microorganisms

MDPI

Review

Transfer RNA Modification Enzymes from Thermophiles and Their Modified Nucleosides in tRNA

Hiroyuki Hori *, Takuya Kawamura, Takako Awai, Anna Ochi, Ryota Yamagami, Chie Tomikawa and Akira Hirata

Department of Materials Science and Biotechnology, Graduate School of Science and Engineering, Ehime University, Bunkyo 3, Matsuyama, Ehime 790-8577, Japan; t.kwmr.0115@gmail.com (T.K.); takak0v0@gmail.com (T.A.); annaoti009@yahoo.co.jp (A.O.); w844038k@yahoo.co.jp (R.Y.); tomikawa.chie.mm@ehime-u.ac.jp (C.T.); hirata.akira.mg@ehime-u.ac.jp (A.H.)

* Correspondence: hori.hiroyuki.my@ehime-u.ac.jp; Tel.: +81-89-927-8548

Received: 12 September 2018; Accepted: 17 October 2018; Published: 20 October 2018

Abstract: To date, numerous modified nucleosides in tRNA as well as tRNA modification enzymes have been identified not only in thermophiles but also in mesophiles. Because most modified nucleosides in tRNA from thermophiles are common to those in tRNA from mesophiles, they are considered to work essentially in steps of protein synthesis at high temperatures. At high temperatures, the structure of unmodified tRNA will be disrupted. Therefore, thermophiles must possess strategies to stabilize tRNA structures. To this end, several thermophile-specific modified nucleosides in tRNA have been identified. Other factors such as RNA-binding proteins and polyamines contribute to the stability of tRNA at high temperatures. *Thermus thermophilus*, which is an extreme-thermophilic eubacterium, can adapt its protein synthesis system in response to temperature changes via the network of modified nucleosides in tRNA and tRNA modification enzymes. Notably, tRNA modification enzymes from thermophiles are very stable. Therefore, they have been utilized for biochemical and structural studies. In the future, thermostable tRNA modification enzymes may be useful as biotechnology tools and may be utilized for medical science.

Keywords: archaea; methylation; pseudouridine; RNA modification; tRNA methyltransferase; tRNA modification

1. Introduction

Transfer RNA is an adaptor molecule required for the conversion of genetic information encoded by nucleic acids into amino acid sequences of proteins [1,2]. Figure 1A shows typically conserved nucleosides in a tRNA molecule, which is represented as a cloverleaf structure (herein, the nucleotide positions in tRNA are numbered, according to Sprinzl et al. [3]). These conserved nucleotides are important for tRNA folding and for stabilization of the L-shaped tRNA structure (Figure 1B) [4–6]. In addition to the standard nucleosides, numerous modified nucleosides in tRNA (for structures, see the MODOMICS and tRNAmodviz databases: http://modomics.genesilico.pl/; http://genesilico.pl/trnamodviz [7]) have been discovered in both thermophilic and mesophilic tRNAs [7,8] (see Supplementary Table S1 for abbreviations of modified nucleosides).

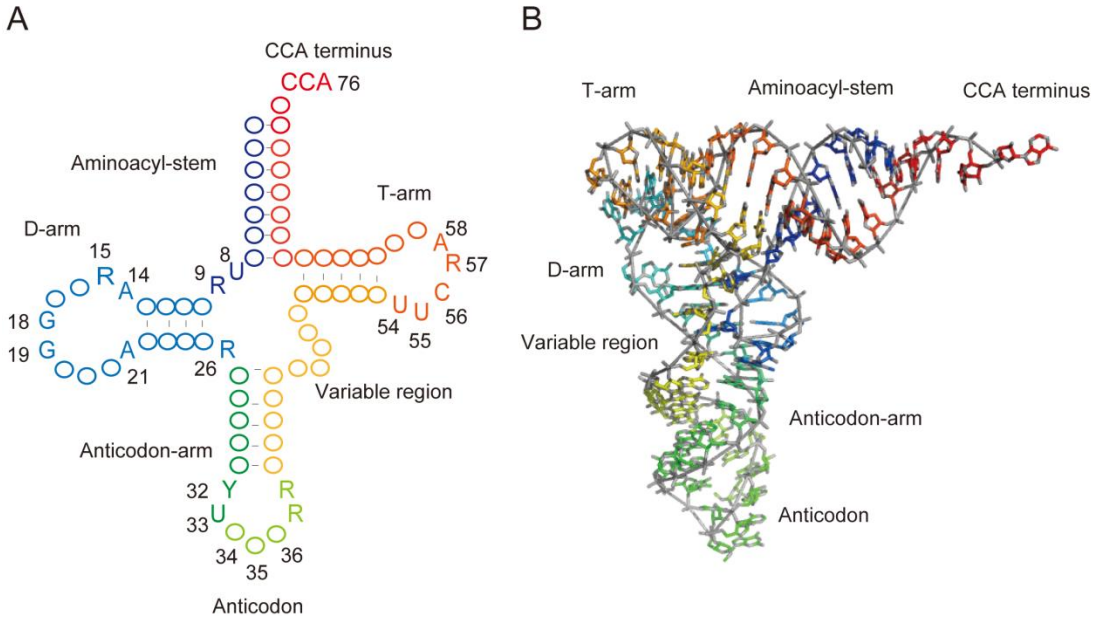

Figure 1. The structure of tRNA. (**A**) Representation of secondary structure of tRNA in a cloverleaf structure. This figure shows tRNA with a short variable region. Conserved nucleosides are shown with position numbers. Abbreviations: R, purine. Y, pyrimidine. (**B**) The L-shaped structure of *Saccharomyces cerevisiae* $tRNA^{Phe}$. The colors of nucleosides correspond to those in (**A**).

A comprehensive review of the modified nucleosides in tRNA from thermophiles and their positions, distribution, predicted (or confirmed) tRNA modification enzymes and structural effects (Table 1) [9–264], which suggests that the majority of modified nucleosides in tRNA from thermophiles are common to those in tRNA from mesophiles. The functions of modified nucleosides in tRNAs have been gradually elucidated by biochemical and structural studies, physicochemical measurements, and analyses of gene disruption strains. The modified nucleosides primarily function in protein synthesis (e.g., stabilization of tRNA structure [88,265–267], correct folding of tRNA [88,265–267], reinforcement, restriction, and/or alteration of codon-anticodon interaction [108,109,114–117,120,124,268–270], recognition by aminoacyl-tRNA synthetases [109,116,117,271], recognition by translation factors [272], and prevention of the frameshift error [122,123,157,158] among others). In short, living organisms cannot synthesize proteins correctly or efficiently without modifications in tRNA.

For some organisms, modifications in tRNA have not been confirmed, but the tRNA modification enzymes have been studied. For example, although no tRNA sequences from *Thermotoga maritima* have been reported, the properties of several tRNA modification enzymes of this hyper-thermophilic eubacterium have been documented and, thus, the nucleoside modifications are predicted. Although many of the functions and biosynthesis pathways of modified nucleosides in tRNA from thermophiles have not yet been investigated, most of them are considered to be basically common to those from mesophiles. However, thermophiles live in extreme environments (e.g., high temperature, anaerobic conditions, extreme pH, and high pressure). Therefore, it is possible that tRNA modifications observed in thermophiles may have novel functions. Furthermore, in some cases, the biosynthesis pathways of some modifications may differ between thermophiles and mesophiles. Moreover, in eukaryotes, tRNA modifications are related to higher biological processes such as cellular transport of tRNA [273–278], RNA quality control [274,279–281], infection [282–286], and the immune response [287–290]. As yet, modified nucleosides in tRNA from thermophilic eukaryotes have not been investigated, but it is possible that a relationship between modified nucleosides in tRNA and these biological phenomena may also be discovered in thermophilic eukaryotes.

In this review, we focus on the modified nucleosides and tRNA modification enzymes from thermophiles including the difficulties in sequencing the rigid and stable tRNAs from thermophiles.

Since the tRNA modifications in moderate thermophiles are essentially similar to those in mesophiles, we describe them separately from extreme-thermophiles and hyper-thermophiles. We focus on the strategies for tRNA stabilization of extreme hyperthermophiles. Furthemrore, we describe the potential effects of these modifications during oxidative and other environmental stresses at high temperatures. Lastly, we describe biotechnological and therapeutic uses for tRNA modification enzymes. To avoid overlap with previous publications, we intentionally refer to representative articles and reviews of modified nucleosides in tRNA and tRNA modification enzymes from mesophiles (main text and Table 1) to aid understanding by the readers. For example, tRNA modifications in archaea including mesophiles have been extensively covered [48,87,291–294] and pseudouridine modifications and methylated nucleosides in tRNA are reviewed elsewhere [87,203,295,296]. Furthermore, the stability of nucleic acids at high temperatures has been reviewed [297]. Other useful publications are pointed out in the appropriate sections throughout the review.

Table 1. Modified nucleosides in tRNA from thermophiles.

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
Am_6	A	Unknown	Stabilization of aminoacyl-stem Enzymatic activity for Am_6 formation has been detected in the cell extract of *Pyrococcus furiosus*	[9]
m^2G_6	B/A	TrmN/Trm14	Stabilization of aminoacyl-stem	[10–15]
U_8	A	CDAT8	Increasing G-C content in tRNA genes In *Methanopyrus kandleri*, U_8 in several tRNA is produced from C_8 by the deamination [16] In *Methanopyrus kandleri*, numerous nucleosides in RNA may be 2-*O*-methylated (see main text) [17]	[16,17]
s^4U_8	B/A	ThiI + IscS/ThiI	UV resistance in *E. coli* and *Salmonella typhimurium* (see main text) Stabilization of D-arm structure in *E. coli* (see main text)	[10,11,18–35]
s^4U_8 and s^4U_9	A	ThiI + α?	UV resistance Stabilization of D-arm structure (see main text) Sulfur-containing modifications in tRNA are reviewed in Reference [35].	[36]
m^1A_9	A	Archaeal Trm10	Stabilization of the D-arm structure Prevention of formation of a Watson–Crick base pair Correct folding of the D-arm region	[37,38]
m^1G_9 and m^1A_9	A	archaeal Trm10	Stabilization of D-arm structure Prevention of formation of a Watson–Crick base pair Correct folding of D-arm region *Thermococcus kodakarensis* Trm10 forms m^1G_9 and m^1A_9, whereas *Sulfolobus acidocaldarius* Trm10 forms only m^1A_9	[37,39]
(m^2G_{10} and) $m^2{}_2G_{10}$	A	archaeal Trm11 (Trm-G_{10}; Trm-$m^2{}_2G_{10}$ enzyme)	Prevention of formation of a Watson-Crick base pair Correct folding of tRNA in *Pyroccocus furiosus* Correct folding of the D-arm region	[40–43]
Ψ_{13}	B/A	TruD/TruD or archaeal Pus7	Stabilization of D-stem structure Archaeal Pus7 generally catalyzes formation of Ψ35 in $tRNA^{Tyr}$, but *Sulfolobus solfaraticus* Pus7 has weak Ψ13 formation activity [46]	[23,44–46]
$G^+{}_{13}$	A	ArcTGT + ArcS?	Stabilization of the D-arm structure *Thermoplasma acidophilum* $tRNA^{Leu}$ exceptionally possesses a $G^+{}_{13}$ modification and *T. acidophilum* ArcTGT acts on positions 13 and 15 in this tRNA [47]	[36,47]

Table 1. *Cont.*

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
G^+_{15}	A	ArcTGT + ArcS or QueF-like protein	Stabilization of interaction between the D-arm and the variable region Several archaea possess a split-type ArcTGT [60,61] Several species in Crenarchaeota possess a QueF-like protein instead of ArcS [60,62,63] G^+ is not found in nucleosides from a *Stetteria hydrogenophila* tRNA mixture [56]	[25,36,47–63]
D_{17}	B	Dus family protein?	Maintenance of D-loop flexibility D_{17} and D_{20} modifications have been reported in *Geobacillus stearothermophilus* tRNA. However, D_{17} and D_{20} are formed by DusB and DusA, respectively, in *Escherichia coli* [65,66] and the *G. stearothermophilus* genome possesses only one *dus*-like gene. This is also observed in *Bacillus subtilis*, which is a mesophilic eubacterium.	[18,19,64–66]
Gm_{18}	B	TrmH	Stabilization of the D-arm and the T-arm interaction. TrmH from thermophiles possess relative broad substrate tRNA specificities as compared with TrmH from *E. coli*. The substrate tRNA specificities of TrmH enzymes differ among thermophiles. TrmH from *Thermus thermophilus* can methylate all tRNA species.	[10,11,20,21,23,24,30,67–81]
D_{20}	B	Dus family protein	Stabilization of local structure of D-loop in *E. coli*? In *A. aeolicus*, the nucleosides at positions 20 and 20a in $tRNA^{Cys}$ are D_{20} and U_{20a}, respectively. Therefore, Dus from *A. aeolicus* may act only on U_{20} in tRNA.	[24,33,65,66,82]
D_{20} and D_{20a}	B	DusA	Stabilization of local structure of the D-loop The melting temperature of a tRNA mixture from the *E. coli dusA* gene disruptant strain is lower than that from the wild-type strain [33]. Therefore, D_{20} and D_{20a} modifications may contribute to stabilize local structure of the D-loop. *Thermus thermophilus* Dus was recently confirmed as a member of the DusA family [65,66,84,85].	[21–23,33,65–67,83–85]
m^1A_{22}	B	TrmK	Prevention of formation of a Watson–Crick base pair	[18,20,86]
Ψ_{22}	A	Unknown	The Ψ_{13}-Ψ_{22} base pair may stabilize D-arm structure [88]	[87,88]

Table 1. *Cont.*

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
m^2G_{26} and $m^2{}_2G_{26}$	A	Trm1	Stabilization of three-dimensional core structure Correct folding of tRNA Recently, it has been reported that $m^2{}_2G_{26}$ modification is required for correct folding of precursor $tRNA^{Ser}$ from *Schizosaccharomyces pombe* [94]. Therefore, a similar phenomenon may take place in thermophiles.	[9,25,44,89–94]
m^2G_{26}, $m^2{}_2G_{26}$, m^2G_{27} and $m^2{}_2G_{27}$	B	Trm1	Stabilization of three-dimensional core structure in *A. aeolicus*. In the case of m^2G_{27} and $m^2{}_2G_{27}$, stabilization of aminoacyl-stem	[24,95]
$m^2{}_2Gm_{26}$	A	Trm1 + unknown MT	Stabilization of three-dimensional core structure The presence of $m^2{}_2Gm$ has been confirmed in nucleosides of a tRNA mixture from several thermophilic archaea [56,97–100]. Although the nucleoside at position 26 in *S. acidocaldarius* $tRNA^{Met}{}_i$ was originally reported as an unidentified G modification [44], it was recently described as $m^2{}_2Gm$ [96]. The MT for 2′-*O*-methylation is unknown.	[44,96]
Cm_{32}	A	archaeal TrmJ	Stabilization of anticodon-loop	[96]
Cm_{32} and Nm_{32}	B	TrmJ	Stabilization of anticodon-loop TrmJ from *E. coli* does not recognize the base at position 32 [96,102]. Um_{32} and Am_{32} have not been reported in tRNAs from thermophilic eubacteria.	[96,101,102]
I_{34}	B	TadA	Alteration of codon–anticodon interaction A-to-I editing in tRNA is reviewed in Reference [107]	[103–107]
k^2C_{34}	B	TilS	Alteration of codon–anticodon interaction (*E. coli* and *B. subtilis*) Change of recognition by aminoacyl-tRNA synthetase (*E. coli* and *B. subtilis*) Decoding of AUA codons by k^2C_{34} and agm^2C_{34} modifications is reviewed in References [114,115].	[108–113]
agm^2C_{34}	A	TiaS	Alteration of codon–anticodon interaction (*Arhaeoglobus fulgidus* and *Haloarcula marismourtui*) Change of recognition by aminoacyl-tRNA synthetase (*Arhaeoglobus fulgidus* and *Haloarcula marismourtui*) Decoding of AUA codons by k^2C_{34} and agm^2C_{34} modifications is reviewed in References [114,115].	[114–120]

Table 1. *Cont.*

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
xm^5U_{34} derivatives	B/A	MnmE + MnmG + MnmC (for mnm^5U_{34} in eubacteria)/Elp3? + α (for cm^5U_{34} in archaea) IscS + TusA + TusBCD + TusE + mnmA (for 2-thiolation in *E. coli*) or YrvO + mnmA (for 2-thiolation in *B. subtilis*) SAMP2 + UbaA + NcsA (for 2-thiolation in *M. maripuludis*)	Reinforcement of codon–anticodon interaction (*E. coli* and other mesophiles) Restriction of wobble base pairing (*E. coli* and other mesophiles) Prevention of frameshift errors (*E. coli* and other mesophiles) Biosynthesis pathways of xm^5U_{34} derivatives are not completely clarified. Although the information on xm^5U_{34} derivatives in tRNA from thermophiles is limited, the biosynthesis pathways may be common with those from mesophiles. For the functions and biosynthesis pathways for xm^5U_{34} derivatives, see References [121–132,136–139,142]. For the thiolation of xm^5s^2U34 derivatives, see References [35,133–135]. *Aquifex aeolicus* exceptionally possesses a DUF752 protein, which is an MT for the xm^5U_{34} modifications without an oxidase domain [136]. A mnm^5U nucleoside has been found in modified nucleosides from unfractionated tRNA in several methane archaea [56]. *Thermoplasma acidophilum* $tRNA^{Leu}$ possesses ncm^5U_{34} [36]. Some thermophiles in Euryarchaea may have a cnm^5U_{34} modification in tRNA [137]. The cm^5U_{34} formation activity of Elp3 from *Methanocaldococcus infernus* has been reported [142]. Several related proteins for synthesis of xm^5U_{34} derivatives from thermophiles have been used for structural studies [136,138–141].	[34–36,56,121–142]
Cm_{34} and $cmnm^5Um_{34}$	B	TrmL	Reinforcement of codon–anticodon interaction (*E. coli*)	[18,143,144]
Gm_{34}	B	Unknown	Reinforcement of codon–anticodon interaction (*G. stearothermophilus*)	[19]
Q_{34} derivatives	B	Tgt + QueA + QueG	Reinforcement of codon–anticodon interaction (*E. coli*) Prevention of frame-shift error (*E. coli*) Biosynthesis pathways and functions of Q derivatives are reviewed in References [152,153]. A crystal structure of QueA from *T. maritima* has been reported [151].	[20,122,145–153]
Cm_{34} and Um_{39} (or Cm_{39})	A	L7Ae + Nop5 + archaeal fibrillarin + Box C/D guide RNA (intron)	Reinforcement of codon–anticodon interaction Reinforcement of anticodon-arm In several archaea, an intron in precursor $tRNA^{Trp}$ functions as a Box C/D guide RNA.	[9,154,155]
Ψ35	A	aPus7 and H/ACA guide RNA system	Reinforcement of codon–anticodon interaction	[46]

Table 1. *Cont.*

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
m^1G_{37}	B/A	TrmD/Trm5	Prevention of frame-shift error (*E. coli* and other mesophiles) Recognition by aminoacyl-tRNA synthetase (*Saccharomyces cerevisiae*)	[36,156–171]
wyosine$_{37}$ derivatives	A	Trm5 + Taw1 + Taw2 + Taw3	Reinforcement of codon–anticodon interaction Prevention of the frame-shift error In several archaea, m^1G_{37} in $tRNA^{Phe}$ is further modified to wyosine derivatives. For the biogenesis pathway of wyosine derivatives, see References [181–183].	[172–183]
t^6A_{37} derivatives	B/A	TsaB, TsaC (TsaC2), TsaD and TsaE/KEOPS complex: Kae1, Bud32, Cgi121 and Pcc1 + Sua5	Reinforcement of codon–anticodon interaction Prevention of frame-shift error Recognition by aminoacyl-tRNA synthetases The biogenesis pathway for t^6A derivatives is reviewed in Reference [191]	[68,184–191]
i^6A_{37} derivatives	B	MiaA + MiaB	Prevention of frame-shift error Reinforcement of codon–anticodon interaction Recognition by aminoacyl-tRNA synthetases i^6A derivatives are reviewed in Reference [197]	[10,11,18–20,24,192–197]
m^6A_{37}	B	YfiC (TrmG?)		[64,198]
Ψ_{38}, Ψ_{39} and Ψ_{40}	B/A	TruA/Pus3	Prevention of frame-shift error (*E. coli*) Reinforcement of anticodon-arm	[10,11,18–20,23,87,199–203]
m^7G_{46}	B	TrmB	Stabilization of three-dimensional core In the case of *T. thermophilus*, m^7G_{46} modification functions a key factor in a network between modified nucleosides in tRNA and tRNA modification enzymes (see main text) [11]	[10,11,19,67,204–208]
m^5C_{48} and m^5C_{49}	A	archaeal Trm4	Stabilization of three-dimensional core	[9,209,210]
m^7G_{49}	A	Unknown		[36]
m^5C_{51}	A	Unknown	Stabilization of T-arm structure	[209]
m^5C_{52}	A	Unknown	Stabilization of T-arm structure	[209]
Ψ_{54} and Ψ_{55}	A	Pus10	Stabilization of D-arm and T-arm interaction	[211–214]
$m^1\Psi_{54}$	A	Pus10 + TrmY	Stabilization of D-arm and T-arm interaction	[215–217]

Table 1. *Cont.*

Modified Nucleoside and Position	Distrib.	Modification Enzyme	Predicted Functions and Additional Information	References
m^5U_{54} + $m^5s^2U_{54}$	B/A	TrmFO + TtuA + TtuB + TtuC + TtuD + IscS/TrmA + TtuA? + TtuB? + α	Stabilization of D-arm and T-arm interaction (see main text) 2-Thiolation of $m^5s^2U_{54}$ in tRNA is reviewed in Reference [239]	[10,11,21–24,67,97,98,134,218–239]
Um_{54}	A	Unknown	Stabilization of D-arm and T-arm interaction	[44]
Ψ_{55}	B/A	TruB/Pus10 or archaeal Cbf5 + α	Stabilization of D-arm and T-arm interaction In the case of *T. thermophilus*, Ψ_{55} is required for low-temperature adaptation (see main text) [248].	[10,11,18–20,23,25,36,44,64,67,211–214,240–248]
Cm_{56}	A	Trm56	Stabilization of D-arm and T-arm interaction	[9,25,36,44,48,89,249–251]
m^2G_{57}	A	Unknown		[44,252]
m^1I_{57}	A	archaeal TrmI + unknown deaminase	Stabilization of T-arm structure	[44,253,254]
m^1A_{57} and m^1A_{58}	A	archaeal TrmI	Stabilization of T-arm structure	[44,255–258]
m^1A_{58}	B	TrmI	Stabilization of T-arm structure	[11,23,67,204,259–264]

This table shows the nucleosides that are modified in tRNA from thermophiles. Most modifications are common to those in tRNA from mesophiles. Several modifications include derivatives and they are summarized as the derivatives (e.g., xm^5U_{34} derivatives). In some cases, only modification enzymes from thermophiles have been reported. For example, although Q derivatives have not been confirmed in tRNA from *T. maritima*, the structure of QueA from *T. maritima* has been reported. In these cases, the modifications are listed here. The references for tRNA modifications and tRNA modification enzymes are mainly those for thermophiles. While there are many references for mesophiles, only representative references are cited. Where available, reviews of a modification and related proteins have been cited. Since modified nucleosides in tRNA from thermophilic eukaryotes have not been reported, modified nucleosides in eukaryotic tRNA have not been included here. The following modified nucleosides have been found in unfractionated tRNA from thermophiles. However, their positions and modified tRNA species are unknown: ac^6A, hn^6A, ms^2hn^6A, methyl-hn^6A, $m^{2,7}Gm$, s^2Um, and ac^4Cm [56,97–100]. Abbreviations are as follows: A, archaea, B, eubacteria, and MT, methyltransferase. The "?" mark indicates the potential function speculated from the structure of the modified nucleosides.

2. Sequencing of tRNA from Thermophiles

The sequence of tRNA provides the most basic information of tRNA. However, as shown in Figure 2, which displays nucleotide sequences of tRNAs from thermophilic eubacteria [10,11,18–24,64,67] and archaea [25,36,44,252], the sequences of only 14 tRNA species have been reported from thermophiles. In addition, in the case of *Aquifex aeolicus* $tRNA^{Cys}$, the sequence has been only partially determined [24].

