987). Plasmid pML1 allows T7 transcription of *T. thermophilus* tRNA^{Asp} (Droogmans et al. 2003). To obtain an in vitro transcript of tRNA^{Phe} of *T. thermophilus*, the corresponding gene was amplified with Pwo polymerase using the oligonucleotides TTC-PHE1 and TTC-PHE2 (Table 1) and genomic *T. thermophilus* DNA. The obtained fragment was cloned into the SmaI site of pUC18 and entirely sequenced. This sequence was in accordance with the genomic sequence but differs with 1 nt (C65 instead of U65) from the reported tRNA sequence (Grawunder et al. 1992). In the resulting plasmid (pML2), the tRNA sequence is flanked by a 5' T7 promoter and a 3' MvaI restriction site.

The reaction mixture for the tRNA-MTase assay (400 μ L) consisted of 50 mM Tris-HCl (pH 8), 5 mM $MgCl_2$, 10^6 counts per minute of the radioactive transcript, 500 μ M AdoMet, and 0.5 μ g of purified enzyme or 100 mg protein of a crude *T. thermophilus* extract. After 30°C of incubation at 60°C for *T. thermophilus* proteins or 70°C for the archaeal proteins, the reaction was stopped by phenol extraction, and the tRNA was ethanol precipitated. The recovered radioactive tRNA was then completely digested by either nuclease P1 (1 μ g) or RNase T2 (0.1 U) in the presence of 5 μ g total yeast tRNA as carrier.

Modified nucleotides were analyzed by 2D TLC on cellulose plates (Merck). The first dimension was with solvent A (isobutyric acid/concentrated NH_4OH /water; 66/1/33; v/v/v); the second dimension was with solvent B (0.1 M sodium phosphate at pH 6.8/ $(NH_4)_2SO_4$ /n-propanol; 100/60/2; v/w/v). The migration pattern was visualized by autoradiography. The nucleotides were identified using a reference map (Grosjean et al. 2004).

Localization of m^2G in *T. thermophilus* tRNA^{Phe}

A radioactive [$\alpha^{32}P$] GTP tRNA^{Phe} transcript from *T. thermophilus* was incubated in the presence of 0.5 μ g of enzyme and 500 μ M AdoMet (Sigma) for 30 min at 60°C (70°C for *PF1002*). As a control, a transcript was incubated in the same conditions but without the enzyme. After incubation, the reaction was stopped by phenol extraction. Transcripts were ethanol precipitated, and the pellet was resuspended in 15 μ L of RNase A buffer (Tris-Cl 10 mM at pH 7.5, 15 mM NaCl) containing 3.5 μ L RNase A. Digestion was carried out for 1 h at 37°C. The radiolabeled fragments were then separated by 30% PAGE and revealed by autoradiography. The slower migrating 5-nt-long fragment was

TABLE 2. Expression cloning strategy of the different ORFs studied in this work

Gene	Expression vector	Used restriction enzyme	Position of His-tag
TTC1157	pET28	NdeI-Sall	N-terminal
PF1002	pET30	NdeI-XhoI	C-terminal
AF2178	pET28	NcoI-Sall	N-terminal
MJ0438	pET30	NdeI-XhoI	C-terminal
MK0969	pET28	NdeI-BamHI	N-terminal
PAB0923	pET28	NdeI-Sall	N-terminal
TK1863	pET28	NcoI-XhoI	N-terminal

isolated from the gel, eluted, and dried. The dry radiolabeled RNA fragment was then dissolved in RNase T2 buffer (50 mM NH₄-acetate at pH 4.5). After RNaseT2 digestion, the nucleotides were separated by 2D TLC (see above) and revealed by autoradiography.

ACKNOWLEDGMENTS

We thank H. Huber (Regensburg University, Germany), H. Grosjean (CNRS, France), and T.J. Santangelo (Ohio State University) for the gift of archaeal genomic DNAs. This work was supported by grants from the Fonds pour le Recherche Fondamentale Collective (grant no. 2.4.520.05F), the Fonds D. et A. Van Buuren, the Fonds J. Brachet, and the FWO (grant G025909N). W.V. is a recipient of an FWO post-doctoral grant. M.F. is a recipient of an IWT predoctoral grant. J.M.B. was supported by the Polish Ministry of Science and Higher Education (grant 188/N-DFG/2008/0), by the European Research Council (StG grant RNA+P=123D), and by the “Ideas for Poland” fellowship from the Foundation for Polish Science.

Received September 19, 2011; accepted December 22, 2011.