In general, sequencing of tRNA from thermophiles is difficult for the following reasons. First, purification of specific tRNA from thermophiles is not easy. Currently, tRNA is purified by the solid DNA probe method [298–300]. In this method, the solid-phase complementary DNA probe is placed in a column and hybridized with the target tRNA and then the target tRNA is eluted from the column. Since the structures of tRNA from thermophiles are very rigid, denaturing the tRNA to allow hybridization is difficult. This problem has been solved by incorporating tetraalkyl-ammonium salt in the hybridization buffer [301]. This salt destabilizes the secondary and tertiary structures of tRNA and promotes formation of the RNA-DNA hetero-duplex. This alteration enabled us to purify *A. aeolicus* $tRNA^{Cys}$ [24], *Thermus thermophilus* $tRNA^{Phe}$ [11], $tRNA^{Met}_f1$ [248] and $tRNA^{Thr}$ [263,302], *Thermoplasma acidophilum* initiator $tRNA^{Met}$ [89], elongator $tRNA^{Met}$ [89], and $tRNA^{Leu}$ [36]. Even with the use of tetraalkyl-ammonium salt, however, the solid DNA probe method is not versatile. For example, because the difference between *T. thermophilus* $tRNA^{Met}_f1$ and $tRNA^{Met}_f2$ is only one G-C base pair in the T-stem (Figure 2H) [21], purification of $tRNA^{Met}_f1$ required its separation from $tRNA^{Met}_f2$ by BD-cellulose column chromatography before the solid DNA probe method could be applied [248].

Second, since the structure of tRNA from thermophiles is rigid, limited cleavage by formamide [303,304] is difficult. Therefore, it is difficult to apply the classical technique used for RNA sequencing to tRNA from thermophiles. Liquid-chromatography/mass-spectrometry (LC/MS) has been found to be the most reliable method to overcome this problem [305,306]. In general, LC/MS requires prior cleavage of tRNA by RNases. However, because the G-C content in the stem regions of tRNA from thermophiles is very high (Figure 2), RNA fragments with the same sequences are often generated by RNase cleavage. Therefore, use of multiple RNases and/or preparation of gene disruptant strains are required to overcome this problem.

Furthermore, given that it is not possible to distinguish uridine and pseudouridine by MS, cyanoethylation of tRNA is generally required to detect this nucleoside [307]. In the sequencing of *T. acidophilum* $tRNA^{Leu}$ [36], we used a combination of the cyanoethylation and classical formamide method for detection of Ψ_{54} because the efficiency of cyanoethylation of Ψ_{54} was low. Thus, specific techniques are required even if an LC/MS system is available.

Third, to determine the modified nucleoside precisely, preparation of a standard compound is often required. For example, it was necessary to prepare the standard ncm^5U nucleoside from the *Saccharomyces cerevisiae trm9* gene disruptant strain [308] to determine the anticodon modification of *T. acidophilum* $tRNA^{Leu}$ [36]. In some cases, synthesis of a standard compound by organic chemistry may be required. Lastly, preparing cultures of thermophiles is not so easy for general biochemical researchers (e.g., under anaerobic conditions at high temperatures).

To overcome these problems, the cooperation of researchers in different fields is required. At present, the solid DNA probe method with tetraalkyl-ammonium coupled with LC/MS is the main method for sequencing tRNA from thermophiles. Therefore, it is anticipated that a large numbers of sequences of tRNA from thermophiles will be reported by using this approach in the future.

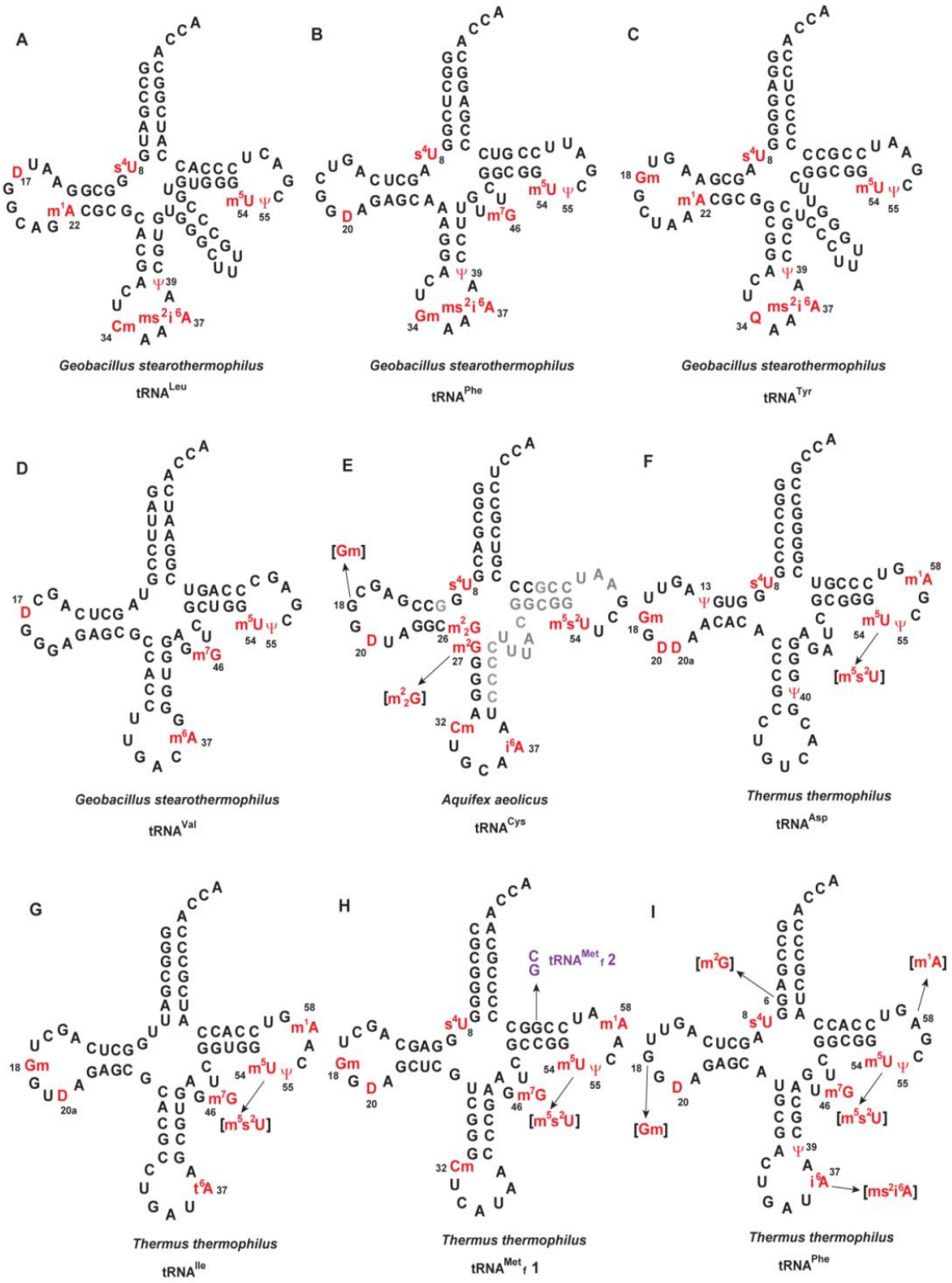

Figure 2. *Cont.*

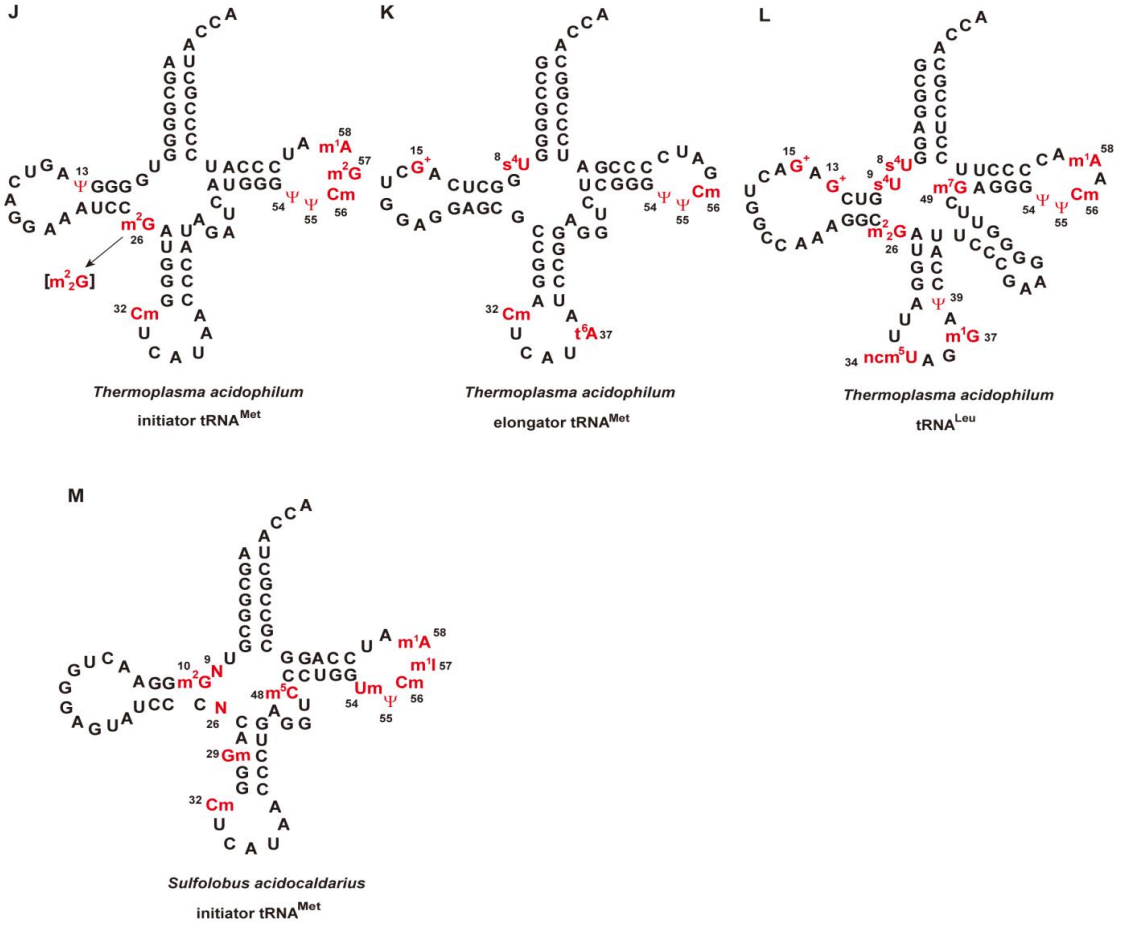

Figure 2. Sequences of tRNA from thermophiles. The modified nucleosides are indicated in red with their positions. Parentheses indicate that a portion of the modified nucleoside is further modified to its derivative. Abbreviations of modified nucleosides are given in Supplementary Table S1. (**A**) *Geobacillus stearothermophilus* $tRNA^{Leu}$. (**B**) *G. stearothermophilus* $tRNA^{Phe}$. (**C**) *G. stearothermophilus* $tRNA^{Tyr}$. (**D**) *G. stearothermophilus* $tRNA^{Val}$. (**E**) *Aquifex aeolicus* $tRNA^{Cys}$. (**F**) *Thermus thermophilus* $tRNA^{Asp}$. (**G**) *T. thermophilus* $tRNA^{Ile}$. (**H**) *T. thermophilus* $tRNA^{Met}{}_f1$. (**I**) *T. thermophilus* $tRNA^{Phe}$. (**J**) *Thermoplasma acidophilum* initiator $tRNA^{Met}$. (**K**) *T. acidophilum* elongator $tRNA^{Met}$. (**L**) *T. acidophilum* $tRNA^{Leu}$. (**M**) *Sulfolobus acidocaldarius* initiator $tRNA^{Met}$ In *A. aeolicus* $tRNA^{Cys}$ (E) the nucleotides shown in gray could not be determined and cyanoethylated $tRNA^{Cys}$ was not analyzed. Therefore, this tRNA may possess additional modifications (e.g., Ψ_{39}, Ψ_{55} and m^1A58). *Thermus thermophilus* possesses two $tRNA^{Met}{}_f$ species. The difference of $tRNA^{Met}{}_f2$ is single G-C base pair, which is indicated in purple in (H). In *S. acidocaldarius* initiator $tRNA^{Met}$ (M), the nucleosides at positions 9 and 26 may be m^1A_9 and $m^2{}_2Gm_{26}$, respectively.

3. Modified Nucleosides in tRNA from Moderate Thermophiles Are Common to Those from Mesophiles

Seven tRNA sequences from moderate thermophiles (*Geobacillus stearothermophilus* and *T. acidophilum*), which live at below 75 °C, have been reported (Figure 2). Furthermore, the modified nucleosides in unfractionated tRNA from moderate thermophiles (*Methanobacterium thermoaggregans*, *Methanobacterium thermoautotrophicum*, and *Methanococcus thermolithotrophicus*) were analyzed [97,99]. These studies have shown that the modified nucleosides in tRNA from moderate thermophiles are typically common to those in tRNA from mesophiles. In summarizing the information on tRNA modifications and tRNA modification enzymes by thermophilic species [8–330] (Table 2), we have separately considered moderate thermophiles, extreme-thermophiles, and hyper-thermophiles. However, there are some differences between moderate thermophiles and mesophiles. For

example, the degree of 2′-*O*-methylation in tRNA from *G. stearothermophilus* is increased at high temperatures [309]. Furthermore, several modifications (Gm_{18}, D modifications, and Gm_{34}) in tRNA from *G. stearothermophilus* cannot be explained by the enzymatic activities of the already-known tRNA modification enzymes, which is described in Table 2. Moreover, *T. acidophilum* possesses several distinct tRNA modifications such as G^{+}_{13} and $m^{7}G_{49}$ (Table 2) [36]. Although these differences are present, thermophile-specific modified nucleosides have not been found in tRNA from moderate thermophiles, which suggests that living organisms can survive at 75 °C via the tRNA modifications in mesophiles.

Table 2. Thermophiles: their tRNA modifications and tRNA modification enzymes.

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
Moderate Thermophiles			
Eubacteria			
Geobacillus stearothermophilus (*Bacillus stearothermophilus*) 30–75 °C		Sequences of $tRNA^{Leu}$ [18], $tRNA^{Phe}$ [19], $tRNA^{Tyr}$ [20], and $tRNA^{Val}2$ [64] have been reported (Figure 2). The majority of modifications in tRNA are similar to those in *B. subtilis*. With increasing culture temperature, the extent of 2′-*O*-methylation in the tRNA mixture increases [309].	
	Gm_{18} (TrmH?)	Although *trmH* is not encoded in the *B. subtilis* genome, a *trmH*-like gene is encoded in the *G. stearothermophilus* genome. Gm_{18} has been found in $tRNA^{Tyr}$ but not in $tRNA^{Leu}$. This modification pattern suggests that the substrate tRNA specificity of *G. stearothermophilus* TrmH may be different from that of other known TrmH enzymes.	[20]
	D_{17}, D_{20} and D_{20a} (Dus family protein?)	In *G. stearothermophilus* tRNA, D_{17}, D_{20}, and D_{20a} modifications have been reported. In *E. coli*, three Dus family proteins known as DusA, DusB, and DusC, produce D_{20} and D_{20a}, D_{17}, and D_{16}, respectively [65,66]. In the *G. stearothermophilus* genome, however, only one gene is annotated as a *dus*-like gene. Therefore, D modifications in *G. stearothermophilus* cannot be explained by the tRNA substrate specificity of the known Dus proteins.	[17,19,64]
	m^1A_{22} (TrmK?)	The m^1A_{22} modification has been found in $tRNA^{Tyr}$ and $tRNA^{Ser}$ from *B. subtilis* and *Mycoplasma capricolum* [310,311]. *G. stearothermophilus* $tRNA^{Leu}$ and $tRNA^{Tyr}$ possess m^1A_{22} [18,20]. The presence of a *trmK*-like gene in the genome of *G. stearothermophilus* has been reported [86].	[20,86]
	Gm_{34} (unknown MT)	*G. stearothermophilus* $tRNA^{Phe}$ possesses Gm_{34} (Figure 2B) [19]. In contrast, the nucleoside at position 34 in *E. coli* $tRNA^{Phe}$ is unmodified G. Given that *E. coli* TrmL acts only on $tRNA^{Leu}$ isoacceptors [143], the 2′-*O*-methylation of G_{34} in $tRNA^{Phe}$ from *G. stearothermophilus* is cannot be simply explained by the activity of known TrmL.	[19]
	m^6A_{37} (YfiC; TrmG?)		[198]
Archaea			
Methanobacterium thermoaggregans Optimum growth temperature 60 °C		Sequences of $tRNA^{Asn}$ and $tRNA^{Gly}$ have been reported [8].	
Methanobacterium thermoautotrophicum 45–75 °C		The modified nucleosides in unfractionated tRNA are essentially common to those in tRNA from mesophilic methane archaea [97].	

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
Methanococcus thermolithotrophicus 17–62 °C		The modified nucleosides in unfractionated tRNA are essentially common to those in tRNA from mesophilic methane archaea [99].	
Thermoplasma acidophilum Optimum growth temperature 55–60 °C		Sequences of $tRNA^{Met}i$ [44,252], $tRNA^{Met}m$ [25], and $tRNA^{Leu}$ [36] have been reported. Several recombinant tRNA modification enzymes have been used for biochemical studies.	
	s^4U_8 and s^4U_9 (ThiI? + α)	The s^4U_9 modification has been found in $tRNA^{Leu}$ [36]. The sulfur donor for s^4U formation is unknown [35].	[36]
	G^+_{13} and G^+_{15} (ArcTGT + ArcS?)	The G^+_{13} modification has been found only in $tRNA^{Leu}$ from *T. acidophilum* [36]. ArcTGT from *T. acidophilum* acts on both G13 and G15 in $tRNA^{Leu}$ [47].	[36,47]
	$m^2{}_2G_{26}$ (Trm1)		[89]
	ncm^5U_{34} (Elp3?)		[36]
	m^1G_{37} (Trm5)		[89]
	m^7G_{49} (unknown MT)		[36]
	Cm_{56} (Trm56)	The presence of unusual *trm56*-like gene in the *T. acidophilum* genome has been reported in a bioinformatics study [250]. The Trm56 enzymatic activity has been confirmed via the recombinant protein [89]. *T. acidophilum* Trm56 exceptionally possesses a long C-terminal region in the SPOUT tRNA MT [312].	[89,250,312]
Extreme-thermophiles and Hyper-thermophiles			
Eubacteria			
Aquifex aeolicus Optimum growth temperature 85–94 °C		The partial sequence of $tRNA^{Cys}$ has been reported [24] (Figure 2E). Several tRNA MT activities have been detected in the *A. aeolicus* cell extract using an *E. coli* tRNA mixture [24]. The tRNA modification enzymes listed below were characterized via recombinant proteins.	
	Gm_{18} (TrmH)		[74,77]
	D_{20} (Dus)	D_{20} exists in $tRNA^{Cys}$. However, the nucleoside at position 20a is unmodified U [24]. Therefore, *A. aeolicus* Dus may act only on U_{20}.	[24,82]
	m^2G_{26}, $m^2{}_2G_{26}$, m^2G_{27} and $m^2{}_2G_{27}$ (Trm1)	*Aquifex aeolicus* exceptionally possesses Trm1 in eubacteria [24].	[24,95]
	I_{34} (TadA)		[104,105]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	mnm^5U_{34} (MnmC2)	MnmC catalyzes the final methylation step of mnm^5U synthesis. *Aquifex aeolicus* MnmC2 comprises only an MT domain.	[136]
	(MnmD; previously called GidA)		[140,141]
	k^2C_{34} (TilS)		[111–113]
	m^1G_{37} (TrmD)	The dimer structure of *A. aeolicus* TrmD is stabilized by inter-subunit disulfide bonds [165].	[160,162,165]
	m^7G_{46} (TrmB)	TrmB proteins from thermophiles (*A. aeolicus* and *T. thermophilus*) possess a long C-terminal region.	[206–208]
	m^5U_{54} and $m^5s^2U_{54}$ (TrmFO)	The presence of *trmFO* gene in *A. aeolicus* genome was initially described in Reference [221].	[24,221]
	m^1A_{58} (TrmI)		[257,262]
Thermotoga maritima 80–90 °C		Sequences of tRNA from *T. maritima* have not been reported. Recombinant proteins have been used for biochemical and structural studies.	
	hn^6A (?)	hn^6A was first identified in modified nucleosides from unfractionated tRNA from *T. maritima* [313]. Because hn^6A was subsequently found in modified nucleosides from psychrophilic archaea [56], it is not a thermophile-specific modification. *Thermotoga maritima* and *Thermodesulfobacterium commune* exceptionally possess hn^6A in eubacteria. The modification position in tRNA, modified tRNA species, and biosynthesis pathway of hn^6A are unknown.	[56,313]
	s^4U_8 (ThiI + IscS)		[31,32]
	oQ_{34} (QueA)		[151]
	mnm^5U_{34} (TrmE)		[138,139]
	t^6A_{37} (TsaB, TsaC/TsaC2, TsaD and TsaE)		[190]
	$ms^2i^6A_{37}$ (MiaB)		[194–196]
	m^1G_{37} (TrmD)		[171]
	m^5U_{54} and $m^5s^2U_{54}$ (TrmFO and TtuA)	The m^5s^2U nucleoside has been found in unfractionated tRNA from *T. maritima* [97].	[97,134,221,222]
	Ψ_{55} (TruD)		[244–247]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	m^1A_{58} (TrmI)		[257]
Thermodesulfobacterium commune Optimum growth temperature 70 °C	hn^6A and ms^2hn^6A (?)	hn^6A and ms^2hn^6A have been found in modified nucleosides from unfractionated tRNA from *T. commune*. The ms^2hn^6A modification may be derived from hn^6A. So far, *T. commune* is the only eubacterium that possesses ms^2hn^6A in tRNA. The modification position in tRNA, modified tRNA species, and biogenesis pathway of hn^6A and ms^2hn^6A are unknown.	[313]
Thermus flavus Optimum growth temperature 70 °C		Partial purification of tRNA m^1A58 MT has been reported: the activity of tRNA m^7G_{46} MT has also been described [204].	
Thermus thermophiles 50–83 °C		Sequences of $tRNA^{Met}f1$ [21], $tRNA^{Met}f2$ [21], $tRNA^{Ile}1$ [67], $tRNA^{Asp}$ [23], and $tRNA^{Phe}$ [10,11] have been reported (Figure 2). Partial sequences of $tRNA^{Ser}_{GGA}$ [259], $tRNA^{Pro}_{GGG}$ [314], and $tRNA^{Pro}_{GGA}$ [314] have been determined. The modification extent of Gm_{18}, $m^5s^2U_{54}$ and m^1A_{58} changes with the culture temperature. At high temperatures (>75 °C), m^7G_{46} [11], $m^5s^2U_{54}$ [230], and m^1A_{58} [260] modifications are essential for survival. At low temperatures (<55 °C), Ψ_{55} is essential for survival [248] and m^5U_{54} supports this effect [225] (see the main text). Recombinant proteins have been used for biochemical and structural studies.	
	m^2G_6 (TrmN)		[10,11,13–15]
	Gm_{18} (TrmH)		[10,11,21,23,30,69–73,75,76,78–81]
	D_{20} and D_{20a} (DusA)		[10,11,23,67,83–85]
	Cm_{34} and $cmnm^5Um_{34}$ (TrmL)		[144]
	Ψ_{39} and Ψ_{40} (TruA)		[10,11,18,23,202]
	m^7G_{46} (TrmB)		[10,11,21,23]
	m^5U_{54} and $m^5s^2U_{54}$ (TrmFO + TtuA + TtuB + TtuC + TtuD + IscS)		[10,11,17,21,23,67,218–236,239]
	Ψ_{55} (TruB)		[10,11,21,23,67,248]
	m^1A_{58} (TrmI)		[11,30,257,259–261,263,264]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
Archaea			
Aerophyrum pernix 80–100 °C	Ψ_{13} and Ψ_{15} (archaeal Pus7 and H/ACA guide RNA system)	A guide RNA for Ψ formation has been predicted based on genome sequencing.	[46]
Archaeoglobus fulgidus 60–95 °C		Modified nucleosides in unfractionated tRNA from *A. fulgidus* have been reported [97].	
	agm^2C_{34} (TiaS)		[116,118,119,315]
Methanocaldcoccus igneus (*Methanococcus igneus*; *Methanotorris igneus*) 45–91 °C		Modified nucleosides in unfractionated tRNA from *M. igneus* have been reported [56,99].	
Methanocaldococcus infernus 55–92 °C	cm^5U_{34} (Elp3)		[142]
Methanocaldcoccus jannashii (*Methanococcus janaschii*) 48–94 °C		Although sequences of tRNA are unknown, the recombinant proteins listed below have been used for biochemical and structural studies.	
	m^2G_6 (Trm14)		[12]
	G^+_{15} (ArcTGT + ArcS)		[51,59]
	Cm_{34} and Um_{39} (L7Ae, Nop5, aFib, Box C/D guide RNA system)		[316]
	m^1G_{37} (Trm5)		[159,161,163,164,166–170]
	$imG2_{37}$ (Trm5b + Taw1)		[173,179]
	$yW\text{-}86_{37}$ (Taw2)		[174]
	m^5C_{48} and m^5C_{49} (archaeal Trm4)		[210]
	Ψ_{54} and Ψ_{55} (Pus10)		[211–214]
	$m^1\Psi_{54}$ (Pus10 + TrmY)		[215–217]
	Ψ_{55} (archaeal Cbf5)		[240]
Methanopyrus kandleri 84–110 °C (Strain 116: up to 122 °C)		Many unique modified nucleosides have been found in unfractionated tRNA [100]. tRNAs likely contain many 2′-*O*-methylated nucleosides derived from the C/D box guide RNA system [17].	

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	ac^6A (?)	The ac^6A nucleoside has been purified from the modified nucleosides in unfractionated tRNA and its structure determined. The modification site, modified tRNA species, and biosynthesis pathway are unknown.	[100]
	U_8 (CDAT8)		[16]
Methanothermus fervidus 80–97 °C		Only tRNA genes were reported in an early study [317].	
Nanoarchaeum equitans 70–98 °C		A unique tRNA processing system has been found [318,319]. The processing of small RNAs in *N. equitans* is reviewed in Reference [320].	
	m^1G_{37} and $imG2_{37}$ (Trm5a)		[176]
	m^5U_{54} (TrmA-like protein)		[237]
Pyrobaculum aerophilum Optimum growth temperature 100 °C	Cm_{56} (L7Ae, Nop5, aFib, Box C/D guide RNA system)	Cm_{56} in tRNA is generally produced by Trm56. However, this modification in *P. aerophilum* is synthesized by the C/D box guide RNA system.	[249]
Pyrobaculum calidifontis 90–95 °C	$G^+{}_{15}$ (ArcTGT + QueF-like protein)	Eubacterial QueF catalyzes the conversion from $preQ_0$ to $preQ_1$. In *P. caldifontis*, however, QueF-like protein catalyzes the conversion from $preQ_0$ at position 15 in tRNA to $G^+{}_{15}$.	[60,62,63]
Pyrobaculum islandicum Optimum growth temperature 100 °C		Modified nucleosides in unfractionated tRNA from *P. islandicum* have been reported [97].	
Pyrococcus abyssi Optimum growth temperature 96 °C		No tRNA sequence has been determined. However, the tRNA modification enzymes listed below have been characterized.	
	m^2G_{10} and $m^2{}_2G_{10}$ (archaeal Trm11, Trm-G10 enzyme, Trm-m22G10 enzyme)		[40,41]
	Ψ_{13} and Ψ_{35} (archaeal Pus7 and H/ACA guide RNA system)		[46]
	Cm_{34} and Um_{39} (L7Ae, Nop5, aFib, and C/D box guide RNA system)	Cm_{34} and Um_{39} in $tRNA^{Trp}$ are formed by the C/D box guide RNA system in which the intron functions as a guide RNA.	[154,155]
	m^1G_{37} (Trm5b)		[180]
	m^1G_{37} and $imG2_{37}$ (Trm5a)		[176,177,179]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	imG-14$_{37}$ (Taw1)		[173,175]
	t^{6}A37 (Kae1)		[185]
	(KEOPS complex)		[184]
	(Sua5 + KEOPS complex)		[187,189]
	m^{5}C$_{48}$ and m^{5}C$_{49}$ (archaeal Trm4 + archaese)		[209]
	m^{5}U$_{54}$ (TrmA-like protein, PAB0719)		[237,238]
	Ψ$_{55}$ (Cbf5 + Nop10)		[241]
	Cm$_{56}$ (Trm56)		[249]
	m^{1}A$_{57}$ and m^{1}A$_{58}$ (archaeal TrmI)		[255–258]
Pyrococcus furiosus Optimum growth temperature 100 °C		Modified nucleosides in unfractionated tRNA from *P. furiosus* have been reported [98]. Activity of several tRNA modification enzymes has been detected in the cell extract of *P. furious* [9].	
	m^{2}G$_{6}$ (Trm14)		[13,15]
	m^{2}G$_{10}$ and m$^{2}{}_{2}$G$_{10}$ (archaeal Trm11, Trm-G10 enzyme, Trm-m22G10 enzyme)		[42]
	G$^{+}{}_{15}$ (ArcTGT)		[57]
	m^{2}G$_{26}$ and m$^{2}{}_{2}$G$_{26}$ (Trm1)		[91,92]
	t^{6}A37 (KEOPS complex)		[188]
	Ψ$_{54}$ and Ψ$_{55}$ (Pus10)		[212,214]
	Ψ$_{55}$ (Cbf5 + Nop10 + Gar1)		[242]
Pyrococcus horikoshii 80–102 °C		The crystal structure of Nop5 in the C/D box guide RNA system from *P. horikoshii* has been solved [321].	
	G$^{+}{}_{15}$ (ArcTGT)		[50,52–55,89]
	m^{2}G$_{26}$ and m$^{2}{}_{2}$G$_{26}$ (Trm1)		[89,93]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	yW-86$_{37}$ (Taw2)		[174]
	m^{5}s^{2}U$_{54}$ (TtuA)		[233]
	Cm$_{56}$ (Trm56)		[251]
Pyrodictium occultum Optimum growth temperature 105 °C		Modified nucleosides in unfractionated tRNA have been analyzed and many 2′-*O*-methylated nucleosides found [97,98]. mimG was originally found among the modified nucleosides in tRNAs from *P. occultum*, *Sulfolobus solfaraticus*, and *Thermoproteus neutrophilus* [322]. Although the melting temperature of *P. occultum* tRNA^{Met}i transcript is only 80 °C and that of native tRNA^{Met}i is more than 100 °C (see main text) [323].	
Pyrolobus fumarii This archaeon can survive at 113 °C.		Modified nucleosides in unfractionated tRNA have been analyzed [324].	
Stetteria hydrogenophila Optimum growth temperature 95 °C		Modified nucleosides in unfractionated tRNA have been analyzed and methyl-hn^{6}A, ms^{2}hn^{6}A, and m^{2,7}Gm identified [56].	
Sulfolobus acidocaldarius Optimum growth temperature 75–80 °C		Sequence of tRNA^{Met}i has been reported [44]. The m^{1}I$_{57}$ modification was originally found in tRNAs from *S. acidocaldarius* and *Haloferax volcanii* [253]. G$^+$ was first isolated from the nucleosides in *S. acidocaldarius* tRNAs and its structure determined [49]. The structures of wyosine derivatives (imG-14 and imG2) have been determined by using the nucleosides from *S. acidocaldarius* tRNAs [325].	
	m^{1}A$_9$ (archaeal Trm10)		[37,38]
	Ψ$_{13}$ and Ψ$_{35}$ (archaeal Pus7 and H/ACA guide RNA system)		[46]
	Cm$_{32}$ (archaeal TrmJ)		[96]
Sulfolobus solfaraticus 55–90 °C		mimG was originally found among the modified nucleosides in tRNAs from *P. occultum*, *S. solfaraticus*, and *Thermoproteus neutrophilus* [322]. The structure of box C/D RNP from *S. solfaraticus* has been reported [326].	
	agm^{2}C (TiaS)	The identification of agm^{2}C$_{34}$ in *Haloarcula marismortui* tRNA^{Ile}2 and the presence of agm^{2}C in *S. solfaraticus* tRNA have been reported.	[117]
	Ψ$_{13}$ and Ψ$_{35}$ (archaeal Pus7 and H/ACA guide RNA system)	Generally, Ψ$_{35}$ in tRNA^{Tyr} is synthesized by archaeal Pus7. However, Pus7 from *S. solfaraticus* possesses weak Ψ$_{13}$ formation activity but not Ψ$_{35}$ formation activity. In *S. solfaraticus* and *A. pernix*, a guide RNA for Ψ$_{35}$ formation exists.	[46]

Table 2. *Cont.*

Species	Predicted Enzyme	Distinct tRNA Modifications and General Information	References
	imG2$_{37}$ (Trm5a; SSO2439 protein)	Trm5a (SSO2439 protein) does not possess m^{1}G$_{37}$ formation activity and is used only for imG2 formation.	[178]
	mimG$_{37}$ (Taw3)		[180]
Sulfolobus tokodaii This archaeon can survive at 87 °C.	Ψ$_{13}$ and Ψ$_{35}$ (archaeal Pus7 and H/ACA guide RNA system)		[46]
	t^{6}A$_{37}$ (Sua5)		[327–329]
Thermococuus celer This archaeon can survive at 85 °C.		Although tRNA genes were analyzed in an early study [330], there is no information on tRNA modifications.	
Thermococcus kodakarensis (*Thermococcus kodakaraensis*; *Pyrococcus kodakarensis*) 65–100 °C	m^{1}A$_9$ and m^{1}G$_9$ (archaeal Trm10)		[37,39]
	m^{2}G$_{10}$ and m$^2{}_2$G$_{10}$ (archaeal Trm11, Trm-G10 enzyme, Trm-m22G10 enzyme)		[43]
	G$^+{}_{15}$ (ArcTGT)		[47]
	m^{5}U$_{54}$ (TrmA-like protein)		[237]
Thermoproteus neutrophilus Optimum growth temperature 85 °C		Modified nucleosides in unfractionated tRNA have been analyzed [97]. mimG was originally found among the modified nucleosides in tRNAs from *P. occultum*, *S. solfaraticus*, and *T. neutrophilus* [322].	

Only distinct modifications that have been investigated are listed by thermophile species. In many cases, only tRNA modification enzymes (rather than modifications) have been studied by using recombinant proteins. For example, the presence of the m^{7}G$_{46}$ modification has not been confirmed in tRNA from *A. aeolicus*, but TrmB (tRNA m^{7}G$_{46}$ MT) has been characterized through the recombinant protein. In this case, m^{7}G$_{46}$ (TrmB) is listed in the section "*Aquifex aeolicus*". The moderate thermophiles and extreme-thermophiles along with hyper-thermophiles are separated. Transfer RNA modifications in thermophilic eukaryotes are unknown. Abbreviation: MT, methyltransferase.

4. Strategies of tRNA Stabilization by Modified Nucleosides in Extreme-Thermophiles and Hyper-Thermophiles

In general, the G-C content in the stem regions of tRNA from thermophiles is very high (Figure 2). However, the stability of tRNA from thermophiles cannot be explained only by the increase in G-C content in the stem region. For example, although the melting temperature of *T. thermophilus* $tRNA^{Phe}$ transcript is 76 °C, that of the native $tRNA^{Phe}$ is 84.5 °C [11]. Thus, modified nucleosides are essentially required for stabilization of tRNA at high temperatures. Modified nucleosides in tRNA from thermophiles have been studied mainly from the view point of tRNA stabilization. So far, only a few modified nucleosides specific to thermophiles have been found (Figure 3). These thermophile-specific modified nucleosides seem to stabilize the tRNA structures at high temperatures. As described below, extreme-thermophiles and hyper-thermophiles possess two strategies of tRNA stabilization by modified nucleosides. One is based on thermophile-specific modification such as $m^5s^2U_{54}$ (Figure 3A) and the other is based on 2′-*O*-methylations at multiple positions in tRNA (Figure 3B–E). Recently, the unknown modified nucleoside at position 26 in *Sulfolobus acidocaldarius* $tRNA^{Met}$ (Figure 2M) was described as $m^2{}_2Gm$ [96]. On the whole, however, the modification site(s), modified tRNA species, and biosynthesis pathways of most thermophile-specific modified nucleosides are unknown. Moreover, these nucleosides may have additional functions at high temperatures beyond their structural effect.

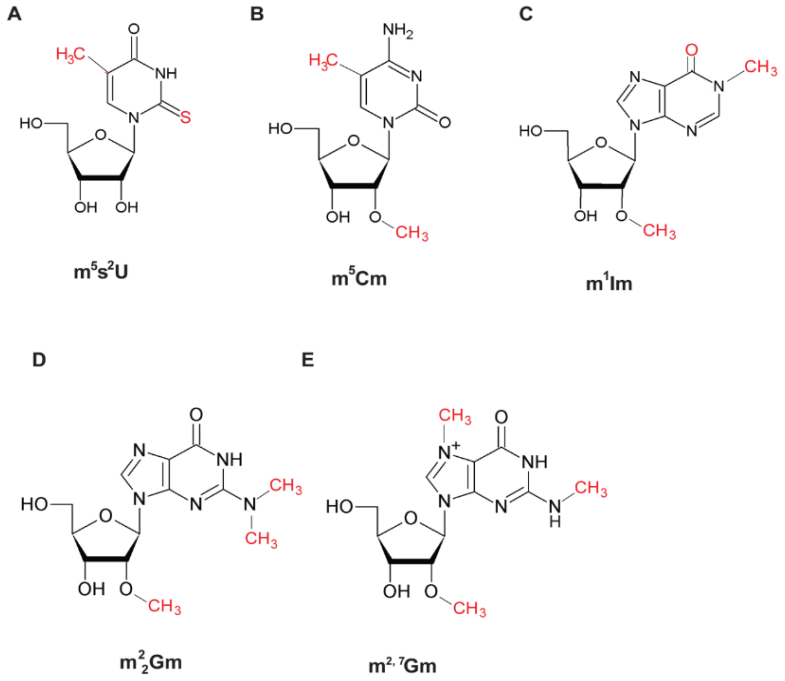

Figure 3. Thermophile-specific modified nucleosides in tRNA. Abbreviations of modified nucleosides are given in Supplementary Table S1. (**A**) m^5s^2U. (**B**) m^5Cm. (**C**) m^1Im. (**D**) $m^2{}_2Gm$. (**E**) $m^{2,7}Gm$. The modifications are indicated in red.

4.1. $m^5s^2U_{54}$ Is a Typical Thermophile-Specific Modified Nucleoside in tRNA

The $m^5s^2U_{54}$ modification was originally found in tRNA from *T. thermophilus* [331]. Subsequently, this modified nucleoside was found in tRNA from *A. aeolicus*, *T. maritima*, *Pyrococcus abyssi*, *Pyorococcus horikoshii*, and *T. kodakarensis* (Table 2) but not from mesophiles. The $m^5s^2U_{54}$ modification forms a reverse Hoogsteen base-pair with A_{58} (or m^1A_{58}) in tRNA and stabilizes the tRNA structure by stacking with the G_{51}–C_{61} base-pair [220]. Because the 2-thio-modification at position 54 increases the melting temperature of tRNA by more than 3 °C [22,218,220], the $m^5s^2U_{54}$ modification contributes to stabilization of the tRNA structure. The degree of $m^5s^2U_{54}$ modification increases with an increasing temperature [22,67,220,229]. At 80 °C, the extent of $m^5s^2U_{54}$ modification in tRNA is

almost 100% [22,67,220,229]. The melting temperature of tRNA mixture is maintained above 85 °C due to the presence of $m^5s^2U_{54}$ modification [229] and *T. thermophilus* can grow at 50 to 83 °C. Thus, living organisms can survive at 80 °C due to the presence of $m^5s^2U_{54}$ modification in tRNA.

4.2. The Network Between Modified Nucleosides in tRNA and tRNA Modification Enzymes in T. thermophilus Adapts Protein Synthesis at Low and High Temperatures

Under natural conditions, the temperature of hot spring water fluctuates for several reasons including an influx of river water, snowfall, and an eruption of hot water. In accordance with these temperature changes, *T. thermophilus* can synthesize proteins efficiently at a wide range of temperatures (50 to 83 °C) by regulating the flexibility (rigidity) of its tRNA [220]. At high temperatures (above 75 °C), three modified nucleosides in tRNA, $m^5s^2U_{54}$ [230], m^1A_{58} [260], and m^7G_{46} [11] are essential for survival of *T. thermophilus*. The m^1A_{58} modification is one of the positive determinants for the two-thiolation system of $m^5s^2U_{54}$. Thus, a *T. thermophilus* disruptant strain of the *trmI* gene encoding the tRNA m^1A_{58} methyltransferase cannot grow at 80 °C [229,260]. The presence of m^7G_{46} modification in tRNA increases the speed of tRNA modification enzymes such as TrmH for Gm_{18}, TrmD for m^1G_{37}, and TrmI for m^1A_{58} [11]. The m^1A_{58} modification further increases the rate of sulfur-transfer to m^5U_{54} by the 2-thiolation system and the introduced modified nucleosides coordinately stabilize the tRNA structure. Thus, the m^7G_{46} modification produced by TrmB is a key factor in the network between modified nucleosides in tRNA and tRNA modification enzymes of *T. thermophilus* at high temperatures. In the *trmB*-gene disruptant starin, $tRNA^{Phe}$ and $tRNA^{Ile}$ were found to be degraded by a temperature shift from 70 °C to 80 °C and heat-shock proteins were not synthesized efficiently [11].

At low temperatures (below 55 °C), in contrast, the Ψ_{55} modification produced by TruB is essential for the survival of *T. thermophilus* [248]. The presence of Ψ_{55} stabilizes both the local structure of the T-arm and the interaction of the T-arm with the D-arm in tRNA. The local rigidity in tRNA caused by Ψ_{55} slows down the speeds of introducing modified nucleosides around Ψ_{55} (Gm_{18}, $m^5s^2U_{54}$ and m^1A_{58}), which maintains the flexibility of tRNA at low temperatures. The presence of m^5U_{54} modification by TrmFO supports this effect of Ψ_{55} [225].

It should be mentioned that D modifications are thought to bring flexibility to tRNA because D does not stack with other bases and brings about the C2′-endo form of ribose [332]. However, a *T. thermophilus* disruptant strain of the *dusA* gene encoding tRNA D_{20}/D_{20a} synthase did not show growth retardation at 50, 60, 70, or 80 °C, and abnormal modifications were not observed in tRNA from this strain [85]. Therefore, the function of D_{20} and D_{20a} modifications is unknown. Since DusA recognizes the interaction of T-arms and D-arms in tRNA [84], the stabilization of the L-shaped tRNA structure by other modified nucleosides is required for the efficient introduction of D_{20} and D_{20a} at high temperatures [85]. Thus, D_{20} and D_{20a} are relatively late modifications in *T. thermophilus* tRNA.

Although the above network is a temperature adaptation system of *T. thermophilus*, it regulates the order in which modified nucleosides are introduced into tRNA. Similar networks have been found in mesophiles [333]. In *Escherichia coli*, for example, the 2′-*O*-methylation at position 34 by TrmL requires an i^6A_{37} modification [334]. However, the network in *T. thermophilus* is distinct because it regulates the structure of a three-dimensional core and many modifications in tRNA are related. One of the advantages of this system is that protein synthesis is not required. The response of the system is very rapid. It is possible that thermophilic archaea possess a similar network between modified nucleosides in tRNA and tRNA modification enzymes because some of them can also grow at a wide range of temperatures.

4.3. Stabilization of tRNA Structure by 2′-O-Methylation

Because 2′-*O*-methylation shifts the equilibrium of ribose puckering to the C3′-endo form and enhances the hydrophobic interaction, this modification, when carried out at multiple positions, brings rigidity of tRNA. Furthermore, 2′-*O*-methylations prevents hydrolysis of phophodiester-bonds in tRNA at high temperatures. Therefore, 2′-*O*-methylations may prolong the half-lives of tRNA. Notably, there

is a living organism in which tRNA is stabilized without $m^5s^2U_{54}$ modification. A hyper-thermophilic archaeon, *Pyrodictium occultum* can grow at 105 °C, and various 2′-*O*-methylted nucleosides such as Ψm, m^1Im, and $m^2{}_2$Gm are present in its tRNA, but s^2U and m^5s^2U are not observed [97,98]. Notably, although the melting temperature of the *P. occultum* $tRNA^{Met}$ transcript is 80 °C, that of the native $tRNA^{Met}$ is more than 100 °C [323]. Thus, the melting temperature of *P. occultum* tRNA is increased by more than 20 °C through a combination of numerous 2′-*O*-methylated nucleosides.

Methanopyrus kandleri can grow at more than 110 °C and tRNAs from this archaeon contain many unique modifications such as U_8 (the product of C_8 to U_8 editing) [16], ac^6A, $m^{2,7}$Gm, and methyl-hn^6A [100]. Furthermore, *M. kandleri* possesses 132 species of C/D-box guide RNAs [17], which suggests that RNAs are highly methylated by the L7Ae, Nop5, aFib, and C/D-box guide RNA system. In the case of *M. kandleri*, therefore, tRNA seems to be stabilized by unique modifications and 2′-*O*-methylations.

These observations suggest that living organisms can survive at more than 100 °C by a combination of 2′-*O*-methylations and other thermophile-specific tRNA modifications.

4.4. Other tRNA Stabilization Factors

RNA binding proteins, polyamines, magnesium ions, and potassium ions are all able to stabilize tRNA in thermophiles. For example, transfer RNA-binding protein 111 (Trbp111) is an RNA-binding protein that is observed only in *A. aeolicus* [335–337]. *A. aeolicus* can grow at 94 °C and modified nucleosides in tRNA of this hyperthermophilic eubacterium are not so different from those in tRNA from *T. thermophilus*, which grows at temperatures below 83 °C. Therefore, Trbp111 may provide more than 10 °C of tRNA stabilization in *A. aeolicus*. The docking model of Trbp111 and tRNA suggests that Trbp111 stabilizes the three-dimensional core of tRNA [336]. Archease is another tRNA-binding protein that can change the methylation site of *P. abyssi* Trm4 [209]. Furthermore, archease promotes the ligation of tRNA exons during tRNA splicing [338,339]. Therefore, it has the potential to stabilize the tRNA structure at high temperatures.

Many tRNA-binding proteins and RNA chaperone proteins have been identified in eukaryotic cells [340,341]. Although these types of protein are unknown in thermophilic eukaryotes, some of them may stabilize the tRNA structure (or help correct folding of tRNA) at high temperatures. Recently, it was revealed that *E. coli* TruB (tRNA Ψ_{55} synthase [243]) possesses an RNA chaperone activity [342,343]. In the case of *T. thermophilus*, although the Ψ_{55} modification is required for survival at low temperatures (below 55 °C), the *truB* gene disruptant strain shows abnormal growth at 80 °C [248]. Therefore, the RNA chaperone effect of TruB may also be expressed at high temperatures in *T. thermophilus*. Furthermore, these observations suggest that other tRNA modification enzymes have the potential to work as RNA chaperones.

In general, polyamines have the potential to interact with nucleic acids and phospholipids because they possess multiple positive charges and hydrophobic areas. There are several studies on the interaction between tRNA and polyamines [344–347]. Thermophiles produce unique polyamines including long and branched polyamines [348–351]. Therefore, polyamines probably contribute to stabilize the tRNA structure at high temperatures. Furthermore, in vitro studies have shown that thermophile-specific long and branched polyamines affect the activities of several tRNA modification enzymes [81,352]. For example, TrmH from *T. thermophilus* methylates tRNA transcript at 80 °C only in the presence of long or branched polyamines [81]. Moreover, the long and branched polyamines are required for the maintenance of several tRNAs and the 70S ribosome and are essential for the survival of *T. thermophilus* at high temperatures [353].