REFERENCES

- Andersen NM, Douthwaite S. 2006. YebU is a m⁵C methyltransferase specific for 16 S rRNA nucleotide 1407. *J Mol Biol* **359**: 777–786.
- Aravind L, Koonin EV. 2001. THUMP: a predicted RNA-binding domain shared by 4-thiouridine, pseudouridine synthases and RNA methylases. *Trends Biochem Sci* **26**: 215–217.
- Armengaud J, Urbonavicius J, Fernandez B, Chaussinand G, Bujnicki JM, Grosjean H. 2004. N²-methylation of guanosine at position 10 in tRNA is catalyzed by a THUMP domain-containing, S-adenosylmethionine-dependent methyltransferase, conserved in Archaea and Eukaryota. *J Biol Chem* **279**: 37142–37152.
- Auffinger P, Westhof E. 1998. Location and distribution of modified nucleotides in tRNA. In *Modification and editing of RNA* (ed. H Grosjean, R Benne), pp. 569–576. ASM Press, Washington.
- Awai T, Takehara T, Takeda H, Hori H. 2005. *Aquifex aeolicus* Trm1 [tRNA (m²₂G26) methyltransferase] has a novel recognition mechanism of the substrate RNA. *Nucleic Acids Symp Ser* **49**: 303–304.
- Awai T, Kimura S, Tomikawa C, Ochi A, Ihsanawati, Bessho Y, Yokoyama S, Ohno S, Nishikawa K, Yokogawa T, et al. 2009. *Aquifex aeolicus* tRNA (N²,N²-guanine)-dimethyltransferase (Trm1) catalyzes transfer of methyl groups not only to guanine 26 but also to guanine 27 in tRNA. *J Biol Chem* **284**: 20467–20478.
- Bujnicki JM, Leach RA, Debski J, Rychlewski L. 2002. Bioinformatic analyses of the tRNA: (guanine 26, N²,N²)-dimethyltransferase (Trm1) family. *J Mol Microbiol Biotechnol* **4**: 405–415.
- Chernyakov I, Whipple JM, Kotelawala L, Grayhack EJ, Phizicky EM. 2008. Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. *Genes Dev* **22**: 1369–1380.
- Constantinesco F, Benachenhou N, Motorin Y, Grosjean H. 1998. The tRNA(guanine-26,N²-N²) methyltransferase (Trm1) from the hyperthermophilic archaeon *Pyrococcus furiosus*: cloning, sequencing of the gene and its expression in *Escherichia coli*. *Nucleic Acids Res* **26**: 3753–3761.
- Constantinesco F, Motorin Y, Grosjean H. 1999. Characterisation and enzymatic properties of tRNA(guanine 26, N², N²)-dimethyltransferase (Trm1p) from *Pyrococcus furiosus*. *J Mol Biol* **291**: 375–392.
- Droogmans L, Grosjean H. 1991. 2'-O-methylation and inosine formation in the wobble position of anticodon-substituted tRNA^{Phe} in a homologous yeast in vitro system. *Biochimie* **73**: 1021–1025.
- Droogmans L, Roovers M, Bujnicki JM, Tricot C, Hartsch T, Stalon V, Grosjean H. 2003. Cloning and characterization of tRNA (m¹A58) methyltransferase (Trm1) from *Thermus thermophilus* HB27, a protein required for cell growth at extreme temperatures. *Nucleic Acids Res* **31**: 2148–2156.
- Edqvist J, Grosjean H, Straby KB. 1992. Identity elements for N²-dimethylation of guanosine-26 in yeast tRNAs. *Nucleic Acids Res* **20**: 6575–6581.
- Edqvist J, Blomqvist K, Straby KB. 1994. Structural elements in yeast tRNAs required for homologous modification of guanosine-26 into dimethylguanosine-26 by the yeast Trm1 tRNA-modifying enzyme. *Biochemistry* **33**: 9546–9551.