Lastly, magnesium ions have been shown to be a tRNA stabilization factor [6,88,354] and are very important when considering the structural effects of several modified nucleosides in tRNA [58,88,354–356]. However, the precise concentration of magnesium ions in thermophile cells is unknown. It may differ depending on the growth environments. Potassium ions also function as RNA stabilization factor [88]. Notably, the interacellular concentration of some hyperthermophilic archaea

(*M. fervidus* and *P. furiosus*) is much higher (700–900 mM) than that of mesophilic archaea [357]. In the case of *Methanothermus sociabilis*, the interacellular potassium concentration reaches 1060 mM [357]. These high concentrations of potassium ions may have effects on the stability of tRNA and the activities of tRNA modification enzymes.

5. tRNA Modifications and Environmental Stresses at High Temperatures

Recent studies have revealed that the modifications in tRNA are stress-resistance and/or stress-response factors [102,358–361]. Furthermore, a high temperature itself can be a stress factor for living organisms because some modified nucleosides (D and m^7G) are liable at high temepratures [297].

5.1. Oxidative Stress

Many thermophiles can grow under aerobic conditions. For example, *Aerophyrum pernix* can grow at 100 °C under aerobic conditions. Under such conditions at high temperatures, living organisms seem to be exposed to heavy oxidative stress, which is a typical environmental stress. The amount of antioxidant enzymes such as superoxide-dismutase, catalase, and peroxidase in *Thermus filiformis*, which is an extreme-thermophilic eubacterium, increases at high temperatures [362].

Among tRNA modification enzymes, both Fe-S cluster proteins [34,130,134,142,150,173,196,236,363] for sulfur-transfer, reduction of base and/or radical S-adenosyl-l-methionine (SAM) reaction, and enzymes with catalytic cysteine residues [141,210,364–366], seem to be easily changed under oxidative stress. In some cases, the substrate (e.g., electron donors and folate derivatives [126,221,227,367]) may be unstable under aerobic conditions at high temperatures. Similarly, several modified nucleosides such as D and s^4U may be labile under oxidative stress at high temperatures. Therefore, aerobic thermophiles need to protect their cellular components from oxidative stress and their tRNA modifications may respond to such stress as in mesophiles. Overall, however, the relationship between oxidative stress and tRNA modifications in thermopiles is unclear. In addition, tRNA modification systems in some thermophiles may utilize aerobic conditions at high temperatures. For example, *A. aeolicus* grows under microaerophilic conditions at high temperatures (80–94 °C) and the dimer structure of *A. aeolicus* TrmD is stabilized by inter-subunit disulfide bonds [165].

5.2. Other Environmental Stresses

Thermophiles often live in severe environments such as extreme pH and high pressure in addition to high temperatures. These environmental stresses may give rise to the diversity of tRNA modifications. At present, however, there are no data to support this viewpoint.

UV-stress is one such environmental stress and the s^4U modification in tRNA is a known UV-stress-resistance factor for *E. coli* [368] and *Salmonella typhimurium* [27]. Thus, the s^4U modification in tRNA is likely to work similarly to a UV-resistant factor in thermophiles. Interestingly, the genomes of *Archaeoglobus fulgidus* and *Methanocaldococcus janaschii*, which were isolated from the oil mines under the sea and deep sea, respectively, contain a *thiI* genes [369] (AF_RS04455 and MJ_RS04985, respectively) encoding tRNA s^4U_8 synthetase. Since sunlight does not reach the environments in which these thermophilic archaea live, the s^4U modification and/or ThiI may have an additional function (e.g., sulfur-metabolism) in these archaea. Furthermore, it was recently reported that the melting temperature of tRNA from an *E. coli thiI*-gene disruptant strain was decreased relative to the wild-type strain [33]. Therefore, the s^4U_8 modification may contribute to stabilize tRNA structure. Furthermore, UV-stress may have an effect on other tRNA modifications via the cross-linking of s^4U in tRNA. For example, the methylation speed of *T. thermophilus* TrmH is decreased when the substrate tRNA is cross-linked [30].

Lastly, the availability of nutrient-factors may have an effect on tRNA modifications in thermophiles. To test this idea, the extent of modifications in tRNA from *T. thermophilus* cells cultured in a nutrient-poor condition was investigated [227]. Contrary to expectation, the extent of the modification of all methylated nucleosides analyzed was normal, which demonstrates that the limited nutrients

were preferentially consumed in the tRNA modification systems [227]. Thus, the findings indicated the importance of tRNA modifications for the survival of *T. thermophilus*.

6. Utilization of tRNA Modification Enzymes from Thermophiles

Given that proteins from thermophiles are heat-resistant and very stable, numerous tRNA modification enzymes have been used in biochemical and structural studies (Tables 1 and 2). In particular, crystal structural studies of thermostable enzymes provided significant information on catalytic mechanisms and RNA-protein interactions. Studies on the crystal structures of tRNA modification enzymes from thermophiles are summarized in Supplementary Table S2. It is anticipated that thermostable proteins will continue to contribute structural studies in the future. Thermostable tRNA modification enzymes can be a tool for molecular and cell biology. For example, *A. fulgidus* TiaS with agmatine analogues has been used for site-specific RNA-labeling in mammalian cells [315]. In addition, thermostable tRNA modification enzymes may be used for healthcare. For example, Gm_{18} modification in tRNA does not stimulate the Toll-like receptor 7 [287,288] and tRNA with Gm_{18} alleviates inflammation [288]. Since TrmH from *T. thermophilus* can methylate all tRNA species [72] and is very stable, it may be useful for preparing tRNAs with Gm_{18} modifications for tRNA therapy.

7. Perspective

Given that the temperature of ancient Earth was very high relative to that of present-day Earth, thermophiles may be remnants of ancient living organisms. Therefore, studies on tRNA modification enzymes and modified nucleosides in tRNA from thermophiles will contribute to the considerations of the evolutionary pathways of living organisms. Furthermore, such studies will continue to shed light on the variety and environmental adaptations of living organisms. Moreover, as outlined above, the thermostable enzymes may be useful as biotechnological and medical tools and may contribute toward the production of valuable materials.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2607/6/4/110/s1, Table S1: Abbreviations of modified nucleosides, Table S2: Crystal structural studies on tRNA modification enzymes from thermophiles.

Author Contributions: All authors determined the concept of this review and collected the information from the references. H.H. wrote the manuscript and all authors revised and approved the manuscript.

Funding: This work was supported by a Grant-in-Aid for Scientific Research (16H04763 to H.H.) from the Japan Society for the Promotion of Science (JSPS).

Acknowledgments: We dedicate this review to Kimitsuna Watanabe who died in 2016. He discovered the $m^5s^2U_{54}$ modification in tRNA and encouraged our studies. We thank previous collaborators.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Crick, F.H. On protein synthesis. *Symp. Soc. Exp. Biol.* **1958**, *12*, 138–163. [PubMed]
2. Grunberger, D.; Weinstein, I.B.; Jacobson, K.B. Codon recognition by enzymatically mischarged valine transfer ribonucleic acid. *Science* **1969**, *166*, 1635–1637. [CrossRef] [PubMed]
3. Sprinzl, M.; Horn, C.; Brown, M.; Ioudovitch, A.; Steinberg, S. Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res.* **1998**, *26*, 148–153. [CrossRef] [PubMed]
4. Robertus, J.D.; Ladner, J.E.; Finch, J.T.; Rhodes, D.; Brown, R.S.; Clark, B.F.C.; Klug, A. Structure of yeast phenylalanine tRNA at 3 Å resolution. *Nature* **1974**, *250*, 546–551. [CrossRef] [PubMed]
5. Kim, S.H.; Suddath, F.L.; Quigley, G.J.; McPherson, A.; Sussan, J.L.; Wang, A.H.J.; Seeman, N.C.; Rich, A. Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. *Science* **1974**, *185*, 435–440. [CrossRef] [PubMed]
6. Shi, H.; Moore, P.B. The crystal structure of yeast phenylalanine tRNA at 1.93 A resolution: A classic structure revisited. *RNA* **2000**, *6*, 1091–1105. [CrossRef] [PubMed]

7. Boccaletto, P.; Machnicka, M.A.; Purta, E.; Piatkowski, P.; Baginski, B.; Wirecki, T.K.; de Crécy-Lagard, V.; Ross, R.; Limbach, P.A.; Kotter, A.; et al. MODOMICS: A database of RNA modification pathways. 2017 update. *Nucleic Acids Res* **2018**, *46*, D303–D307. [CrossRef] [PubMed]
8. Juhling, F.; Morl, M.; Hartmann, R.K.; Sprinzl, M.; Stadler, P.F.; Putz, J. tRNAdb 2009: Compilation of tRNA sequences and tRNA genes. *Nucleic Acids Res.* **2009**, *37*, D159–D162. [CrossRef] [PubMed]
9. Constantinesco, F.; Motorin, Y.; Grosjean, H. Transfer RNA modification enzymes from *Pyrococcus furiosus*: Detection of the enzymatic activities in vitro. *Nucleic Acids Res.* **1999**, *27*, 1308–1315. [CrossRef] [PubMed]
10. Grawunder, U.; Schön, A.; Sprintzl, M. Sequence and base modifications of two phenylalanine-tRNAs from *Thermus thermophilus* HB8. *Nucleic Acids Res.* **1992**, *20*, 137. [CrossRef] [PubMed]
11. Tomikawa, C.; Yokogawa, T.; Kanai, T.; Hori, H. N^7-Methylguanine at position 46 (m^7G46) in tRNA from *Thermus thermophilus* is required for cell viability through a tRNA modification network. *Nucleic Acids Res.* **2010**, *38*, 942–957. [CrossRef] [PubMed]
12. Menezes, S.; Gaston, K.W.; Krivos, K.L.; Apolinario, E.E.; Reich, N.O.; Sowers, K.R.; Limbach, P.A.; Perona, J.J. Formation of m^2G6 in *Methanocaldococcus jannaschii* tRNA catalyzed by the novel methyltransferase Trm14. *Nucleic Acids Res.* **2011**, *39*, 7641–7655. [CrossRef] [PubMed]
13. Fislage, M.; Roovers, M.; Münnich, S.; Droogmans, L.; Versées, W. Crystallization and preliminary X-ray crystallographic analysis of putative tRNA-modification enzymes from *Pyrococcus furiosus* and *Thermus thermophilus*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2011**, *67*, 1432–1435. [CrossRef] [PubMed]
14. Roovers, M.; Oudjama, Y.; Fislage, M.; Bujnicki, J.M.; Versées, W.; Droogmans, L. The open reading frame TTC1157 of *Thermus thermophilus* HB27 encodes the methyltransferase forming N^2-methylguanosine at position 6 in tRNA. *RNA* **2012**, *18*, 815–824. [CrossRef] [PubMed]
15. Fislage, M.; Roovers, M.; Tuszynska, I.; Bujnicki, J.M.; Droogmans, L.; Versées, W. Crystal structures of the tRNA:m^2G6 methyltransferase Trm14/TrmN from two domains of life. *Nucleic Acids Res.* **2012**, *40*, 5149–5161. [CrossRef] [PubMed]
16. Randau, L.; Stanley, B.J.; Kohlway, A.; Mechta, S.; Xiong, Y.; Söll, D. A cytidine deaminase edits C to U in transfer RNAs in Archaea. *Science* **2009**, *324*, 657–659. [CrossRef] [PubMed]
17. Su, A.A.; Tripp, V.; Randau, L. RNA-Seq analyses reveal the order of tRNA processing events and the maturation of C/D box and CRISPR RNAs in the hyperthermophile *Methanopyrus kandleri*. *Nucleic Acids Res.* **2013**, *41*, 6250–6258. [CrossRef] [PubMed]
18. Pixa, G.; Dirheimer, G.; Keith, G. Sequence of $tRNA^{Leu}$ CmAA from *Bacillus stearothermophilus*. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 578–585. [CrossRef]
19. Keith, G.; Guerrier-Takada, C.; Grosjean, H.; Dirheimer, G. A revised sequence for *Bacillus stearothermophilus* phenylalanine tRNA. *FEBS Lett.* **1977**, *84*, 241–243. [CrossRef]
20. Brown, R.S.; Rubin, J.R.; Rhodes, D.; Guilley, H.; Simoncsits, A.; Brownlee, G.G. The nucleoside sequence of tyrosine tRNA from *Bacillus stearothermophilus*. *Nucleic Acids Res.* **1978**, *5*, 23–36. [CrossRef] [PubMed]
21. Watanabe, K.; Kuchino, Y.; Yamaizumi, Z.; Kato, M.; Oshima, T.; Nishimura, S. Nucleotide sequence of formylmethionine tRNA from an extreme thermophile, *Thermus thermophilus* HB8. *J. Biochem.* **1979**, *86*, 893–905. [CrossRef] [PubMed]
22. Watanabe, K.; Oshima, T.; Hansske, F.; Ohta, T. Separation and comparison of 2-thioribothymidine-containing transfer ribonucleic acid and the ribothymidine-containing counterpart from cells of *Thermus thermophilus* HB 8. *Biochemistry* **1983**, *22*, 98–102. [CrossRef] [PubMed]
23. Keith, G.; Yusupov, M.; Briand, C.; Moras, D.; Kern, D.; Brion, C. Sequence of $tRNA^{Asp}$ from *Thermus thermophilus* HB8. *Nucleic Acids Res.* **1993**, *21*, 4399. [CrossRef] [PubMed]
24. Awai, T.; Kimura, S.; Tomikawa, C.; Ochi, A.; Ihsanawati, I.S.; Bessho, Y.; Yokoyama, S.; Ohno, S.; Nishikawa, K.; Yokogawa, T.; et al. *Aquifex aeolicus* tRNA (N^2, N^2-guanine)-dimethyltransferase (Trm1) catalyzes transfer of methyl groups not only to guanine 26 but also to guanine 27 in tRNA. *J. Biol. Chem.* **2009**, *284*, 20467–20478. [CrossRef] [PubMed]
25. Kilpatrick, M.W.; Walker, R.T. The nucleotide sequence of the $tRNA_M^{Met}$ from the archaebacterium *Thermoplasma acidophilum*. *Nucleic Acids Res.* **1981**, *9*, 4387–4390. [CrossRef] [PubMed]
26. Caldeira de Araujo, A.; Favre, A. Induction of size reduction in *Escherichia coli* by near-ultraviolet light. *Eur. J. Biochem.* **1985**, *146*, 605–610. [CrossRef] [PubMed]

27. Kramer, G.F.; Baker, J.C.; Ames, B.N. Near-UV stress in *Salmonella typhimurium*: 4-thiouridine in tRNA, ppGpp, and ApppGpp as components of an adaptive response. *J. Bacteriol.* **1988**, *170*, 2344–2351. [CrossRef] [PubMed]
28. Mueller, E.G.; Buck, C.J.; Palenchar, P.M.; Barnhart, L.E.; Paulson, J.L. Identification of a gene involved in the generation of 4-thiouridine in tRNA. *Nucleic Acids Res.* **1998**, *26*, 2060–2610. [CrossRef]
29. Kambampati, R.; Lauhon, C.T. IscS is a sulfurtransferase for the in vitro biosynthesis of 4-thiouridine in *Escherichia coli* tRNA. *Biochemistry* **1999**, *38*, 16561–16568. [CrossRef] [PubMed]
30. Hori, H.; Saneyoshi, M.; Kumagai, I.; Miura, K.; Watanabe, K. Effects of modification of 4-thiouridine in *E. coli* $tRNA^{Met}_{f}$ on its methyl acceptor activity by thermostable Gm-methylases. *J. Biochem.* **1989**, *106*, 798–802. [CrossRef] [PubMed]
31. Naumann, P.T.; Lauhon, C.T.; Ficner, R. Purification, crystallization and preliminary crystallographic analysis of a 4-thiouridine synthetase-RNA complex. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2013**, *69*, 421–424. [CrossRef] [PubMed]
32. Neumann, P.; Lakomek, K.; Naumann, P.T.; Erwin, W.M.; Lauhon, C.T.; Ficner, R. Crystal structure of a 4-thiouridine synthetase-RNA complex reveals specificity of tRNA U8 modification. *Nucleic Acids Res.* **2014**, *42*, 6673–6685. [CrossRef] [PubMed]
33. Nomura, Y.; Ohno, S.; Nishikawa, K.; Yokogawa, T. Correlation between the stability of tRNA tertiary structure and the catalytic efficiency of a tRNA-modifying enzyme, archaeal tRNA-guanine transglycosylase. *Genes Cells* **2016**, *21*, 41–52. [CrossRef] [PubMed]
34. Liu, Y.; Vinyard, D.J.; Reesbeck, M.E.; Suzuki, T.; Manakongtreecheep, K.; Holland, P.L.; Brudvig, G.W.; Söll, D. A [3Fe-4S] cluster is required for tRNA thiolation in archaea and eukaryotes. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 12703–12708. [CrossRef] [PubMed]
35. Čavužić, M.; Liu, Y. Biosynthesis of sulfur-containing tRNA modifications: A comparison of bacterial, archaeal, and eukaryotic pathways. *Biomolecules* **2017**, *7*, 27. [CrossRef] [PubMed]
36. 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*, 3537–3580. [CrossRef] [PubMed]
37. Kempenaers, M.; Roovers, M.; Oudjama, Y.; Tkaczuk, K.L.; Bujnicki, J.M.; Droogmans, L. New archaeal methyltransferases forming 1-methyladenosine or 1-methyladenosine and 1-methylguanosine at position 9 of tRNA. *Nucleic Acids Res.* **2010**, *38*, 6533–6543. [CrossRef] [PubMed]
38. Van Laer, B.; Roovers, M.; Wauters, L.; Kasprzak, J.M.; Dyzma, M.; Deyaert, E.; Kumar Singh, R.; Feller, A.; Bujnicki, J.M.; Droogmans, L.; et al. Structural and functional insights into tRNA binding and adenosine N^1-methylation by an archaeal Trm10 homologue. *Nucleic Acids Res.* **2016**, *44*, 940–953. [CrossRef] [PubMed]
39. Singh, R.K.; Feller, A.; Roovers, M.; Van Elder, D.; Wauters, L.; Droogmans, L.; Versées, W. Structural and biochemical analysis of the dual-specificity Trm10 enzyme from *Thermococcus kodakaraensis* prompts reconsideration of its catalytic mechanism. *RNA* **2018**, *24*, 1080–1092. [CrossRef] [PubMed]
40. Armengaud, J.; Urbonavicius, J.; Fernandez, B.; Chaussinand, G.; Bujnicki, J.M.; Grosjean, H. N^2-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.* **2004**, *279*, 37142–37152. [CrossRef] [PubMed]
41. Gabant, G.; Auxilien, S.; Tuszynska, I.; Locard, M.; Gajda, M.J.; Chaussinand, G.; Fernandez, B.; Dedieu, A.; Grosjean, H.; Golinelli-Pimpaneau, B.; et al. THUMP from archaeal tRNA:$m^2{}_2$G10 methyltransferase, a genuine autonomously folding domain. *Nucleic Acids Res.* **2006**, *34*, 2483–2494. [CrossRef] [PubMed]
42. Urbonavicius, J.; Armengaud, J.; Grosjean, H. 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.* **2006**, *357*, 387–399. [CrossRef] [PubMed]
43. Hirata, A.; Nishiyama, S.; Tamura, T.; Yamauchi, A.; Hori, H. Structural and functional analyses of the archaeal tRNA $m^2G/m^2{}_2G10$ methyltransferase aTrm11 provide mechanistic insights into site specificity of a tRNA methyltransferase that contains common RNA-binding modules. *Nucleic Acids Res.* **2016**, *44*, 6377–6390. [CrossRef] [PubMed]
44. Kuchino, Y.; Ihara, M.; Yabusaki, Y.; Nishimura, S. Initiator tRNAs from archaebacteria show common unique sequence characteristics. *Nature* **1982**, *298*, 684–685. [CrossRef] [PubMed]

45. Kaya, Y.; Ofengand, J. A novel unanticipated type of pseudouridine synthase with homologs in bacteria, archaea, and eukarya. *RNA* **2003**, *9*, 711–721. [CrossRef] [PubMed]
46. 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*, 1308–1322. [CrossRef] [PubMed]
47. Kawamura, T.; Hirata, A.; Ohno, S.; Nomura, Y.; Nagano, T.; Nameki, N.; Yokogawa, T.; Hori, H. Multisite-specific archaeosine tRNA-guanine transglycosylase (ArcTGT) from *Thermoplasma acidophilum*, a thermo-acidophilic archaeon. *Nucleic Acids Res.* **2016**, *44*, 1894–1908. [CrossRef] [PubMed]
48. Gupta, R. *Halobacterium volcanii* tRNAs. Identification of 41 tRNAs covering all amino acids, and the sequences of 33 class I tRNAs. *J. Biol. Chem.* **1984**, *259*, 9461–9471. [PubMed]
49. Gregson, J.M.; Crain, P.F.; Edmonds, C.G.; Gupta, R.; Hashizume, T.; Phillipson, D.W.; McCloskey, J.A. Structure of the archaeal transfer RNA nucleoside G*-15 (2-amino-4,7-dihydro-4-oxo-7-beta-D-ribofuranosyl-1H-pyrrolo[2,3-d]pyrimidine-5-carboximi dam ide (archaeosine)). *J. Biol. Chem.* **1993**, *268*, 10076–10086. [PubMed]
50. Watanabe, M.; Matsuo, M.; Tanaka, S.; Akimoto, H.; Asahi, S.; Nishimura, S.; Katze, J.R.; Hashizume, T.; Crain, P.F.; McCloskey, J.A.; et al. Biosynthesis of archaeosine, a novel derivative of 7-deazaguanosine specific to archaeal tRNA, proceeds via a pathway involving base replacement on the tRNA polynucleotide chain. *J. Biol. Chem.* **1997**, *272*, 20146–20151. [CrossRef] [PubMed]
51. Bai, Y.; Fox, D.T.; Lacy, J.A.; Van Lanen, S.G.; Iwata-Reuyl, D. Hypermodification of tRNA in Thermophilic archaea. Cloning, overexpression, and characterization of tRNA-guanine transglycosylase from *Methanococcus jannaschii*. *J. Biol. Chem.* **2000**, *275*, 28731–28738. [CrossRef] [PubMed]
52. Watanabe, M.; Nameki, N.; Matsuo-Takasaki, M.; Nishimura, S.; Okada, N. tRNA recognition of tRNA-guanine transglycosylase from a hyperthermophilic archaeon, *Pyrococcus horikoshii*. *J. Biol. Chem.* **2001**, *276*, 2387–2394. [CrossRef] [PubMed]
53. Ishitani, R.; Nureki, O.; Kijimoto, T.; Watanabe, M.; Kondo, H.; Nameki, N.; Okada, N.; Nishimura, S.; Yokoyama, S. Crystallization and preliminary X-ray analysis of the archaeosine tRNA-guanine transglycosylase from *Pyrococcus horikoshii*. *Acta Crystallogr. D Biol. Crystallogr.* **2001**, *57*, 1659–1662. [CrossRef] [PubMed]
54. Ishitani, R.; Nureki, O.; Fukai, S.; Kijimoto, T.; Nameki, N.; Watanabe, M.; Kondo, H.; Sekine, M.; Okada, N.; Nishimura, S.; et al. Crystal structure of archaeosine tRNA-guanine transglycosylase. *J. Mol. Biol.* **2002**, *318*, 665–677. [CrossRef]
55. Ishitani, R.; Nureki, O.; Fukai, S.; Kijimoto, T.; Nameki, N.; Watanabe, M.; Kondo, H.; Sekine, M.; Okada, N.; Nishimura, S.; et al. Alternative tertiary structure of tRNA for recognition by a posttranscriptional modification enzyme. *Cell* **2003**, *113*, 383–394. [CrossRef]
56. Noon, K.R.; Guymon, R.; Crain, P.F.; McCloskey, J.A.; Thomm, M.; Lim, J.; Cavicchioli, R. Influence of temperature on tRNA modification in archaea: *Methanococcoides burtonii* (optimum growth temperature [Topt], 23 degrees C) and *Stetteria hydrogenophila* (Topt, 95 degrees C). *J. Bacteriol.* **2003**, *185*, 5483–5490. [CrossRef] [PubMed]
57. Sabina, J.; Söll, D. The RNA-binding PUA domain of archaeal tRNA-guanine transglycosylase is not required for archaeosine formation. *J. Biol. Chem.* **2006**, *281*, 6993–7001. [CrossRef] [PubMed]
58. Oliva, R.; Tramontano, A.; Cavallo, L. Mg^{2+} binding and archaeosine modification stabilize the G15 C48 Levitt base pair in tRNAs. *RNA* **2007**, *13*, 1427–1436. [CrossRef] [PubMed]
59. Phillips, G.; Chikwana, V.M.; Maxwell, A.; El-Yacoubi, B.; Swairjo, M.A.; Iwata-Reuyl, D.; de Crécy-Lagard, V. Discovery and characterization of an amidinotransferase involved in the modification of archaeal tRNA. *J. Biol. Chem.* **2010**, *285*, 12706–12713. [CrossRef] [PubMed]
60. Phillips, G.; Swairjo, M.A.; Gaston, K.W.; Bailly, M.; Limbach, P.A.; Iwata-Reuyl, D.; de Crécy-Lagard, V. Diversity of archaeosine synthesis in crenarchaeota. *ACS Chem. Biol.* **2012**, *7*, 300–305. [CrossRef] [PubMed]
61. Nomura, Y.; Onda, Y.; Ohno, S.; Taniguchi, H.; Ando, K.; Oka, N.; Nishikawa, K.; Yokogawa, T. Purification and comparison of native and recombinant tRNA-guanine transglycosylases from Methanosarcina acetivorans. *Protein Expr. Purif.* **2013**, *88*, 13–19. [CrossRef] [PubMed]
62. Mei, X.; Alvarez, J.; Bon Ramos, A.; Samanta, U.; Iwata-Reuyl, D.; Swairjo, M.A. Crystal structure of the archaeosine synthase QueF-like-Insights into amidino transfer and tRNA recognition by the tunnel fold. *Proteins* **2017**, *85*, 103–116. [CrossRef] [PubMed]

63. Bon Ramos, A.; Bao, L.; Turner, B.; de Crécy-Lagard, V.; Iwata-Reuyl, D. QueF-Like, a Non-homologous archaeosine synthase from the crenarchaeota. *Biomolecules* **2017**, *7*, 36. [CrossRef] [PubMed]
64. Takada-Guerrier, C.; Grosjean, H.; Dirheimer, G.; Keith, G. The primary structure of $tRNA_2^{Val}$ from *Bacillus stearothermophilus*. *FEBS Lett.* **1976**, *62*, 1–3. [CrossRef]
65. Bishop, A.C.; Xu, J.; Johnson, R.C.; Schimmel, P.; de Crécy-Lagard, V. Identification of the tRNA-dihydrouridine synthase family. *J. Biol. Chem.* **2002**, *277*, 25090–25095. [CrossRef] [PubMed]
66. Bou-Nader, C.; Montémont, H.; Guérineau, V.; Jean-Jean, O.; Brégeon, D.; Hamdane, D. Unveiling structural and functional divergences of bacterial tRNA dihydrouridine synthases: Perspectives on the evolution scenario. *Nucleic Acids Res.* **2018**, *46*, 1386–1394. [CrossRef] [PubMed]
67. Horie, N.; Hara-Yokoyama, M.; Yokoyama, S.; Watanabe, K.; Kuchino, Y.; Nishimura, S.; Miyazawa, T. Two $tRNA^{Ile}1$ species from an extreme thermophile, *Thermus thermophilus* HB8: Effect of 2-thiolation of ribothymidine on the thermostability of tRNA. *Biochemistry* **1985**, *24*, 5711–5715. [CrossRef] [PubMed]
68. Persson, B.C.; Jäger, G.; Gustafsson, C. The spoU gene of *Escherichia coli*, the fourth gene of the spoT operon, is essential for tRNA (Gm18) 2′-*O*-methyltransferase activity. *Nucleic Acids Res.* **1997**, *25*, 4093–4097. [CrossRef] [PubMed]
69. Kumagai, I.; Watanabe, K.; Oshima, T. A thermostable tRNA (guanosine-2′)-methyltransferase from *Thermus. thermophilus* HB27 and the effect of ribose methylation on the conformational stability of tRNA. *J. Biol. Chem.* **1982**, *257*, 7388–7395. [PubMed]
70. Matsumoto, T.; Ohta, T.; Kumagai, I.; Oshima, T.; Murao, K.; Hasegawa, T.; Ishikura, H.; Watanabe, K. A thermostable Gm-methylase recognizes the tertiary structure of tRNA. *J. Biochem.* **1987**, *101*, 1191–1198. [CrossRef] [PubMed]
71. Matsumoto, T.; Nishikawa, K.; Hori, H.; Ohta, T.; Miura, K.; Watanabe, K. Recognition sites of tRNA by a thermostable tRNA(guanosine-2′-)-methyltransferase from *Thermus thermophilus* HB27. *J. Biochem.* **1990**, *107*, 331–338. [CrossRef] [PubMed]
72. Hori, H.; Yamazaki, N.; Matsumoto, T.; Watanabe, Y.; Ueda, T.; Nishikawa, K.; Kumagai, I.; Watanabe, K. Substrate recognition of tRNA (Guanosine-2′-)-methyltransferase from *Thermus thermophilus* HB27. *J. Biol. Chem.* **1998**, *273*, 25721–25727. [CrossRef] [PubMed]
73. Hori, H.; Suzuki, T.; Sugawara, K.; Inoue, Y.; Shibata, T.; Kuramitsu, S.; Yokoyama, S.; Oshima, T.; Watanabe, K. Identification and characterization of tRNA (Gm18) methyltransferase from *Thermus thermophilus* HB8: Domain structure and conserved amino acid sequence motifs. *Genes Cells* **2002**, *7*, 259–272. [CrossRef] [PubMed]
74. Hori, H.; Kubota, S.; Watanabe, K.; Kim, J.M.; Ogasawara, T.; Sawasaki, T.; Endo, Y. *Aquifex aeolicus* tRNA (Gm18) methyltransferase has unique substrate specificity: tRNA recognition mechanism of the enzyme. *J. Biol. Chem.* **2003**, *278*, 25081–25090. [CrossRef] [PubMed]
75. Nureki, O.; Watanabe, K.; Fukai, S.; Ishii, R.; Endo, Y.; Hori, H.; Yokoyama, S. Deep knot structure for construction of active site and cofactor binding site of tRNA modification enzyme. *Structure* **2004**, *12*, 593–604. [CrossRef] [PubMed]
76. Watanabe, K.; Nureki, O.; Fukai, S.; Ishii, R.; Okamoto, H.; Yokoyama, S.; Endo, Y.; Hori, H. Roles of conserved amino acid sequence motifs in the SpoU (TrmH) RNA methyltransferase family. *J. Biol. Chem.* **2005**, *280*, 10368–10377. [CrossRef] [PubMed]
77. Pleshe, E.; Truesdell, J.; Batey, R.T. Structure of a class II TrmH tRNA-modifying enzyme from *Aquifex aeolicus*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2005**, *61*, 722–728. [CrossRef] [PubMed]
78. Watanabe, K.; Nureki, O.; Fukai, S.; Endo, Y.; Hori, H. Functional categorization of the conserved basic amino acid residues in TrmH (tRNA (Gm18) methyltransferase) enzymes. *J. Biol. Chem.* **2006**, *281*, 34630–34639. [CrossRef] [PubMed]
79. Ochi, A.; Makabe, K.; Kuwajima, K.; Hori, H. Flexible recognition of the tRNA G18 methylation target site by TrmH methyltransferase through first binding and induced fit processes. *J. Biol. Chem.* **2010**, *285*, 9018–9029. [CrossRef] [PubMed]
80. Ochi, A.; Makabe, K.; Yamagami, R.; Hirata, A.; Sakaguchi, R.; Hou, Y.M.; Watanabe, K.; Nureki, O.; Kuwajima, K.; Hori, H. The catalytic domain of topological knot tRNA methyltransferase (TrmH) discriminates between substrate tRNA and nonsubstrate tRNA via an induced-fit process. *J. Biol. Chem.* **2013**, *288*, 25562–25574. [CrossRef] [PubMed]

81. Hori, H.; Terui, Y.; Nakamoto, C.; Iwashita, C.; Ochi, A.; Watanabe, K.; Oshima, T. Effects of polyamines from *Thermus thermophilus*, an extreme-thermophilic eubacterium, on tRNA methylation by tRNA (Gm18) methyltransferase (TrmH). *J. Biochem.* **2016**, *159*, 509–517. [CrossRef] [PubMed]
82. Savage, D.F.; de Crécy-Lagard, V.; Bishop, A.C. Molecular determinants of dihydrouridine synthase activity. *FEBS Lett.* **2006**, *580*, 5198–5202. [CrossRef] [PubMed]
83. Yu, F.; Tanaka, Y.; Yamamoto, S.; Nakamura, A.; Kita, S.; Hirano, N.; Tanaka, I.; Yao, M. Crystallization and preliminary X-ray crystallographic analysis of dihydrouridine synthase from *Thermus thermophilus* and its complex with tRNA. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2011**, *67*, 685–688. [CrossRef] [PubMed]
84. Yu, F.; Tanaka, Y.; Yamashita, K.; Suzuki, T.; Nakamura, A.; Hirano, N.; Suzuki, T.; Yao, M.; Tanaka, I. Molecular basis of dihydrouridine formation on tRNA. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 19593–19598. [CrossRef] [PubMed]
85. Kusuba, H.; Yoshida, T.; Iwasaki, E.; Awai, T.; Kazayama, A.; Hirata, A.; Tomikawa, C.; Yamagami, R.; Hori, H. In vitro dihydrouridine formation by tRNA dihydrouridine synthase from *Thermus thermophilus*, an extreme-thermophilic eubacterium. *J. Biochem.* **2015**, *158*, 513–521. [PubMed]
86. Roovers, M.; Kaminska, K.H.; Tkaczuk, K.L.; Gigot, D.; Droogmans, L.; Bujnicki, J.M. The YqfN protein of *Bacillus subtilis* is the tRNA: M^1A22 methyltransferase (TrmK). *Nucleic Acids Res.* **2008**, *36*, 3252–3262. [CrossRef] [PubMed]
87. Blaby, I.K.; Majumder, M.; Chatterjee, K.; Jana, S.; Grosjean, H.; de Crécy-Lagard, V.; Gupta, R. Pseudouridine formation in archaeal RNAs: The case of *Haloferax volcanii*. *RNA* **2011**, *17*, 1367–1380. [CrossRef] [PubMed]
88. Lorenz, C.; Lünse, C.E.; Mörl, M. tRNA modifications: Impact on structure and thermal adaptation. *Biomolecules* **2017**, *7*, 35. [CrossRef] [PubMed]
89. Kawamura, T.; Anraku, R.; Hasegawa, T.; Tomikawa, C.; Hori, H. Transfer RNA methyltransferases from *Thermoplasma acidophilum*, a thermoacidophilic archaeon. *Int. J. Mol. Sci.* **2014**, *16*, 91–113. [CrossRef] [PubMed]
90. Phillips, J.H.; Kjellin-Straby, K. Studies on microbial ribonucleic acid. IV. Two mutants of *Saccharomyces cerevisiae* lacking N-2-dimethylguanine in soluble ribonucleic acid. *J. Mol. Biol.* **1967**, *26*, 509–518. [CrossRef]
91. Constantinesco, F.; Benachenhou, N.; Motorin, Y.; Grosjean, H. The tRNA(guanine-26,N^2-N^2) methyltransferase (Trm1) from the hyperthermophilic archaeon *Pyrococcus furiosus*: Cloning, sequencing of the gene and its expression in *Escherichia coli*. *Nucleic Acids Res.* **1998**, *26*, 3753–3761. [CrossRef] [PubMed]
92. Constantinesco, F.; Motorin, Y.; Grosjean, H. Characterisation and enzymatic properties of tRNA (guanine 26, N (2), N (2))-dimethyltransferase (Trm1p) from *Pyrococcus furiosus*. *J. Mol. Biol.* **1999**, *291*, 375–392. [CrossRef] [PubMed]
93. Ihsanawati; Nishimoto, M.; Higashijima, K.; Shirouzu, M.; Grosjean, H.; Bessho, Y.; Yokoyama, S. Crystal structure of tRNA N^2,N^2-guanosine dimethyltransferase Trm1 from *Pyrococcus horikoshii*. *J. Mol. Biol.* **2008**, *383*, 871–884. [CrossRef] [PubMed]
94. Vakiloroayaei, A.; Shah, N.S.; Oeffinger, M.; Bayfield, M.A. The RNA chaperone La promotes pre-tRNA maturation via indiscriminate binding of both native and misfolded targets. *Nucleic Acids Res.* **2017**, *45*, 11341–11355. [CrossRef] [PubMed]
95. Awai, T.; Ochi, A.; Ihsanawati; Sengoku, T.; Hirata, A.; Bessho, Y.; Yokoyama, S.; Hori, H. Substrate tRNA recognition mechanism of a multisite-specific tRNA methyltransferase, *Aquifex aeolicus* Trm1, based on the X-ray crystal structure. *J. Biol. Chem.* **2011**, *286*, 35236–35246. [CrossRef] [PubMed]
96. Somme, J.; Van Laer, B.; Roovers, M.; Steyaert, J.; Versées, W.; Droogmans, L. Characterization of two homologous 2′-*O*-methyltransferases showing different specificities for their tRNA substrates. *RNA* **2014**, *20*, 1257–1271. [CrossRef] [PubMed]
97. Edmonds, C.G.; Crain, P.F.; Gupta, R.; Hashizume, T.; Hocart, C.H.; Kowalak, J.A.; Pomerantz, S.C.; Stetter, K.O.; McCloskey, J.A. Posttranscriptional modification of tRNA in *Thermophilic archaea* (Archaebacteria). *J. Bacteriol.* **1991**, *173*, 3138–3148. [CrossRef] [PubMed]
98. Kowalak, J.A.; Dalluge, J.J.; McCloskey, J.A.; Stetter, K.O. The role of posttranscriptional modification in stabilization of transfer RNA from *Hyperthermophiles*. *Biochemistry* **1994**, *33*, 7869–7876. [CrossRef] [PubMed]

99. McCloskey, J.A.; Graham, D.E.; Zou, S.; Crain, P.F.; Ibba, M.; Konisky, J.; Soll, D.; Olsen, G.J. Post-transcriptional modification in archaeal tRNAs: Identities and phylogenetic relations of nucleotides from mesophilic and hyperthermophilic Methanococcales. *Nucleic Acids Res.* **2001**, *29*, 4299–4706. [CrossRef]
100. Sauerwald, A.; Sitaramaiah, D.; McCloskey, J.A.; Söll, D.; Crain, P.F. N^6-Acetyladenosine: A new modified nucleoside from *Methanopyrus kandleri* tRNA. *FEBS Lett.* **2005**, *579*, 2807–2810. [CrossRef] [PubMed]
101. Purta, E.; van Vliet, F.; Tkaczuk, K.L.; Dunin-Horkawicz, S.; Mori, H.; Droogmans, L.; Bujnicki, J.M. The *yfhQ* gene of *Escherichia coli* encodes a tRNA: Cm32/Um32 methyltransferase. *BMC Mol. Biol.* **2006**, *7*, 23. [CrossRef] [PubMed]
102. Jaroensuk, J.; Atichartpongkul, S.; Chionh, Y.H.; Wong, Y.H.; Liew, C.W.; McBee, M.E.; Thongdee, N.; Prestwich, E.G.; DeMott, M.S.; Mongkolsuk, S.; et al. Methylation at position 32 of tRNA catalyzed by TrmJ alters oxidative stress response in *Pseudomonas aeruginosa*. *Nucleic Acids Res.* **2016**, *44*, 10834–10848. [CrossRef] [PubMed]
103. Wolf, J.; Gerber, A.P.; Keller, W. *tadA*, an essential tRNA-specific adenosine deaminase from *Escherichia coli*. *EMBO J.* **2002**, *21*, 3841–3851. [CrossRef] [PubMed]
104. Elias, Y.; Huang, R.H. Biochemical and structural studies of A-to-I editing by tRNA: A34 deaminases at the wobble position of transfer RNA. *Biochemistry* **2005**, *44*, 12057–12065. [CrossRef] [PubMed]
105. 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*, 16002–16008. [CrossRef] [PubMed]
106. Yokobori, S.; Kitamura, A.; Grosjean, H.; Bessho, Y. Life without tRNAArg-adenosine deaminase TadA: Evolutionary consequences of decoding the four CGN codons as arginine in Mycoplasmas and other Mollicutes. *Nucleic Acids Res.* **2013**, *41*, 6531–6543. [CrossRef] [PubMed]
107. Torres, A.G.; Piñeyro, D.; Filonava, L.; Stracker, T.H.; Batlle, E.; Ribas de Pouplana, L. A-to-I editing on tRNAs: Biochemical, biological and evolutionary implications. *FEBS Lett.* **2014**, *588*, 4279–4286. [CrossRef] [PubMed]
108. Muramatsu, T.; Yokoyama, S.; Horie, N.; Matsuda, A.; Ueda, T.; Yamaizumi, Z.; Kuchino, Y.; Nishimura, S.; Miyazawa, T. A novel lysine-substituted nucleoside in the first position of the anticodon of minor isoleucine tRNA from *Escherichia coli*. *J. Biol. Chem.* **1988**, *263*, 9261–9267. [PubMed]
109. Muramatsu, T.; Nishikawa, K.; Nemoto, F.; Kuchino, Y.; Nishimura, S.; Miyazawa, T.; Muramatsu, T.; Nishikawa, K.; Nemoto, F.; Kuchino, Y.; et al. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. *Nature* **1988**, *336*, 179–181. [CrossRef] [PubMed]
110. Soma, A.; Ikeuchi, Y.; Kanemasa, S.; Kobayashi, K.; Ogasawara, N.; Ote, T.; Kato, J.; Watanabe, K.; Sekine, Y.; Suzuki, T. An RNA-modifying enzyme that governs both the codon and amino acid specificities of isoleucine tRNA. *Mol. Cell* **2003**, *12*, 689–698. [CrossRef]
111. Nakanishi, K.; Fukai, S.; Ikeuchi, Y.; Soma, A.; Sekine, Y.; Suzuki, T.; Nureki, O. Structural basis for lysidine formation by ATP pyrophosphatase accompanied by a lysine-specific loop and a tRNA-recognition domain. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7487–7492. [CrossRef] [PubMed]
112. Kuratani, M.; Yoshikawa, Y.; Bessho, Y.; Higashijima, K.; Ishii, T.; Shibata, R.; Takahashi, S.; Yutani, K.; Yokoyama, S. Structural basis of the initial binding of tRNA(Ile) lysidine synthetase TilS with ATP and L-lysine. *Structure* **2007**, *15*, 1642–1653. [CrossRef] [PubMed]
113. Nakanishi, K.; Bonnefond, L.; Kimura, S.; Suzuki, T.; Ishitani, R.; Nureki, O. Structural basis for translational fidelity ensured by transfer RNA lysidine synthetase. *Nature* **2009**, *461*, 1144–1148. [CrossRef] [PubMed]
114. Suzuki, T.; Numata, T. Convergent evolution of AUA decoding in bacteria and archaea. *RNA Biol.* **2014**, *11*, 1586–1596. [CrossRef] [PubMed]
115. Numata, T. Mechanisms of the tRNA wobble cytidine modification essential for AUA codon decoding in prokaryotes. *Biosci. Biotechnol. Biochem.* **2015**, *79*, 347–353. [CrossRef] [PubMed]
116. Ikeuchi, Y.; Kimura, S.; Numata, T.; Nakamura, D.; Yokogawa, T.; Ogata, T.; Wada, T.; Suzuki, T.; Suzuki, T. Agmatine-conjugated cytidine in a tRNA anticodon is essential for AUA decoding in archaea. *Nat. Chem. Biol.* **2010**, *6*, 277–282. [CrossRef] [PubMed]
117. Mandal, D.; Köhrer, C.; Su, D.; Russell, S.P.; Krivos, K.; Castleberry, C.M.; Blum, P.; Limbach, P.A.; Söll, D.; RajBhandary, U.L. Agmatidine, a modified cytidine in the anticodon of archaeal tRNA(Ile), base pairs with adenosine but not with guanosine. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 2872–2877. [CrossRef] [PubMed]

118. Terasaka, N.; Kimura, S.; Osawa, T.; Numata, T.; Suzuki, T. Biogenesis of 2-agmatinylcytidine catalyzed by the dual protein and RNA kinase TiaS. *Nat. Struct. Mol. Biol.* **2011**, *18*, 1268–1274. [CrossRef] [PubMed]
119. Osawa, T.; Kimura, S.; Terasaka, N.; Inanaga, H.; Suzuki, T.; Numata, T. Structural basis of tRNA agmatinylation essential for AUA codon decoding. *Nat. Struct. Mol. Biol.* **2011**, *18*, 1275–1280. [CrossRef] [PubMed]
120. Voorhees, R.M.; Mandal, D.; Neubauer, C.; Köhrer, C.; RajBhandary, U.L.; Ramakrishnan, V. The structural basis for specific decoding of AUA by isoleucine tRNA on the ribosome. *Nat. Struct. Mol. Biol.* **2013**, *20*, 641–643. [CrossRef] [PubMed]
121. Yamanaka, K.; Hwang, J.; Inouye, M. Characterization of GTPase activity of TrmE, a member of a novel GTPase superfamily, from *Thermotoga maritima*. *J. Bacteriol.* **2000**, *182*, 7078–7082. [CrossRef] [PubMed]
122. Urbonavicius, J.; Qian, Q.; Durand, J.M.; Hagervall, T.G.; Björk, G.R. Improvement of reading frame maintenance is a common function for several tRNA modifications. *EMBO J.* **2001**, *20*, 4863–4873. [CrossRef] [PubMed]
123. Urbonavicius, J.; Stahl, G.; Durand, J.M.; Ben Salem, S.N.; Qian, Q.; Farabaugh, P.J.; Björk, G.R. Transfer RNA modifications that alter +1 frameshifting in general fail to affect -1 frameshifting. *RNA* **2003**, *9*, 760–768. [CrossRef] [PubMed]
124. Takai, K.; Yokoyama, S. Roles of 5-substituents of tRNA wobble uridines in the recognition of purine-ending codons. *Nucleic Acids Res.* **2003**, *31*, 6383–6391. [CrossRef] [PubMed]
125. Bujnicki, J.M.; Oudjama, Y.; Roovers, M.; Owczarek, S.; Caillet, J.; Droogmans, L. Identification of a bifunctional enzyme MnmC involved in the biosynthesis of a hypermodified uridine in the wobble position of tRNA. *RNA* **2004**, *18*, 1236–1242. [CrossRef] [PubMed]
126. Armengod, M.E.; Moukadiri, I.; Prado, S.; Ruiz-Partida, R.; Benítez-Páez, A.; Villarroya, M.; Lomas, R.; Garzón, M.J.; Martínez-Zamora, A.; Meseguer, S.; Navarro-González, C. Enzymology of tRNA modification in the bacterial MnmEG pathway. *Biochimie* **2012**, *94*, 1510–1520. [CrossRef] [PubMed]
127. Moukadiri, I.; Garzón, M.J.; Björk, G.R.; Armengod, M.E. The output of the tRNA modification pathways controlled by the *Escherichia coli* MnmEG and MnmC enzymes depends on the growth conditions and the tRNA species. *Nucleic Acids Res.* **2014**, *42*, 2602–2623. [CrossRef] [PubMed]
128. Armengod, M.E.; Meseguer, S.; Villarroya, M.; Prado, S.; Moukadiri, I.; Ruiz-Partida, R.; Garzón, M.J.; Navarro-González, C.; Martínez-Zamora, A. Modification of the wobble uridine in bacterial and mitochondrial tRNAs reading NNA/NNG triplets of 2-codon boxes. *RNA Biol.* **2014**, *11*, 1495–1507. [CrossRef] [PubMed]
129. Kalhor, H.R.; Clarke, S. Novel methyltransferase for modified uridine residues at the wobble position of tRNA. *Mol. Cell Biol.* **2003**, *23*, 9283–9292. [CrossRef] [PubMed]
130. Huang, B.; Johansson, M.J.; Byström, A.S. An early step in wobble uridine tRNA modification requires the Elongator complex. *RNA* **2005**, *11*, 424–436. [CrossRef] [PubMed]
131. Begley, U.; Dyavaiah, M.; Patil, A.; Rooney, J.P.; DiRenzo, D.; Young, C.M.; Conklin, D.S.; Zitomer, R.S.; Begley, T.J. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Mol. Cell* **2007**, *28*, 860–870. [CrossRef] [PubMed]
132. Mazauric, M.H.; Dirick, L.; Purushothaman, S.K.; Björk, G.R.; 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*, 18505–18515. [CrossRef] [PubMed]
133. Chavarria, N.E.; Hwang, S.; Cao, S.; Fu, X.; Holman, M.; Elbanna, D.; Rodriguez, S.; Arrington, D.; Englert, M.; Uthandi, S.; et al. Archaeal Tuc1/Ncs6 homolog required for wobble uridine tRNA thiolation is associated with ubiquitin-proteasome, translation, and RNA processing system homologs. *PLoS ONE* **2014**, *9*, e99104. [CrossRef] [PubMed]
134. Arragain, S.; Bimai, O.; Legrand, P.; Caillat, S.; Ravanat, J.L.; Touati, N.; Binet, L.; Atta, M.; Fontecave, M.; Golinelli-Pimpaneau, B. Nonredox thiolation in tRNA occurring via sulfur activation by a [4Fe-4S] cluster. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 7355–7360. [CrossRef] [PubMed]
135. Black, K.A.; Dos Satos, P.C. Abbreviated Pathway for Biosynthesis of 2-Thiouridine in *Bacillus subtilis*. *J. Bacteriol.* **2015**, *197*, 1952–1962. [CrossRef] [PubMed]