- Ellis SR, Morales MJ, Li JM, Hopper AK, Martin NC. 1986. Isolation and characterization of the TRM1 locus, a gene essential for the N²,N²-dimethylguanosine modification of both mitochondrial and cytoplasmic tRNA in *Saccharomyces cerevisiae*. *J Biol Chem* **261**: 9703–9709.
- Fislage M, Roovers M, Munnich S, Droogmans L, Versées W. 2011. Crystallization and preliminary X-ray crystallographic analysis of putative tRNA-modification enzymes from *Pyrococcus furiosus* and *Thermus thermophilus*. *Acta Crystallogr* **67**: 1432–1435.
- Fislage M, Roovers M, Tuszynska I, Bujnicki JM, Droogmans L, Versées W. 2012. Crystal structures of the tRNA:m²G6 methyltransferase Trm14/TrmN from two domains of life. *Nucleic Acids Res* (in press).
- Gabant G, Auxilien S, Tuszynska I, Locard M, Gajda MJ, Chaussinand G, Fernandez B, Dedieu A, Grosjean H, Golinelli-Pimpaneau B, et al. 2006. THUMP from archaeal tRNA:m²G10 methyltransferase, a genuine autonomously folding domain. *Nucleic Acids Res* **34**: 2483–2494.
- Grawunder U, Schon A, Sprinzl M. 1992. Sequence and base modifications of two phenylalanine-tRNAs from *Thermus thermophilus* HB8. *Nucleic Acids Res* **20**: 137.
- Grosjean H, Sprinzl M, Steinberg S. 1995. Posttranscriptionally modified nucleosides in transfer RNA: their locations and frequencies. *Biochimie* **77**: 139–141.
- Grosjean H, Keith G, Droogmans L. 2004. Detection and quantification of modified nucleotides In *RNA using thin-layer chromatography*. In *RNA interference, editing, and modification. Methods and protocols* (ed. JM Gott), pp. 357–391. Humana Press, Totowa, NJ.
- Hopper AK, Furukawa AH, Pham HD, Martin NC. 1982. Defects in modification of cytoplasmic and mitochondrial transfer RNAs are caused by single nuclear mutations. *Cell* **28**: 543–550.
- Ihsanawati, Nishimoto M, Higashijima K, Shirouzu M, Grosjean H, Bessho Y, Yokoyama S. 2008. Crystal structure of tRNA N²,N²-guanosine dimethyltransferase Trm1 from *Pyrococcus horikoshii*. *J Mol Biol* **383**: 871–884.
- Ishida K, Kunibayashi T, Tomikawa C, Ochi A, Kanai T, Hirata A, Iwashita C, Hori H. 2011. Pseudouridine at position 55 in tRNA controls the contents of other modified nucleotides for low-temperature adaptation in the extreme-thermophilic eubacterium *Thermus thermophilus*. *Nucleic Acids Res* **39**: 2304–2318.
- Koyama Y, Hoshino T, Tomizuka N, Furukawa K. 1986. Genetic transformation of the extreme thermophile *Thermus thermophilus* and of other *Thermus* spp. *J Bacteriol* **166**: 338–340.
- Lesnyak DV, Sergiev PV, Bogdanov AA, Dontsova OA. 2006. Identification of *Escherichia coli* m²G methyltransferases: I. the ycbY gene encodes a methyltransferase specific for G2445 of the 23 S rRNA. *J Mol Biol* **364**: 20–25.
- Lesnyak DV, Osipiuk J, Skarina T, Sergiev PV, Bogdanov AA, Edwards A, Savchenko A, Joachimiak A, Dontsova OA. 2007. Methyltransferase that modifies guanine 966 of the 16 S rRNA: functional identification and tertiary structure. *J Biol Chem* **282**: 5880–5887.
- Liao H, McKenzie T, Hageman R. 1986. Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. *Proc Natl Acad Sci* **83**: 576–580.
- Liu J, Straby KB. 2000. The human tRNA(m²G₂₆)dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. *Nucleic Acids Res* **28**: 3445–3451.