136. Kitamura, A.; Nishimoto, M.; Sengoku, T.; Shibata, R.; Jäger, G.; Björk, G.R.; Grosjean, H.; Yokoyama, S.; Bessho, Y. Characterization and structure of the *Aquifex aeolicus* protein DUF752: A bacterial tRNA-methyltransferase (MnmC2) functioning without the usually fused oxidase domain (MnmC1). *J. Biol. Chem.* **2012**, *287*, 43950–43960. [CrossRef] [PubMed]
137. Mandal, D.; Köhrer, C.; Su, D.; Babu, I.R.; Chan, C.T.; Liu, Y.; Söll, D.; Blum, P.; Kuwahara, M.; Dedon, P.C.; Rajbhandary, U.L. Identification and codon reading properties of 5-cyanomethyl uridine, a new modified nucleoside found in the anticodon wobble position of mutant haloarchaeal isoleucine tRNAs. *RNA* **2014**, *20*, 177–188. [CrossRef] [PubMed]
138. Scrima, A.; Vetter, I.R.; Armengod, M.E.; Wittinghofer, A. The structure of the TrmE GTP-binding protein and its implications for tRNA modification. *EMBO J.* **2005**, *24*, 23–33. [CrossRef] [PubMed]
139. Scrima, A.; Wittinghofer, A. Dimerisation-dependent GTPase reaction of MnmE: How potassium acts as GTPase-activating element. *EMBO J.* **2006**, *25*, 2940–2951. [CrossRef] [PubMed]
140. Osawa, T.; Inanaga, H.; Numata, T. Crystallization and preliminary X-ray diffraction analysis of the tRNA-modification enzyme GidA from *Aquifex aeolicus*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2009**, *65*, 508–511. [CrossRef] [PubMed]
141. Osawa, T.; Ito, K.; Inanaga, H.; Nureki, O.; Tomita, K.; Numata, T. Conserved cysteine residues of GidA are essential for biogenesis of 5-carboxymethylaminomethyluridine at tRNA anticodon. *Structure* **2009**, *17*, 713–724. [CrossRef] [PubMed]
142. Selcadurai, K.; Wang, P.; Seimetz, J.; Huang, R.H. Archaeal Elp3 catalyzes tRNA wobble uridine modification at C5 via a radical mechanism. *Nat. Chem. Biol.* **2014**, *10*, 810–812. [CrossRef] [PubMed]
143. Benítez-Páez, A.; Villarroya, M.; Douthwaite, S.; Gabaldón, T.; Armengod, M.E. *YibK* is the 2′-*O*-methyltransferase TrmL that modifies the wobble nucleotide in *Escherichia coli* tRNA(Leu) isoacceptors. *RNA* **2010**, *16*, 2131–2143. [CrossRef] [PubMed]
144. Pang, P.; Deng, X.; Wang, Z.; Xie, W. Structural and biochemical insights into the 2′-*O*-methylation of pyrimidines 34 in tRNA. *FEBS J.* **2017**, *284*, 2251–2263. [CrossRef] [PubMed]
145. Okada, N.; Noguchi, S.; Nishimura, S.; Ohgi, T.; Goto, T.; Crain, P.F.; McCloskey, J.A. Structure determination of a nucleoside Q precursor isolated from *E. coli* tRNA: 7-(aminomethyl)-7-deazaguanosine. *Nucleic Acids Res.* **1978**, *5*, 2289–2296. [CrossRef] [PubMed]
146. Okada, N.; Noguchi, S.; Kasai, H.; Shindo-Okada, N.; Ohgi, T.; Goto, T.; Nishimura, S. Novel mechanism of post-transcriptional modification of tRNA. Insertion of bases of Q precursors into tRNA by a specific tRNA transglycosylase reaction. *J. Biol. Chem.* **1979**, *254*, 3067–3073. [PubMed]
147. Nakanishi, S.; Ueda, T.; Hori, H.; Yamazaki, N.; Okada, N.; Watanabe, K. A UGU sequence in the anticodon loop is a minimum requirement for recognition by Escherichia coli tRNA-guanine transglycosylase. *J. Biol. Chem.* **1994**, *269*, 32221–32225. [PubMed]
148. Slany, R.K.; Bösl, M.; Kersten, H. Transfer and isomerization of the ribose moiety of AdoMet during the biosynthesis of queuosine tRNAs, a new unique reaction catalyzed by the QueA protein from *Escherichia coli*. *Biochimie* **1994**, *76*, 389–393. [CrossRef]
149. Van Lanen, S.G.; Kinzie, S.D.; Matthieu, S.; Link, T.; Culp, J.; Iwata-Reuyl, D. tRNA modification by S-adenosylmethionine: tRNA ribosyltransferase-isomerase. Assay development and characterization of the recombinant enzyme. *J. Biol. Chem.* **2003**, *278*, 10491–10499. [CrossRef] [PubMed]
150. Miles, Z.D.; Myers, W.K.; Kincannon, W.M.; Britt, R.D.; Bandarian, V. Biochemical and Spectroscopic Studies of Epoxyqueuosine Reductase: A Novel Iron-Sulfur Cluster- and Cobalamin-Containing Protein Involved in the Biosynthesis of Queuosine. *Biochemistry* **2015**, *54*, 4927–4935. [CrossRef] [PubMed]
151. Mathews, I.; Schwarzenbacher, R.; McMullan, D.; Abdubek, P.; Ambing, E.; Axelrod, H.; Biorac, T.; Canaves, J.M.; Chiu, H.J.; Deacon, A.M.; et al. Crystal structure of S-adenosylmethionine: tRNA ribosyltransferase-isomerase (QueA) from *Thermotoga maritima* at 2.0 A resolution reveals a new fold. *Proteins* **2005**, *59*, 869–974. [CrossRef] [PubMed]
152. Vinayak, M.; Pathak, C. Queuosine modification of tRNA: Its divergent role in cellular machinery. *Biosci. Rep.* **2009**, *30*, 135–148. [CrossRef] [PubMed]
153. Hutinet, G.; Swarjo, M.A.; de Crécy-Lagard, V. Deazaguanine derivatives, examples of crosstalk between RNA and DNA modification pathways. *RNA Biol.* **2017**, *14*, 1175–1184. [CrossRef] [PubMed]

154. Clouet d'Orval, B.; Bortolin, M.L.; Gaspin, C.; Bachellerie, J.P. Box C/D RNA guides for the ribose methylation of archaeal tRNAs. The $tRNA^{Trp}$ intron guides the formation of two ribose-methylated nucleosides in the mature $tRNA^{Trp}$. *Nucleic Acids Res.* **2001**, *29*, 4518–4529. [CrossRef] [PubMed]
155. Bortolin, M.L.; Bachellerie, J.P.; Clouet-d'Orval, B. In vitro RNP assembly and methylation guide activity of an unusual box C/D RNA, cis-acting archaeal pre-$tRNA^{Trp}$. *Nucleic Acids Res.* **2003**, *31*, 6524–6535. [CrossRef] [PubMed]
156. Byström, A.S.; Björk, G.R. Chromosomal location and cloning of the gene (*trmD*) responsible for the synthesis of tRNA (m^1G) methyltransferase in *Escherichia coli* K-12. *Mol. Gen. Genet.* **1982**, *188*, 440–446. [CrossRef] [PubMed]
157. Björk, G.R.; Wikstrom, P.M.; Byström, A.S. Prevention of translational frameshifting by the modified nucleoside 1-methylguanosine. *Science* **1989**, *244*, 986–989. [CrossRef] [PubMed]
158. Farabaugh, P.J.; Björk, G.R. How translational accuracy influences reading frame maintenance. *EMBO J.* **1999**, *18*, 1427–1434. [CrossRef] [PubMed]
159. Björk, G.R.; Jacobsson, K.; Nilsson, K.; Johansson, M.J.; Byström, A.S.; Persson, O.P. A primordial tRNA modification required for the evolution of life? *EMBO J.* **2001**, *20*, 231–239. [CrossRef] [PubMed]
160. Liu, J.; Wang, W.; Shin, D.H.; Yokota, H.; Kim, R.; Kim, S.H. Crystal structure of tRNA (m^1G37) methyltransferase from *Aquifex aeolicus* at 2.6 A resolution: A novel methyltransferase fold. *Proteins* **2003**, *53*, 326–328. [CrossRef] [PubMed]
161. Christian, T.; Evilia, C.; Williams, S.; Hou, Y.M. Distinct origins of tRNA(m^1G37) methyltransferase. *J. Mol. Biol.* **2004**, *339*, 707–719. [CrossRef] [PubMed]
162. Takeda, H.; Toyooka, T.; Ikeuchi, Y.; Yokobori, S.; Okadome, K.; Takano, F.; Oshima, T.; Suzuki, T.; Endo, Y.; Hori, H. The substrate specificity of tRNA (m^1G37) methyltransferase (TrmD) from *Aquifex aeolicus*. *Genes Cells* **2006**, *11*, 1353–1365. [CrossRef] [PubMed]
163. Christian, T.; Evilia, C.; Hou, Y.M. Catalysis by the second class of tRNA(m^1G37) methyl transferase requires a conserved proline. *Biochemistry* **2006**, *45*, 7463–7473. [CrossRef] [PubMed]
164. Christian, T.; Hou, Y.M. Distinct determinants of tRNA recognition by the TrmD and Trm5 methyl transferases. *J. Mol. Biol.* **2007**, *373*, 623–632. [CrossRef] [PubMed]
165. Toyooka, T.; Awai, T.; Kanai, T.; Imanaka, T.; Hori, H. Stabilization of tRNA (m^1G37) methyltransferase [TrmD] from *Aquifex aeolicus* by an intersubunit disulfide bond formation. *Genes Cells* **2008**, *13*, 807–816. [CrossRef] [PubMed]
166. Goto-Ito, S.; Ito, T.; Ishii, R.; Muto, Y.; Bessho, Y.; Yokoyama, S. Crystal structure of archaeal tRNA(m(1)G37)methyltransferase aTrm5. *Proteins* **2008**, *72*, 1274–1289. [CrossRef] [PubMed]
167. Goto-Ito, S.; Ito, T.; Kuratani, M.; Bessho, Y.; Yokoyama, S. Tertiary structure checkpoint at anticodon loop modification in tRNA functional maturation. *Nat. Struct. Mol. Biol.* **2009**, *16*, 1109–1115. [CrossRef] [PubMed]
168. Lahoud, G.; Goto-Ito, S.; Yoshida, K.; Ito, T.; Yokoyama, S.; Hou, Y.M. Differentiating analogous tRNA methyltransferases by fragments of the methyl donor. *RNA* **2011**, *17*, 1236–1246. [CrossRef] [PubMed]
169. Sakaguchi, R.; Giessing, A.; Dai, Q.; Lahoud, G.; Liutkeviciute, Z.; Klimasauskas, S.; Piccirilli, J.; Kirpekar, F.; Hou, Y.M. Recognition of guanosine by dissimilar tRNA methyltransferases. *RNA* **2012**, *18*, 1687–1701. [CrossRef] [PubMed]
170. Christian, T.; Gamper, H.; Hou, Y.M. Conservation of structure and mechanism by Trm5 enzymes. *RNA* **2013**, *19*, 1192–1199. [CrossRef] [PubMed]
171. Ito, T.; Masuda, I.; Yoshida, K.; Goto-Ito, S.; Sekine, S.; Suh, S.W.; Hou, Y.M.; Yokoyama, S. Structural basis for methyl-donor-dependent and sequence-specific binding to tRNA substrates by knotted methyltransferase TrmD. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E4197–4205. [CrossRef] [PubMed]
172. Goto-Ito, S.; Ishii, R.; Ito, T.; Shibata, R.; Fusatomi, E.; Sekine, S.I.; Bessho, Y.; Yokoyama, S. Structure of an archaeal TYW1, the enzyme catalyzing the second step of wye-base biosynthesis. *Acta Crystallogr. D Biol. Crystallogr.* **2007**, *63*, 1059–1068. [CrossRef] [PubMed]
173. Suzuki, Y.; Noma, A.; Suzuki, T.; Senda, M.; Senda, T.; Ishitani, R.; Nureki, O. Crystal structure of the radical SAM enzyme catalyzing tricyclic modified base formation in tRNA. *J. Mol. Biol.* **2007**, *372*, 1204–1214. [CrossRef] [PubMed]

174. Umitsu, M.; Nishimasu, H.; Noma, A.; Suzuki, T.; Ishitani, R.; Nureki, O. Structural basis of AdoMet-dependent aminocarboxypropyl transfer reaction catalyzed by tRNA-wybutosine synthesizing enzyme, TYW2. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15616–15621. [CrossRef] [PubMed]
175. Perche-Letuvée, P.; Kathirvelu, V.; Berggren, G.; Clemancey, M.; Latour, J.M.; Maurel, V.; Douki, T.; Armengaud, J.; Mulliez, E.; Fontecave, M.; et al. 4-Demethylwyosine synthase from *Pyrococcus abyssi* is a radical-S-adenosyl-L-methionine enzyme with an additional [4Fe-4S](+2) cluster that interacts with the pyruvate co-substrate. *J. Biol. Chem.* **2012**, *287*, 41174–41185. [CrossRef] [PubMed]
176. Urbonavičius, J.; Rutkienė, R.; Lopato, A.; Tauraitė, D.; Stankevičiūtė, J.; Aučynaitėm, A.; Kaliniene, L.; van Tilbeurgh, H.; Meškys, R. Evolution of $tRNA^{Phe}$: imG2 methyltransferases involved in the biosynthesis of wyosine derivatives in Archaea. *RNA* **2016**, *22*, 1871–1883.
177. Wang, C.; Jia, Q.; Chen, R.; Wei, Y.; Li, J.; Ma, J.; Xie, W. Crystal structures of the bifunctional tRNA methyltransferase Trm5a. *Sci. Rep.* **2016**, *6*, 33553. [CrossRef] [PubMed]
178. Currie, M.A.; Brown, G.; Wong, A.; Ohira, T.; Sugiyama, K.; Suzuki, T.; Yakunin, A.F.; Jia, Z. Structural and functional characterization of the TYW3/Taw3 class of SAM-dependent methyltransferases. *RNA* **2017**, *23*, 346–354. [CrossRef] [PubMed]
179. Wang, C.; Jia, Q.; Zeng, J.; Chen, R.; Xie, W. Structural insight into the methyltransfer mechanism of the bifunctional Trm5. *Sci. Adv.* **2017**, *3*, e1700195. [CrossRef] [PubMed]
180. Wu, J.; Jia, Q.; Wu, S.; Zeng, H.; Sun, Y.; Wang, C.; Ge, R.; Xie, W. The crystal structure of the *Pyrococcus abyssi* mono-functional methyltransferase PaTrm5b. *Biochem. Biophys. Res. Commun.* **2017**, *493*, 240–245. [CrossRef] [PubMed]
181. de Crécy-Lagard, V.; Brochier-Armanet, C.; Urbonavicius, J.; Fernandez, B.; Phillips, G.; Lyons, B.; Noma, A.; Alvarez, S.; Droogmans, L.; Armengaud, J.; et al. Biosynthesis of wyosine derivatives in tRNA: An ancient and highly diverse pathway in Archaea. *Mol. Biol. Evol.* **2010**, *27*, 2062–2077. [CrossRef] [PubMed]
182. Urbonavičius, J.; Meškys, R.; Grosjean, H. Biosynthesis of wyosine derivatives in tRNA(Phe) of Archaea: Role of a remarkable bifunctional tRNA(Phe):m1G/imG2 methyltransferase. *RNA* **2014**, *20*, 747–753. [CrossRef] [PubMed]
183. Perche-Letuvée, P.; Molle, T.; Forouhar, F.; Mulliez, E.; Atta, M. Wybutosine biosynthesis: Structural and mechanistic overview. *RNA Biol.* **2014**, *11*, 1508–1518. [CrossRef] [PubMed]
184. Mao, D.Y.; Neculai, D.; Downey, M.; Orlicky, S.; Haffani, Y.Z.; Ceccarelli, D.F.; Ho, J.S.; Szilard, R.K.; Zhang, W.; Ho, C.S.; et al. Atomic structure of the KEOPS complex: An ancient protein kinase-containing molecular machine. *Mol. Cell* **2008**, *32*, 259–275. [CrossRef] [PubMed]
185. Hecker, A.; Lopreiato, R.; Graille, M.; Collinet, B.; Forterre, P.; Libri, D.; van Tilbeurgh, H. Structure of the archaeal Kae1/Bud32 fusion protein MJ1130: A model for the eukaryotic EKC/KEOPS subcomplex. *EMBO J.* **2008**, *27*, 2340–2351. [CrossRef] [PubMed]
186. Hecker, A.; Graille, M.; Madec, E.; Gadelle, D.; Le Cam, E.; van Tilbergh, H.; Forterre, P. The universal Kae1 protein and the associated Bud32 kinase (PRPK), a mysterious protein couple probably essential for genome maintenance in Archaea and Eukarya. *Biochem. Soc. Trans.* **2009**, *37*, 29–35. [CrossRef] [PubMed]
187. 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 t^6A tRNA modification in Archaea and Eukarya. *Nucleic Acids Res.* **2013**, *41*, 1953–1964. [CrossRef] [PubMed]
188. Wan, L.C.; Pillon, M.C.; Thevakumaran, N.; Sun, Y.; Chakrabartty, A.; Guarné, A.; Kurinov, I.; Durocher, D.; Sicheri, F. Structural and functional characterization of KEOPS dimerization by Pcc1 and its role in t^6A biosynthesis. *Nucleic Acids Res.* **2016**, *44*, 6971–6980. [CrossRef] [PubMed]
189. Pichard-Kostuch, A.; Zhang, W.; Liger, D.; Daugeron, M.C.; Letoquart, J.; Li de la Sierra-Gallay, I.; Forterre, P.; Collinet, B.; van Tilbeurgh, H.; Basta, T. Structure-function analysis of Sua5 protein reveals novel functional motifs required for the biosynthesis of the universal t^6A tRNA modification. *RNA* **2018**, *24*, 926–938. [CrossRef] [PubMed]
190. Luthra, A.; Swinehart, W.; Bayooz, S.; Phan, P.; Stec, B.; Iwata-Reuyl, D.; Swairjo, M.A. Structure and mechanism of a bacterial t^6A biosynthesis system. *Nucleic Acids Res.* **2018**, *46*, 1395–1411. [CrossRef] [PubMed]
191. Thiaville, P.C.; Iwata-Reuyl, D.; de Crécy-Lagard, V. Diversity of the biosynthesis pathway for threonylcarbamoyladenosine (t^6A), a universal modification of tRNA. *RNA Biol.* **2014**, *11*, 1529–1539. [CrossRef] [PubMed]

192. Caillet, J.; Droogmans, L. Molecular cloning of the *Escherichia coli miaA* gene involved in the formation of delta 2-isopentenyl adenosine in tRNA. *J. Bacteriol.* **1988**, *170*, 4147–4152. [CrossRef] [PubMed]
193. Esberg, B.; Leung, H.C.; Tsui, H.C.; Björk, G.R.; Winkler, M.E. Identification of the *miaB* gene, involved in methylthiolation of isopentenylated A37 derivatives in the tRNA of *Salmonella typhimurium* and *Escherichia coli*. *J. Bacteriol.* **1999**, *181*, 7256–7265. [PubMed]
194. Pierrel, F.; Hernandez, H.L.; Johnson, M.K.; Fontecave, M.; Atta, M. MiaB protein from *Thermotoga maritima*. Characterization of an extremely thermophilic tRNA-methylthiotransferase. *J. Biol. Chem.* **2003**, *278*, 29515–29524. [CrossRef] [PubMed]
195. Pierrel, F.; Douki, T.; Fontecave, M.; Atta, M. MiaB protein is a bifunctional radical-S-adenosylmethionine enzyme involved in thiolation and methylation of tRNA. *J. Biol. Chem.* **2004**, *279*, 47555–47563. [CrossRef] [PubMed]
196. Hernández, H.L.; Pierrel, F.; Elleingand, E.; García-Serres, R.; Huynh, B.H.; Johnson, M.K.; Fontecave, M.; Atta, M. MiaB, a bifunctional radical-S-adenosylmethionine enzyme involved in the thiolation and methylation of tRNA, contains two essential [4Fe-4S] clusters. *Biochemistry* **2007**, *46*, 5140–5147. [CrossRef] [PubMed]
197. Schweizer, U.; Bohleber, S.; Fradejas-Villar, N. The modified base isopentenyladenosine and its derivatives in tRNA. *RNA Biol.* **2017**, *14*, 1197–1208. [CrossRef] [PubMed]
198. Golovina, A.Y.; Sergiev, P.V.; Golovin, A.V.; Serebryakova, M.V.; Demina, I.; Govorun, V.M.; Dontsova, O.A. The *yfiC* gene of *E. coli* encodes an adenine-N^6 methyltransferase that specifically modifies A37 of $tRNA_1^{Val}(cmo^5UAC)$. *RNA* **2009**, *15*, 1134–1141. [CrossRef] [PubMed]
199. Allaudeen, H.S.; Yang, S.K.; Söll, D. Leucine tRNA(1) from HisT mutant of *Salmonella typhimurium* lacks two pseudouridines. *FEBS Lett.* **1972**, *28*, 205–208. [CrossRef]
200. Kammen, H.O.; Marvel, C.C.; Hardy, L.; Penhoet, E.E. Purification, structure, and properties of *Escherichia coli* tRNA pseudouridine synthase I. *J. Biol. Chem.* **1988**, *263*, 2255–2263. [PubMed]
201. Lecointe, F.; Simos, G.; Sauer, A.; Hurt, E.C.; Motorin, Y.; Grosjean, H. Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of psi 38 and psi 39 in tRNA anticodon loop. *J. Biol. Chem.* **1998**, *273*, 1316–1323. [CrossRef] [PubMed]
202. Dong, X.; Bessho, Y.; Shibata, R.; Nishimoto, M.; Shirouzu, M.; Kuramitsu, S.; Yokoyama, S. Crystal structure of tRNA pseudouridine synthase TruA from *Thermus thermophilus* HB8. *RNA Biol.* **2006**, *3*, 115–122. [CrossRef] [PubMed]
203. Spenkuch, F.; Motorin, Y.; Helm, M. Pseudouridine: Still mysterious, but never a fake (uridine)! *RNA Biol.* **2014**, *11*, 1540–1554. [CrossRef] [PubMed]
204. Morozov, I.A.; Gambaryan, A.S.; Lvova, T.N.; Nedospasov, A.A.; Venkstern, T.V. Purification and characterization of tRNA (adenine-1-)-methyltransferase from *Thermus flavus* strain 71. *Eur. J. Biochem.* **1982**, *129*, 429–436. [CrossRef] [PubMed]
205. De Bie, L.G.; Roovers, M.; Oudjama, Y.; Wattiez, R.; Tricot, C.; Stalon, V.; Droogmans, L.; Bujnicki, J.M. The *yggH* gene of *Escherichia coli* encodes a tRNA (m^7G46) methyltransferase. *J. Bacteriol.* **2003**, *185*, 3238–3243. [CrossRef] [PubMed]
206. Okamoto, H.; Watanabe, K.; Ikeuchi, Y.; Suzuki, T.; Endo, Y.; Hori, H. Substrate tRNA recognition mechanism of tRNA (m^7G46) methyltransferase from *Aquifex aeolicus*. *J. Biol. Chem.* **2004**, *279*, 49151–49159. [CrossRef] [PubMed]
207. Tomikawa, C.; Ochi, A.; Hori, H. The C-terminal region of thermophilic tRNA (m^7G46) methyltransferase (TrmB) stabilizes the dimer structure and enhances fidelity of methylation. *Proteins* **2008**, *71*, 1400–1408. [CrossRef] [PubMed]
208. Tomikawa, C.; Takai, K.; Hori, H. Kinetic characterization of substrate-binding sites of thermostable tRNA methyltransferase (TrmB). *J. Biochem.* **2018**, *163*, 133–142. [CrossRef] [PubMed]
209. Auxilien, S.; El Khadali, F.; Rasmussen, A.; Douthwaite, S.; Grosjean, H. Archease from *Pyrococcus abyssi* improves substrate specificity and solubility of a tRNA m^5C methyltransferase. *J. Biol. Chem.* **2007**, *282*, 18711–18721. [CrossRef] [PubMed]
210. Kuratani, M.; Hirano, M.; Goto-Ito, S.; Itoh, Y.; Hikida, Y.; Nishimoto, M.; Sekine, S.; Bessho, Y.; Ito, T.; Grosjean, H.; et al. Crystal structure of *Methanocaldococcus jannaschii* Trm4 complexed with sinefungin. *J. Mol. Biol.* **2010**, *401*, 323–333. [CrossRef] [PubMed]

211. Roovers, M.; Hale, C.; Tricot, C.; Terns, M.P.; Terns, R.M.; Grosjean, H.; Droogmans, L. Formation of the conserved pseudouridine at position 55 in archaeal tRNA. *Nucleic Acids Res.* **2006**, *34*, 4293–4301. [CrossRef] [PubMed]
212. Gurha, P.; Gupta, R. Archaeal Pus10 proteins can produce both pseudouridine 54 and 55 in tRNA. *RNA* **2008**, *14*, 2521–2527. [CrossRef] [PubMed]
213. Joardar, A.; Jana, S.; Fitzek, E.; Gurha, P.; Majumder, M.; Chatterjee, K.; Geisler, M.; Gupta, R. Role of forefinger and thumb loops in production of Ψ54 and Ψ55 in tRNAs by archaeal Pus10. *RNA* **2013**, *19*, 1279–1294. [CrossRef] [PubMed]
214. Kamalampeta, R.; Keffer-Wilkes, L.C.; Kothe, U. tRNA binding, positioning, and modification by the pseudouridine synthase Pus10. *J. Mol. Biol.* **2013**, *425*, 3863–3874. [CrossRef] [PubMed]
215. Chen, H.Y.; Yuan, Y.A. Crystal structure of Mj1640/DUF358 protein reveals a putative SPOUT-class RNA methyltransferase. *J. Mol. Cell. Biol.* **2010**, *2*, 366–374. [CrossRef] [PubMed]
216. Wurm, J.P.; Griese, M.; Bahr, U.; Held, M.; Heckel, A.; Karas, M.; Soppa, J.; Wöhnert, J. Identification of the enzyme responsible for N^1-methylation of pseudouridine 54 in archaeal tRNAs. *RNA* **2012**, *18*, 412–420. [CrossRef] [PubMed]
217. Chatterjee, K.; Blaby, I.K.; Thiaville, P.C.; Majumder, M.; Grosjean, H.; Yuan, Y.A.; Gupta, R.; de Crécy-Lagard, V. The archaeal COG1901/DUF358 SPOUT-methyltransferase members, together with pseudouridine synthase Pus10, catalyze the formation of 1-methylpseudouridine at position 54 of tRNA. *RNA* **2012**, *18*, 421–433. [CrossRef] [PubMed]
218. Davanloo, P.; Sprinzl, M.; Watanabe, K.; Albani, M.; Kersten, H. Role of ribothymidine in the thermal stability of transfer RNA as monitored by proton magnetic resonance. *Nucleic Acids Res.* **1979**, *6*, 1571–1581. [CrossRef] [PubMed]
219. Watanabe, K.; Himeno, H.; Ohta, T. Selective utilization of 2-thioribothymidine- and ribothymidine-containing tRNAs by the protein synthetic systems of *Thermus thermophilus* HB 8 depending on the environmental temperature. *J. Biochem.* **1984**, *96*, 1625–1632. [CrossRef] [PubMed]
220. Yokoyama, S.; Watanabe, K.; Miyazawa, T. Dynamic structures and functions of transfer ribonucleic acids from extreme thermophiles. *Adv. Biophys.* **1987**, *23*, 115–147. [CrossRef]
221. Urbonavicius, J.; Skouloubris, S.; Myllykallio, H.; Grosjean, H. Identification of a novel gene encoding a flavin-dependent tRNA:m^5U methyltransferase in bacteria—evolutionary implications. *Nucleic Acids Res.* **2005**, *33*, 3955–3964. [CrossRef] [PubMed]
222. Cicmil, N. Crystallization and preliminary X-ray crystallographic characterization of TrmFO, a folate-dependent tRNA methyltransferase from *Thermotoga maritima*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2008**, *64*, 193–195. [CrossRef] [PubMed]
223. Nishimasu, H.; Ishitani, R.; Yamashita, K.; Iwashita, C.; Hirata, A.; Hori, H.; Nureki, O. Atomic structure of a folate/FAD-dependent tRNA T54 methyltransferase. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8180–8185. [CrossRef] [PubMed]
224. Yamagami, R.; Yamashita, K.; Nishimasu, H.; Tomikawa, C.; Ochi, A.; Iwashita, C.; Hirata, A.; Ishitani, R.; Nureki, O.; Hori, H. The tRNA recognition mechanism of folate/FAD-dependent tRNA methyltransferase (TrmFO). *J. Biol. Chem.* **2012**, *287*, 42480–42494. [CrossRef] [PubMed]
225. Yamagami, R.; Tomikawa, C.; Shigi, N.; Kazayama, A.; Asai, S.; Takuma, H.; Hirata, A.; Fourmy, D.; Asahara, H.; Watanabe, K.; et al. The folate/FAD-dependent tRNA methyltransferase (TrmFO) from *Thermus thermophilus* regulates the other modifications in tRNA at low temperatures. *Genes Cells* **2016**, *21*, 740–754. [CrossRef] [PubMed]
226. Hamdane, D.; Grosjean, H.; Fontecave, M. Flavin-Dependent Methylation of RNAs: Complex Chemistry for a Simple Modification. *J. Mol. Biol.* **2016**, *428*, 4867–4881. [CrossRef] [PubMed]
227. Yamagami, R.; Miyake, R.; Fukumoto, A.; Nakashima, M.; Hori, H. Consumption of N^5, N^{10}-methylenetetrahydrofolate in *Thermus thermophilus* under nutrient-poor condition. *J. Biochem.* **2018**, *164*, 141–152. [CrossRef] [PubMed]
228. Shigi, N.; Suzuki, T.; Tamakoshi, M.; Oshima, T.; Watanabe, K. Conserved bases in the TPsi C loop of tRNA are determinants for thermophile-specific 2-thiouridylation at position 54. *J. Biol. Chem.* **2002**, *277*, 39128–39135. [CrossRef] [PubMed]

229. Shigi, N.; Suzuki, T.; Terada, T.; Shirouzu, M.; Yokoyama, S.; Watanabe, K. Temperature-dependent biosynthesis of 2-thioribothymidine of *Thermus thermophilus* tRNA. *J. Biol. Chem.* **2006**, *281*, 2104–2113. [CrossRef] [PubMed]
230. Shigi, N.; Sakaguchi, Y.; Suzuki, T.; Watanabe, K. Identification of two tRNA thiolation genes required for cell growth at extremely high temperatures. *J. Biol. Chem.* **2006**, *281*, 14296–14306. [CrossRef] [PubMed]
231. Shigi, N.; Sakaguchi, Y.; Asai, S.; Suzuki, T.; Watanabe, K. Common thiolation mechanism in the biosynthesis of tRNA thiouridine and sulphur-containing cofactors. *EMBO J.* **2008**, *27*, 3267–3278. [CrossRef] [PubMed]
232. Shigi, N. Posttranslational modification of cellular proteins by a ubiquitin-like protein in bacteria. *J. Biol. Chem.* **2012**, *287*, 17568–17577. [CrossRef] [PubMed]
233. Nakagawa, H.; Kuratani, M.; Goto-Ito, S.; Ito, T.; Katsura, K.; Terada, T.; Shirouzu, M.; Sekine, S.; Shigi, N.; Yokoyama, S. Crystallographic and mutational studies on the tRNA thiouridine synthetase TtuA. *Proteins* **2013**, *81*, 1232–1244. [CrossRef] [PubMed]
234. Chen, M.; Narai, S.; Omura, N.; Shigi, N.; Chimnaronk, S.; Tanaka, Y.; Yao, M. Crystallographic study of the 2-thioribothymidine-synthetic complex TtuA-TtuB from *Thermus thermophilus*. *Acta Crystallogr. F Struct. Biol. Commun.* **2016**, *72*, 777–781. [CrossRef] [PubMed]
235. Shigi, N.; Asai, S.-I.; Watanabe, K. Identification of a rhodanese-like protein involved in thiouridine biosynthesis in *Thermus thermophilus* tRNA. *FEBS Lett.* **2016**, *590*, 4628–4637. [CrossRef] [PubMed]
236. Chen, M.; Asai, S.-I.; Narai, S.; Nambu, S.; Omura, N.; Sakaguchi, Y.; Suzuki, T.; Ikeda-Saito, M.; Watanabe, K.; Yao, M.; et al. Biochemical and structural characterization of oxygen-sensitive 2-thiouridine synthesis catalyzed by an iron-sulfur protein TtuA. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 4954–4959. [CrossRef] [PubMed]
237. Urbonavicius, J.; Auxilien, S.; Walbott, H.; Trachana, K.; Golinelli-Pimpaneau, B.; Brochier-Armanet, C.; Grosjean, H. Acquisition of a bacterial RumA-type tRNA(uracil-54, C5)-methyltransferase by Archaea through an ancient horizontal gene transfer. *Mol. Microbiol.* **2008**, *67*, 323–335. [CrossRef] [PubMed]
238. Walbott, H.; Leulliot, N.; Grosjean, H.; Golinelli-Pimpaneau, B. The crystal structure of *Pyrococcus abyssi* tRNA (uracil-54, C5)-methyltransferase provides insights into its tRNA specificity. *Nucleic Acids Res.* **2008**, *36*, 4929–4940. [CrossRef] [PubMed]
239. Shigi, N. Biosynthesis and functions of sulfur modifications in tRNA. *Front. Genet.* **2014**, *5*, 67. [CrossRef] [PubMed]
240. Gurha, P.; Joardar, A.; Chaurasia, P.; Gupta, R. Differential roles of archaeal box H/ACA proteins in guide RNA-dependent and independent pseudouridine formation. *RNA Biol.* **2007**, *4*, 101–109. [CrossRef] [PubMed]
241. Muller, S.; Fourmann, J.B.; Loegler, C.; Charpentier, B.; Branlant, C. Identification of determinants in the protein partners aCBF5 and aNOP10 necessary for the tRNA: Psi55-synthase and RNA-guided RNA: Psi-synthase activities. *Nucleic Acids Res.* **2007**, *35*, 5610–5624. [CrossRef] [PubMed]
242. Kamalampeta, R.; Kothe, U. Archaeal proteins Nop10 and Gar1 increase the catalytic activity of Cbf5 in pseudouridylating tRNA. *Sci. Rep.* **2012**, *2*, 663. [CrossRef] [PubMed]
243. Nurse, K.; Wrzesinski, J.; Bakin, A.; Lane, B.G.; Ofengand, J. Purification, cloning, and properties of the tRNA psi 55 synthase from *Escherichia coli*. *RNA* **1995**, *1*, 102–112. [PubMed]
244. Pan, H.; Agarwalla, S.; Moustakas, D.T.; Finer-Moore, J.; Stroud, R.M. Structure of tRNA pseudouridine synthase TruB and its RNA complex: RNA recognition through a combination of rigid docking and induced fit. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 12648–12653. [CrossRef] [PubMed]
245. Wouters, J.; Tricot, C.; Durbecq, V.; Roovers, M.; Stalon, V.; Droogmans, L. Preliminary X-ray crystallographic analysis of tRNA pseudouridine 55 synthase from the thermophilic eubacterium *Thermotoga maritima*. *Acta Crystallogr. D Biol. Crystallogr.* **2003**, *59*, 152–154. [CrossRef] [PubMed]
246. Phannachet, K.; Huang, R.H. Conformational change of pseudouridine 55 synthase upon its association with RNA substrate. *Nucleic Acids Res.* **2004**, *32*, 1422–1429. [CrossRef] [PubMed]
247. Phannachet, K.; Elias, Y.; Huang, R.H. Dissecting the roles of a strictly conserved tyrosine in substrate recognition and catalysis by pseudouridine 55 synthase. *Biochemistry* **2005**, *44*, 15488–15494. [CrossRef] [PubMed]

248. Ishida, K.; Kunibayashi, T.; Tomikawa, C.; Ochi, A.; Kanai, T.; Hirata, A.; Iwashita, C.; Hori, H. 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.* **2011**, *39*, 2304–2318. [CrossRef] [PubMed]
249. Renalier, M.H.; Joseph, N.; Gaspin, C.; Thebault, P.; Mougin, A. The Cm56 tRNA modification in archaea is catalyzed either by a specific 2′-O-methylase, or a C/D sRNP. *RNA* **2005**, *11*, 1051–1063. [CrossRef] [PubMed]
250. Tkaczuk, K.L.; Dunin-Horkawicz, S.; Purta, E.; Bujnicki, J.M. Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases. *BMC Bioinfor.* **2007**, *8*, 73. [CrossRef] [PubMed]
251. Kuratani, M.; Bessho, Y.; Nishimoto, M.; Grosjean, H.; Yokoyama, S. Crystal structure and mutational study of a unique SpoU family archaeal methylase that forms 2′-*O*-methylcytidine at position 56 of tRNA. *J. Mol. Biol.* **2008**, *375*, 1064–1075. [CrossRef] [PubMed]
252. Walker, R.T. Mycoplasma evolution: A review of the use of ribosomal and transfer RNA nucleotide sequences in the determination of phylogenetic relationships. *Yale J. Biol. Med.* **1983**, *56*, 367–372. [PubMed]
253. Yamaizumi, Z.; Ihara, M.; Kuchino, Y.; Gupta, R.; Woese, C.R.; Nishimura, S. Archaebacterial tRNA contains 1-methylinosine at residue 57 in T psi C-loop. *Nucleic Acids Symp. Ser.* **1982**, *11*, 209–213.
254. Grosjean, H.; Constantinesco, F.; Foiret, D.; Benachenhou, N. A novel enzymatic pathway leading to 1-methylinosine modification in *Haloferax volcanii* tRNA. *Nucleic Acids Res.* **1995**, *23*, 4312–4319. [CrossRef] [PubMed]
255. Roovers, M.; Wouters, J.; Bujnicki, J.M.; Tricot, C.; Stalon, V.; Grosjean, H.; Droogmans, L. A primordial RNA modification enzyme: The case of tRNA (m^1A) methyltransferase. *Nucleic Acids Res.* **2004**, *32*, 465–476. [CrossRef] [PubMed]
256. Guelorget, A.; Roovers, M.; Guérineau, V.; Barbey, C.; Li, X.; Golinelli-Pimpaneau, B. Insights into the hyperthermostability and unusual region-specificity of archaeal *Pyrococcus abyssi* tRNA $m^1A57/58$ methyltransferase. *Nucleic Acids Res.* **2010**, *38*, 6206–6218. [CrossRef] [PubMed]
257. Guelorget, A.; Barraud, P.; Tisné, C.; Golinelli-Pimpaneau, B. Structural comparison of tRNA m(1)A58 methyltransferases revealed different molecular strategies to maintain their oligomeric architecture under extreme conditions. *BMC Struct. Biol.* **2011**, *11*, 48. [CrossRef] [PubMed]
258. Hamdane, D.; Guelorget, A.; Guérineau, V.; Golinelli-Pimpaneau, B. Dynamics of RNA modification by a multi-site-specific tRNA methyltransferase. *Nucleic Acids Res.* **2014**, *42*, 11697–11706. [CrossRef] [PubMed]
259. Biou, V.; Yaremchuk, A.; Tukalo, M.; Cusack, S. The 2.9 A crystal structure of *T. thermophilus* seryl-tRNA synthetase complexed with tRNA(Ser). *Science* **1994**, *263*, 1404–1410. [CrossRef] [PubMed]
260. Droogmans, L.; Roovers, M.; Bujnicki, J.M.; Tricot, C.; Hartsch, T.; Stalon, V.; Grosjean, H. Cloning and characterization of tRNA (m^1A58) methyltransferase (TrmI) from *Thermus thermophilus* HB27, a protein required for cell growth at extreme temperatures. *Nucleic Acids Res.* **2003**, *31*, 2148–2156. [CrossRef] [PubMed]
261. Barraud, P.; Golinelli-Pimpaneau, B.; Atmanene, C.; Sanglier, S.; Van Dorsselaer, A.; Droogmans, L.; Dardel, F.; Tisné, C. Crystal structure of *Thermus thermophilus* tRNA m^1A58 methyltransferase and biophysical characterization of its interaction with tRNA. *J. Mol. Biol.* **2008**, *377*, 535–550. [CrossRef] [PubMed]
262. Kuratani, M.; Yanagisawa, T.; Ishii, R.; Matsuno, M.; Si, S.Y.; Katsura, K.; Ushikoshi-Nakayama, R.; Shibata, R.; Shirouzu, M.; Bessho, Y.; et al. Crystal structure of tRNA m^1A58 methyltransferase TrmI from *Aquifex aeolicus* in complex with S-adenosyl-L-methionine. *J. Struct. Funct. Genomics* **2014**, *15*, 173–180. [CrossRef] [PubMed]
263. Takuma, H.; Ushio, N.; Minoji, M.; Kazayama, A.; Shigi, N.; Hirata, A.; Ochi, A.; Hori, H. Substrate tRNA recognition mechanism of eubacterial tRNA (m^1A58) methyltransferase (TrmI). *J. Biol. Chem.* **2015**, *290*, 5912–5925. [CrossRef] [PubMed]
264. Dégut, C.; Ponchon, L.; Folly-Klan, M.; Barraud, P.; Tisné, C. The m^1A58 modification in eubacterial tRNA: An overview of tRNA recognition and mechanism of catalysis by TrmI. *Biophys. Chem.* **2016**, *210*, 27–34. [CrossRef] [PubMed]
265. Väre, V.Y.; Eruysal, E.R.; Narendran, A.; Sarachan, K.L.; Agris, P.F. Chemical and Conformational Diversity of Modified Nucleosides Affects tRNA Structure and Function. *Biomolecules* **2017**, *7*, 29. [CrossRef] [PubMed]
266. Motorin, Y.; Helm, M. tRNA stabilization by modified nucleotides. *Biochemistry* **2010**, *49*, 4934–4944. [CrossRef] [PubMed]
267. El Yacoubi, B.; Bailly, M.; de Crécy-Lagard, V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. *Annu. Rev. Genet.* **2012**, *46*, 69–95. [CrossRef] [PubMed]

268. Manickam, N.; Joshi, K.; Bhatt, M.J.; Farabaugh, P.J. Effects of tRNA modification on translational accuracy depend on intrinsic codon-anticodon strength. *Nucleic Acids Res.* **2016**, *44*, 1871–1881. [CrossRef] [PubMed]
269. Grosjean, H.; Westhof, E. An integrated, structure- and energy-based view of the genetic code. *Nucleic Acids Res.* **2016**, *44*, 8020–8040. [CrossRef] [PubMed]
270. Agris, P.F.; Eruysal, E.R.; Narendran, A.; Väre, V.Y.P.; Vangaveti, S.; Ranganathan, S.V. Celebrating wobble decoding: Half a century and still much is new. *RNA Biol.* **2017**, *16*, 1–17. [CrossRef] [PubMed]
271. Perret, V.; Garcia, A.; Grosjean, H.; Ebel, J.P.; Florentz, C.; Giegé, R. Relaxation of a transfer RNA specificity by removal of modified nucleotides. *Nature* **1990**, *344*, 787–789. [CrossRef] [PubMed]
272. Aström, S.U.; Byström, A.S. Rit1, a tRNA backbone-modifying enzyme that mediates initiator and elongator tRNA discrimination. *Cell* **1994**, *79*, 535–546. [CrossRef]
273. Kaneko, T.; Suzuki, T.; Kapushoc, S.T.; Rubio, M.A.; Ghazvini, J.; Watanabe, K.; Simpson, L.; Suzuki, T. Wobble modification differences and subcellular localization of tRNAs in *Leishmania tarentolae*: Implication for tRNA sorting mechanism. *EMBO J.* **2003**, *22*, 657–667. [CrossRef] [PubMed]
274. Anderson, J.; Phan, L.; Cuesta, R.; Carison, B.A.; Pak, M.; Asano, K.; Björk, G.R.; Tamame, M.; Hinnebusch, A.G. The essential Gcd10p-Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA. *Genes Dev.* **1998**, *12*, 3650–3652. [CrossRef] [PubMed]
275. Anderson, J.; Phan, L.; Hinnebusch, A.G. The Gcd10p/Gcd14p complex is the essential two-subunit tRNA(1-methyladenosine) methyltransferase of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5173–5178. [CrossRef] [PubMed]
276. Ohira, T.; Suzuki, T. Retrograde nuclear import of tRNA precursors is required for modified base biogenesis in yeast. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 10502–10507. [CrossRef] [PubMed]
277. Ohira, T.; Suzuki, T. Precursors of tRNAs are stabilized by methylguanosine cap structures. *Nat. Chem. Biol.* **2016**, *12*, 648–655. [CrossRef] [PubMed]
278. Chatterjee, K.; Nostramo, R.T.; Wan, Y.; Hopper, A.K. tRNA dynamics between the nucleus, cytoplasm and mitochondrial surface: Location, location, location. *Biochim. Biophys. Acta* **2018**, *1861*, 373–386. [CrossRef] [PubMed]
279. Kadaba, S.; Krueger, A.; Trice, T.; Krecic, A.M.; Hinnebusch, A.G.; Anderson, J. Nuclear surveillance and degradation of hypomodified initiator $tRNA^{Met}$ in *S. cerevisiae*. *Genes Dev.* **2004**, *18*, 1227–1240. [CrossRef] [PubMed]
280. Alexandrov, A.; Chernyakov, I.; Gu, W.; Hiley, S.L.; Hughes, T.R.; Grayhack, E.J.; Phizicky, E.M. Rapid tRNA decay can result from lack of nonessential modifications. *Mol. Cell* **2006**, *21*, 87–96. [CrossRef] [PubMed]
281. Phizicky, E.M.; Hopper, A.K. tRNA biology charges to the front. *Genes Dev.* **2010**, *24*, 1832–1860. [CrossRef] [PubMed]
282. Durand, J.M.; Okada, N.; Tobe, T.; Watarai, M.; Fukuda, I.; Suzuki, T.; Nakata, N.; Komatsu, K.; Yoshikawa, M.; Sasakawa, C. vacC, a virulence-associated chromosomal locus of Shigella flexneri, is homologous to tgt, a gene encoding tRNA-guanine transglycosylase (Tgt) of Escherichia coli K-12. *J. Bacteriol.* **1994**, *176*, 4627–4634. [CrossRef] [PubMed]
283. Saga, A.E.; Vasil, A.I.; Vasil, M.L. Molecular characterization of mutants affected in the osmoprotectant-dependent induction of phspholipase C in *Pseudomonas aeruginosa* PAO1. *Mol. Microbiol.* **1997**, *23*, 43–56. [CrossRef]
284. Takano, Y.; Takayanagi, N.; Hori, H.; Ikeuchi, Y.; Suzuki, T.; Kimura, A.; Okuno, T. A gene involved in modifying transfer RNA is required for fungal pathogenicity and stress tolerance of *Colletotrichum lagenarium*. *Mol. Microbiol.* **2006**, *60*, 81–92. [CrossRef] [PubMed]
285. Sleiman, D.; Goldschmidt, V.; Barraud, P.; Marquet, R.; Paillart, J.C.; Tisné, C. Initiation of HIV-1 reverse transcription and functional role of nucleocapsid-mediated tRNA/viral genome interactions. *Virus Res.* **2012**, *169*, 324–339. [CrossRef] [PubMed]
286. Saadatmand, J.; Kleiman, L. Aspects of HIV-1 assembly that promote primer tRNA (Lys3) annealing to viral RNA. *Virus Res.* **2012**, *169*, 340–348. [CrossRef] [PubMed]
287. Gehrig, S.; Eberle, M.-E.; Botschen, F.; Rimbach, K.; Eberle, F.; Eigenbrod, T.; Kaiser, S.; Holmes, W.M.; Erdmann, V.A.; Sprinzl, M.; et al. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. *J. Exp. Med.* **2012**, *209*, 225–233. [CrossRef] [PubMed]