- Liu J, Zhou GQ, Straby KB. 1999. *Caenorhabditis elegans* ZC376.5 encodes a tRNA (m^2G_{26})dimethyltransferase in which 246 arginine is important for the enzyme activity. *Gene* **226**: 73–81.
- Mazauric MH, Dirick L, Purushothaman SK, Bjork GR, Lapeyre B. 2010. Trm112p is a 15-kDa zinc finger protein essential for the activity of two tRNA and one protein methyltransferases in yeast. *J Biol Chem* **285**: 18505–18515.
- Menezes S, Gaston KW, Krivos KL, Apolinario EE, Reich NO, Sowers KR, Limbach PA, Perona JJ. 2011. Formation of m^2G_6 in *Methanocaldococcus jannaschii* tRNA catalyzed by the novel methyltransferase Trm14. *Nucleic Acids Res* **39**: 7641–7655.
- Niederberger C, Graub R, Costa A, Desgres J, Schweingruber ME. 1999. The tRNA N^2,N^2 -dimethylguanosine-26 methyltransferase encoded by gene *trm1* increases efficiency of suppression of an ochre codon in *Schizosaccharomyces pombe*. *FEBS Lett* **464**: 67–70.
- Okada K, Muneyoshi Y, Endo Y, Hori H. 2009. Production of yeast (m^2G_{10}) methyltransferase (Trm11 and Trm112 complex) in a wheat germ cell-free translation system. *Nucleic Acids Symp Ser* **53**: 303–304.
- Pallan PS, Kreutz C, Bosio S, Micura R, Egli M. 2008. Effects of N^2,N^2 -dimethylguanosine on RNA structure and stability: crystal structure of an RNA duplex with tandem $m^2G:A$ pairs. *RNA* **14**: 2125–2135.
- Purushothaman SK, Bujnicki JM, Grosjean H, Lapeyre B. 2005. Trm11p and Trm112p are both required for the formation of 2-methylguanosine at position 10 in yeast tRNA. *Mol Cell Biol* **25**: 4359–4370.
- Ramseier TM, Negre D, Cortay JC, Scarabel M, Cozzone AJ, Saier MH Jr. 1993. In vitro binding of the pleiotropic transcriptional regulatory protein, FruR, to the *fru*, *pps*, *ace*, *pts* and *icd* operons of *Escherichia coli* and *Salmonella typhimurium*. *J Mol Biol* **234**: 28–44.
- Reinhart MP, Lewis JM, Leboy PS. 1986. A single tRNA (guanine)-methyltransferase from *Tetrahymena* with both mono- and dimethylating activity. *Nucleic Acids Res* **14**: 1131–1148.
- Reyes VM, Abelson J. 1987. A synthetic substrate for tRNA splicing. *Anal Biochem* **166**: 90–106.
- Roovers M, Hethke C, Legrain C, Thomm M, Glansdorff N. 1997. Isolation of the gene encoding *Pyrococcus furiosus* ornithine carbamoyltransferase and study of its expression profile in vivo and in vitro. *Eur J Biochem* **247**: 1038–1045.
- Sergiev PV, Lesnyak DV, Bogdanov AA, Dontsova OA. 2006. Identification of *Escherichia coli* m^2G methyltransferases: II. The *ygjO* gene encodes a methyltransferase specific for G1835 of the 23 S rRNA. *J Mol Biol* **364**: 26–31.
- Sergiev PV, Bogdanov AA, Dontsova OA. 2007. Ribosomal RNA guanine- (N^2)-methyltransferases and their targets. *Nucleic Acids Res* **35**: 2295–2301.
- Sprinzi M, Vassilenko KS. 2005. Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res* **33**: D139–D140.
- Sunita S, Purta E, Durawa M, Tkaczuk KL, Swaathi J, Bujnicki JM, Sivaraman J. 2007. Functional specialization of domains tandemly duplicated within 16S rRNA methyltransferase RsmC. *Nucleic Acids Res* **35**: 4264–4274.
- Takeda H, Hori H, Endo Y. 2002. Identification of *Aquifex aeolicus* tRNA (m^2G_{26}) methyltransferase gene. *Nucleic Acids Res Suppl* **2002**: 229–230.
- Tomikawa C, Yokogawa T, Kanai T, Hori H. 2010. N^7 -Methylguanine at position 46 (m^7G_{46}) in tRNA from *Thermus thermophilus* is required for cell viability at high temperatures through a tRNA modification network. *Nucleic Acids Res* **38**: 942–957.
- Tscherne JS, Nurse K, Popienick P, Ofengand J. 1999. Purification, cloning, and characterization of the 16 S RNA m^2G_{1207} methyltransferase from *Escherichia coli*. *J Biol Chem* **274**: 924–929.
- Urbonavicius J, Armengaud J, Grosjean H. 2006. Identity elements required for enzymatic formation of N^2,N^2 -dimethylguanosine from N^2 -monomethylated derivative and its possible role in avoiding alternative conformations in archaeal tRNA. *J Mol Biol* **357**: 387–399.

RNA

A PUBLICATION OF THE RNA SOCIETY

The open reading frame TTC1157 of *Thermus thermophilus* HB27 encodes the methyltransferase forming *N*²-methylguanosine at position 6 in tRNA

Martine Roovers, Yamina Oudjama, Marcus Fislage, et al.

RNA 2012 18: 815-824 originally published online February 15, 2012

Access the most recent version at doi:[10.1261/rna.030411.111](https://doi.org/10.1261/rna.030411.111)

References

This article cites 45 articles, 11 of which can be accessed free at:
<http://rnajournal.cshlp.org/content/18/4/815.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to RNA go to:

<http://rnajournal.cshlp.org/subscriptions>