288. Jöckel, S.; Nees, G.; Sommer, R.; Zhao, Y.; Cherkasov, D.; Hori, H.; Ehm, G.; Schnare, M.; Nain, M.; Kaufmann, A.; Bauer, S. The 2′-*O*-methylation status of a single guanosine controls transfer RNA-mediated Toll-like receptor 7 activation or inhibition. *J. Exp. Med.* **2012**, *209*, 235–241. [CrossRef] [PubMed]
289. Schmitt, F.C.F.; Freund, I.; Weigand, M.A.; Helm, M.; Dalpke, A.H.; Eigenbrod, T. Identification of an optimized 2′-*O*-methylated trinucleotide RNA motif inhibiting Toll-like receptors 7 and 8. *RNA* **2017**, *23*, 1344–1351. [CrossRef] [PubMed]
290. Keller, P.; Freund, I.; Marchand, V.; Bec, G.; Huang, R.; Motorin, Y.; Eigenbrod, T.; Dalpke, A.; Helm, M. Double methylation of tRNA-U54 to 2′-*O*-methylthymidine (Tm) synergistically decreases immune response by Toll-like receptor 7. *Nucleic Acids Res.* **2018**. [CrossRef] [PubMed]
291. Gu, X.R.; Nicoghosian, K.; Cedergren, R.J.; Wong, J.T. Sequences of halobacterial tRNAs and the paucity of U in the first position of their anticodons. *Nucleic Acids Res.* **1983**, *11*, 5433–5442. [CrossRef] [PubMed]
292. Grosjean, H.; Gaspin, C.; Marck, C.; Decatur, W.A.; de Crecy-Lagard, V. RNomics and Modomics in the halophilic archaea *Haloferax volcanii*: Identification of RNA modification genes. *BMC Genom.* **2008**, *9*, 470. [CrossRef] [PubMed]
293. Phillips, G.; de Crécy-Lagard, V. Biosynthesis and function of tRNA modifications in Archaea. *Curr. Opin. Microbiol.* **2011**, *14*, 335–341. [CrossRef] [PubMed]
294. Grosjean, H.; Gupta, R.; Maxwell, S. Modified nucleotides in arhcaeal RNAs. In *Archaea: New Models for Prokaryotic Biology*; Blum, P., Ed.; Caister Academic Press: Portland, OR, USA, 2008; pp. 171–196.
295. Rintala-Dempsey, A.C.; Kothe, U. Eukaryotic stand-alone pseudouridine synthases–RNA modifying enzymes and emerging regulators of gene expression? *RNA Biol.* **2017**, *14*, 1185–1196. [CrossRef] [PubMed]
296. Hori, H. Methylated nucleosides in tRNA and tRNA methyltransferases. *Front. Genet.* **2014**, *5*, 144. [CrossRef] [PubMed]
297. Grosjean, H.; Oshima, T. How nucleic acids cope with high temperature. In *Physiology and Biochemistry of Extremophiles*; Gerday, C., Glansdorff, N., Eds.; ASM Press: Washington, DC, USA, 2007; pp. 39–56.
298. Kumazawa, Y.; Yokogawa, T.; Tsurui, H.; Miura, K.; Watanabe, K. Effect of the higher-order structure of tRNAs on the stability of hybrids with oligodeoxyribonucleotides: Separation of tRNA by an efficient solution hybridization. *Nucleic Acids Res.* **1992**, *20*, 2223–2232. [CrossRef] [PubMed]
299. Tsurui, H.; Kumazawa, Y.; Sanokawa, R.; Watanabe, Y.; Kuroda, T.; Wada, A.; Watanabe, K.; Shirai, T. Batchwise purification of specific tRNAs by a solid-phase DNA probe. *Anal. Biochem.* **1994**, *221*, 166–172. [CrossRef] [PubMed]
300. Suzuki, T.; Ueda, T.; Watanabe, K. A new method for identifying the amino acid attached to a particular RNA in the cell. *FEBS Lett.* **1996**, *381*, 195–198. [CrossRef]
301. Yokogawa, T.; Kitamura, Y.; Nakamura, D.; Ohno, S.; Nishikawa, K. Optimization of the hybridization-based method for purification of thermostable tRNAs in the presence of tetraalkylammonium salts. *Nucleic Acids Res.* **2010**, *38*, e89. [CrossRef] [PubMed]
302. Kazayama, A.; Yamagami, R.; Yokogawa, T.; Hori, H. Improved solid-phase DNA probe method for tRNA purification: Large-scale preparation and alteration of DNA fixation. *J. Biochem.* **2015**, *157*, 411–418. [CrossRef] [PubMed]
303. Stanley, J.; Vassilenko, S. A different approach to RNA sequencing. *Nature* **1978**, *274*, 87–89. [CrossRef] [PubMed]
304. Kuchino, Y.; Kato, M.; Sugisaki, H.; Nishimura, S. Nucleotide sequence of starfish initiator tRNA. *Nucleic Acids Res.* **1979**, *6*, 3459–3469. [CrossRef] [PubMed]
305. Suzuki, T.; Ikeuchi, Y.; Noma, A.; Suzuki, T.; Sakaguchi, Y. Mass spectrometric identification and characterization of RNA-modifying enzymes. *Methods Enzymol.* **2007**, *425*, 211–229. [PubMed]
306. Suzuki, T.; Suzuki, T. A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. *Nucleic Acids Res.* **2014**, *42*, 7346–7357. [CrossRef] [PubMed]
307. Mengel-Jorgensen, J.; Kirpekar, F. Detection of pseudouridine and other modifications in tRNA by cyanoethylation and MALDI mass spectrometry. *Nucleic Acids Res* **2002**, *30*, e135. [CrossRef] [PubMed]
308. Chen, C.; Huang, B.; Anderson, J.T.; Byström, A.S. 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.
309. Agris, P.F.; Koh, H.; Söll, D. The effect of growth temperatures on the in vivo ribose methylation of *Bacillus stearothermophilus* transfer RNA. *Arch Biochem Biophys.* **1973**, *154*, 277–282. [CrossRef]

310. Andachi, Y.; Yamao, F.; Muto, A.; Osawa, S. Codon recognition patterns as deduced from sequences of the complete set of transfer RNA species in *Mycoplasma capricolum*. Resemblance to mitochondria. *J. Mol. Biol.* **1989**, *209*, 37–54. [CrossRef]
311. Matsugi, J.; Jia, H.T.; Murao, K.; Ishikura, H. Nucleotide sequences of serine tRNAs from *Bacillus subtilis*. *Biochem. Biophys. Acta* **1992**, *1130*, 333–335. [CrossRef]
312. Hori, H. Transfer RNA methyltransferases with a SpoU-TrmD (SPOUT) fold and their modified nucleosides in tRNA. *Biomolecules* **2017**, *7*, 23. [CrossRef] [PubMed]
313. Reddy, D.M.; Crain, P.F.; Edmonds, C.G.; Gupta, R.; Hashizume, T.; Stetter, K.O.; Widdel, F.; McCloskey, J.A. Structure determination of two new amino acid-containing derivatives of adenosine from tRNA of thermophilic bacteria and archaea. *Nucleic Acids Res.* **1992**, *20*, 5607–5615. [CrossRef] [PubMed]
314. Cusack, S.; Yaremchuk, A.; Krikliviy, I.; Tukalo, M. $tRNA^{Pro}$ anticodon recognition by *Thermus thermophilus* prolyl-tRNA synthetase. *Structure* **1998**, *6*, 101–108. [CrossRef]
315. Li, F.; Dong, J.; Hu, X.; Gong, W.; Li, J.; Shen, J.; Tian, H.; Wang, J. A covalent approach for site-specific RNA labeling in Mammalian cells. *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 4597–4602. [CrossRef] [PubMed]
316. Singh, S.K.; Gurha, P.; Tran, E.J.; Maxwell, E.S.; Gupta, R. Sequential 2′-*O*-methylation of archaeal pre-$tRNA^{Trp}$ nucleotides is guided by the intron-encoded but trans-acting box C/D ribonucleoprotein of pre-tRNA. *J. Biol. Chem.* **2004**, *279*, 47661–47671. [CrossRef] [PubMed]
317. Haas, E.S.; Daniels, C.J.; Reeve, J.N. Genes encoding 5S rRNA and tRNAs in the extremely thermophilic archaebacterium *Methanothermus fervidus*. *Gene* **1989**, *77*, 253–263. [CrossRef]
318. Randau, L.; Münch, R.; Hohn, M.J.; Jahn, D.; Söll, D. *Nanoarchaeum equitans* creates functional tRNAs from separate genes for their 5′- and 3′-halves. *Nature* **2005**, *433*, 537–541. [CrossRef] [PubMed]
319. Randau, L.; Pearson, M.; Söll, D. The complete set of tRNA species in *Nanoarchaeum equitans*. *FEBS Lett.* **2005**, *579*, 2945–2947. [CrossRef] [PubMed]
320. Richter, H.; Mohr, S.; Randau, L. C/D box sRNA, CRISPR RNA and tRNA processing in an archaeon with a minimal fragmented genome. *Biochem. Soc. Trans.* **2013**, *41*, 411–415. [CrossRef] [PubMed]
321. Hardin, J.W.; Reyes, F.E.; Batey, R.T. Analysis of a critical interaction within the archaeal box C/D small ribonucleoprotein complex. *J. Biol. Chem.* **2009**, *284*, 15317–15324. [CrossRef] [PubMed]
322. McCloskey, J.A.; Crain, P.F.; Edmonds, C.G.; Gupta, R.; Hashizume, T.; Phillipson, D.W.; Stetter, K.O. Structure determination of a new fluorescent tricyclic nucleoside from archaebacterial tRNA. *Nucleic Acids Res.* **1987**, *15*, 683–693. [CrossRef] [PubMed]
323. Ushida, C.; Muramatsu, T.; Mizushima, H.; Ueda, T.; Watanabe, K.; Stetter, K.O.; Crain, P.F.; McCloskey, J.A.; Kuchino, Y. Structural feature of the initiator tRNA gene from *Pyrodictium occultum* and the thermal stability of its gene product, tRNA(imet). *Biochimie* **1996**, *79*, 847–855. [CrossRef]
324. McCloskey, J.A.; Liu, X.H.; Crain, P.F.; Bruenger, E.; Guymon, R.; Hashizume, T.; Stetter, K.O. Posttranscriptional modification of transfer RNA in the submarine hyperthermophile *Pyrolobus fumarii*. *Nucleic Acids Symp. Ser.* **2000**, *44*, 267–268. [CrossRef]
325. Zhou, S.; Sitaramaiah, D.; Noon, K.R.; Guymon, R.; Hashizume, T.; McCloskey, J.A. Structures of two new "minimalist" modified nucleosides from archaeal tRNA. *Bioorg. Chem.* **2004**, *32*, 82–91. [CrossRef] [PubMed]
326. Yang, Z.; Lin, J.; Ye, K. Box C/D guide RNAs recognize a maximum of 10 nt of substrates. *Proc. Natl. Acad. Sci. USA* **2016**, *27*, 10878–10883. [CrossRef] [PubMed]
327. Agari, Y.; Sato, S.; Wakamatsu, T.; Bessho, Y.; Ebihara, A.; Yokoyama, S.; Kuramitsu, S.; Shinkai, A. X-ray crystal structure of a hypothetical Sua5 protein from *Sulfolobus tokodaii* strain 7. *Proteins* **2008**, *70*, 1108–1111. [CrossRef] [PubMed]
328. Kuratani, M.; Kasai, T.; Akasaka, R.; Higashijima, K.; Terada, T.; Kigawa, T.; Shinkai, A.; Bessho, Y.; Yokoyama, S. Crystal structure of *Sulfolobus tokodaii* Sua5 complexed with L-threonine and AMPPNP. *Proteins* **2011**, *79*, 2065–2075. [CrossRef] [PubMed]
329. Parthier, C.; Goerlich, S.; Jaenecke, F.; Breithaupt, C.; Brauer, U.; Fandrich, U.; Clausnitzer, D.; Wehmeier, U.F.; Bottcher, C.; Scheel, D.; et al. The O-carbamoyltransferase TobZ catalyzes an ancient enzymatic reaction. *Angew. Chem. Int. Ed. Engl.* **2012**, *51*, 4046–4052. [CrossRef] [PubMed]
330. Klenk, H.P.; Schwass, V.; Zillig, W. Nucleotide sequence of the genes encoding proline tRNA(UGG) and threonine tRNA(GGU) and consensus promoter model of *Thermococcus celer*. *Biochim. Biophys. Acta* **1993**, *1172*, 236–238. [CrossRef]

331. Watanabe, K.; Oshima, T.; Saneyoshi, M.; Nishimura, S. Replacement of ribothymidine by 5-methyl-2-thiouridine in sequence GT psi C in tRNA of an extreme thermophile. *FEBS Lett.* **1974**, *43*, 59–63. [CrossRef]
332. Dalluge, J.J.; Hashizume, T.; Sopchik, A.E.; McCloskey, J.A.; Davis, D.R. Conformational flexibility in RNA: The role of dihydrouridine. *Nucleic Acids Res.* **1996**, *24*, 1073–1079. [CrossRef] [PubMed]
333. Sokołowski, M.; Klassen, R.; Bruch, A.; Schaffrath, R.; Glatt, S. Cooperativity between different tRNA modifications and their modification pathways. *Biochem. Biophys. Acta* **2018**, *1861*, 409–418. [CrossRef] [PubMed]
334. Liu, R.J.; Zhou, M.; Fang, Z.P.; Wang, M.; Zhou, X.L.; Wang, E.D. The tRNA recognition mechanism of the minimalist SPOUT methyltransferase, TrmL. *Nucleic Acids Res.* **2013**, *41*, 7828–7842. [CrossRef] [PubMed]
335. Morales, A.J.; Swairjo, M.A.; Schimmel, P. Structure-specific tRNA-binding protein from the extreme thermophile *Aquifex aeolicus*. *EMBO J.* **1999**, *18*, 3475–3483. [CrossRef] [PubMed]
336. Swairjo, M.A.; Morales, A.J.; Wang, C.C.; Ortiz, A.R.; Schimmel, P. Crystal structure of trbp111: A structure-specific tRNA-binding protein. *EMBO J.* **2000**, *19*, 6278–6298. [CrossRef] [PubMed]
337. Kushiro, T.; Schimmel, P. Trbp111 selectively binds a noncovalently assembled tRNA-like structure. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16631–16635. [CrossRef] [PubMed]
338. Desai, K.K.; Cheng, C.L.; Bingman, C.A.; Phillips, G.N., Jr.; Raines, R.T. A tRNA splicing operon: Archease endows RtcB with dual GTP/ATP cofactor specificity and accelerates RNA ligation. *Nucleic Acids Res.* **2014**, *42*, 3931–3942. [CrossRef] [PubMed]
339. Popow, J.; Jurkin, J.; Schleiffer, A.; Martinez, J. Analysis of orthologous groups reveals archease and DDX1 as tRNA splicing factors. *Nature* **2014**, *511*, 104–107. [CrossRef] [PubMed]
340. Maraia, R.J.; Arimbasseri, A.G. Factors That Shape Eukaryotic tRNAomes: Processing, Modification and Anticodon-Codon Use. *Biomolecules* **2017**, *7*, 26. [CrossRef] [PubMed]
341. Blewett, N.H.; Maraia, R.J. La involvement in tRNA and other RNA processing events including differences among yeast and other eukaryotes. *Biochim. Biophys. Acta* **2018**, *1861*, 361–372. [CrossRef] [PubMed]
342. Gutgsell, N.; Englund, N.; Niu, L.; Kaya, Y.; Lane, B.G.; Ofengand, J. Deletion of the Escherichia coli pseudouridine synthase gene truB blocks formation of pseudouridine 55 in tRNA in vivo, does not affect exponential growth, but confers a strong selective disadvantage in competition with wild-type cells. *RNA* **2000**, *6*, 1870–1881. [CrossRef] [PubMed]
343. Keffer-Wilkes, L.C.; Veerareddygari, G.R.; Kothe, U. RNA modification enzyme TruB is a tRNA chaperone. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 14306–14311. [CrossRef] [PubMed]
344. Quigley, G.J.; Teeter, M.M.; Rich, A. Structural analysis of spermine and magnesium ion binding to yeast phenylalanine transfer RNA. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 64–68. [CrossRef] [PubMed]
345. Frydman, B.; de los Santos, C.; Frydman, R.B. A ^{13}C NMR study of [5,8-$^{13}C_2$]spermidine binding to tRNA and to *Escherichia coli* macromolecules. *J. Biol. Chem.* **1990**, *265*, 20874–20878. [PubMed]
346. Terui, Y.; Ohnuma, M.; Hiraga, K.; Kawashima, E.; Oshima, T. Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*. *Biochem. J.* **2005**, *388*, 427–433. [CrossRef] [PubMed]
347. Ouameur, A.A.; Bourassa, P.; Tajmir-Riahi, H.A. Probing tRNA interaction with biogenic polyamines. *RNA* **2010**, *16*, 1968–1979. [CrossRef] [PubMed]
348. Oshima, T. Unique polyamines produced by an extreme thermophiles, *Thermus thermophilus*. *Amino Acids* **2007**, *33*, 367–372. [CrossRef] [PubMed]
349. Oshima, T.; Moriya, T.; Terui, Y. Identification, chemical synthesis, and biological functions of unusual polyamines produced by extreme thermophiles. *Methods Mol. Biol.* **2011**, *720*, 81–111. [PubMed]
350. Hamana, K.; Niitsu, M.; Samejima, K.; Matsuzaki, S. Polyamine distributions in thermophilic eubacteria belonging to *Thermus* and *Acidothermus*. *J. Biochem.* **1991**, *109*, 444–449. [CrossRef] [PubMed]
351. Hamana, K.; Tanaka, T.; Hosoya, R.; Niitsu, M.; Itoh, T. Cellular polyamines of the acidophilic, thermophilic and thermoacidophilic archaebacteria, *Acidilobus*, *Ferroplasma*, *Pyrobaculum*, *Pyrococcus*, *Staphylothermus*, *Thermococcus*, *Thermodiscus* and *Vulcanisaeta*. *J. Gen. Appl. Microbiol.* **2003**, *49*, 287–293. [CrossRef] [PubMed]
352. Hayrapetyan, A.; Grosjean, H.; Helm, M. Effect of a quaternary pentamine on RNA stabilization and enzymatic methylation. *Biol. Chem.* **2009**, *390*, 851–861. [CrossRef] [PubMed]
353. Nakashima, M.; Yamagami, R.; Tomikawa, C.; Ochi, Y.; Moriya, T.; Asahara, H.; Fourmy, D.; Yoshizawa, S.; Oshima, T.; Hori, H. Long and branched polyamines are required for maintenance of the ribosome, $tRNA^{His}$ and $tRNA^{Tyr}$ in *Thermus thermophilus* cells at high temperatures. *Genes Cells* **2017**, *22*, 628–645. [CrossRef] [PubMed]

354. Yue, D.; Kintanar, A.; Horowitz, J. Nucleoside modifications stabilize Mg^{2+} binding in *Escherichia coli* tRNA(Val): An imino proton NMR investigation. *Biochemistry* **1994**, *33*, 8905–8911. [CrossRef] [PubMed]
355. Agris, P.F. The importance of being modified: Roles of modified nucleosides and Mg^{2+} in RNA structure and function. *Prog. Nucleic Acid Res. Mol. Biol.* **1996**, *53*, 79–129. [PubMed]
356. Nobles, K.N.; Yarian, C.S.; Liu, G.; Guenther, R.H.; Agris, P.F. Highly conserved modified nucleosides influence Mg^{2+}-dependent tRNA folding. *Nucleic Acids Res.* **2002**, *30*, 4751–4760. [CrossRef] [PubMed]
357. Hensel, R.; Konig, H. Thermoadaptation of metanogenic bacteria by interacellular ion concentration. *FEMS Microbiol. Lett.* **1988**, *49*, 75–79. [CrossRef]
358. Nawrot, B.; Sochacka, E.; Duchler, M. tRNA structural and functional changes induced by oxidative stress. *Cell Mol. Life Sci.* **2011**, *68*, 4023–4032. [CrossRef] [PubMed]
359. Preston, M.A.; D'Silva, S.; Kon, Y.; Phizicky, E.M. $tRNA^{His}$ 5-methylcytidine levels increase in response to several growth arrest conditions in *Saccharomyces cerevisiae*. *RNA* **2013**, *19*, 243–256. [CrossRef] [PubMed]
360. Endres, L.; Dedon, P.C.; Begley, T.J. Codon-biased translation can be regulated by wobble-base tRNA modification systems during cellular stress responses. *RNA Biol.* **2015**, *12*, 603–614. [CrossRef] [PubMed]
361. Chan, C.T.; Deng, W.; Li, F.; DeMott, M.S.; Babu, I.R.; Begley, T.J.; Dedon, P.C. Highly Predictive Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. *Chem. Res. Toxicol.* **2015**, *28*, 978–988. [CrossRef] [PubMed]
362. Polikarpov, I.; Prade, R.; Caldana, C.; Paes Leme, A.F.; Mercadante, A.Z.; Riaño-Pachón, D.M.; Squina, F.M. Thermal adaptation strategies of the extremophile bacterium *Thermus filiformis* based on multi-omics analysis. *Extremophiles* **2017**, *21*, 775–788.
363. Kimura, S.; Suzuki, T. Iron-sulfur proteins responsible for RNA modifications. *Biochim. Biophys. Acta* **2015**, *1853*, 1272–1283. [CrossRef] [PubMed]
364. Ramamurthy, V.; Swann, S.L.; Spedaliere, C.J.; Mueller, E.G. Role of cysteine residues in pseudouridine synthases of different families. *Biochemistry* **1999**, *38*, 13106–13111. [CrossRef] [PubMed]
365. Urbonavicius, J.; Jäger, G.; Björk, G.R. Amino acid residues of the *Escherichia coli* tRNA(m^5U54)methyltransferase (TrmA) critical for stability, covalent binding of tRNA and enzymatic activity. *Nucleic Acids Res.* **2007**, *35*, 3297–3305. [CrossRef] [PubMed]
366. Alian, A.; Lee, T.T.; Griner, S.L.; Stroud, R.M.; Finer-Moore, J. Structure of a TrmA-RNA complex: A consensus RNA fold contributes to substrate selectivity and catalysis in m^5U methyltransferases. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6876–6881. [CrossRef] [PubMed]
367. Byrne, R.T.; Jenkins, H.T.; Peters, D.T.; Whelan, F.; Stowell, J.; Aziz, N.; Kasatsky, P.; Rodnina, M.V.; Koonin, E.V.; Konevega, A.L.; et al. Major reorientation of tRNA substrates defines specificity of dihydrouridine synthases. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 6033–6037. [CrossRef] [PubMed]
368. Ryals, J.; Hsu, R.Y.; Lipsett, M.N.; Bremer, H. Isolation of single-site *Escherichia coli* mutants deficient in thiamine and 4-thiouridine syntheses: Identification of a *nuvC* mutant. *J. Bacteriol.* **1982**, *151*, 899–904. [PubMed]
369. Liu, Y.; Zhu, X.; Nakamura, A.; Orlando, R.; Söll, D.; Whitman, W.B. Biosynthesis of 4-thiouridine in tRNA in the methanogenic archaeon *Methanococcus maripaludis*. *J. Biol. Chem.* **2012**, *287*, 36683–36692. [CrossRef] [PubMed]

© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).