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Original article

Genome-wide analysis revealed novel molecular features and evolution of Anti-codons in cyanobacterial tRNAs

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ABSTRACT

Transfer RNAs (tRNAs) play important roles to decode the genetic information contained in mRNA in the process of translation. The tRNA molecules possess conserved nucleotides at specific position to regulate the unique function. However, several nucleotides at different position of the tRNA undergo modification to maintain proper stability and function. The major modifications include the presence of pseudouridine (Ψ) residue instead of uridine and the presence of m^5-methylation sites. We found that, Ψ13 is conserved in D-stem, whereas Ψ38 & Ψ39 were conserved in the anti-codon loop (AL) and anti-codon arm (ACA), respectively. Furthermore, Ψ55 found to be conserved in the Ψ loop. Although, fourteen possible methylation sites can be found in the tRNA, cyanobacterial tRNAs were found to possess conserved G9, m^3C_{32}, C_{36}, A_{37}, m^5C_{38} and U_{54} methylation sites. The presence of multiple conserved methylation sites might be responsible for providing necessary stability to the tRNA. The evolutionary study revealed, $tRNA^{Met}$ and $tRNA^{Ile}$ were evolved earlier than other tRNA isotypes and their evolution is date back to at least 4000 million years ago. The presence of novel pseudouridination and m^5-methylation sites in the cyanobacterial tRNAs are of particular interest for basic biology. Further experimental study can delineate their functional significance in protein translation.

1. Introduction

tRNAs are central molecules that brings protein translation event in the cell. The molecular structure (clover leaf-like) of the tRNA was proposed by Robert Holly and reported to contain three loops (D-loop, anti-codon loop, and pseudouridine loop) and four arms (acceptor arm, D-arm, anticodon arm, and pseudouridine arm). In addition, it also contains a variable region in between the anti-codon arm and pseudouridine arm. The anti-codon loop makes bridge with the mRNA and facilitate the protein translation event. The adaptor molecule tRNA binds the amino acids at the 3′-end and encodes to the specific codons of the mRNA. The positions of triplet anti-codon are numbered as 34, 35 and 36 (Kirchner and Ignatova, 2014; Mohanta and Bae, 2017). During the interaction of the codon and the anti-codon, 2nd and 3rd letter (positions 35 and 36) of the anti-codon base-pair with the 2nd and 1st position of the codon, respectively. The G nucleotides at the 34th position (G34) of the anti-codon can base-pair with the U3 and C3 nucleotide of the codon. Thus, all the codons ending with pyrimidine (UUU, UUC) will translate to the same amino acid. This irregular base-pairing is known as "wobble" pairing (Charette and Gray, 2000). Therefore, tRNA modified nucleotides are sometimes found in the wobble position (34th position). Wobble modifications plays important roles in regulating codon restrictions, altering, or expanding the

Abbreviations: tRNA, transfer RNA; Ψ, Pseudouridine; U, Uridine; A, Adenine; G, Guanine; C, Cytosine.

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decoding properties of the tRNA. Unlike wobble modification, tRNA undergoes several other modifications as well in its nucleotides at different positions. Modification at the 34th and 37th position of the tRNA is critical for translational maintenance of accuracy in the reading frame (Hou et al., 2015). In $tRNA^{Lys/UUU}$, $tRNA^{Gln/UUG}$, $tRNA^{Glu/UUC}$, and $tRNA^{Arg/UCU}$ U34 base of the anti-codon modified to 5-methoxycarbonyl-methyl-2-thiouridine (mcm^5S^2U34) (Hou et al., 2015). The $tRNA^{Lys/UUU}$ can read AAA and AAG codons of $tRNA^{Lys}$ and base pairs with A-U and G-U, thus having very weak interactions. Thus, modification of U34 by mcm^5S^2 leads to stabilization of A-U and G-U base paring (Charette and Gray, 2000). Unlike, U34 base modifications, tRNA bring modifications at several nucleotides. The major modifications are the presence of pseudouridine and m^5-methylation at different parts of the tRNA. The genomic detail of the pseudouridine and m^5-methylation site is not reported so far. Therefore, we have deciphered the genomic details of the pseudouridine and m^5-methylation event in cyanobacterial tRNA.

To identify the conserved nucleotide position, all the tRNAs were grouped separately according to their distinct tRNA family and analyzed using Multalin software (http://multalin.toulouse.inra.fr/multalin/). Multiple sequence alignment was performed using Multalin software (http://multalin.toulouse.inra.fr/multalin/) (Corpet, 1988). The conserved nucleotide position in tRNA alignment was analyzed and corroborated with nucleotide position of individual tRNAs to find the putative pseudouridine and m^5-methylation sites.

2. Materials and methods

2.1. Identification and in-silico analysis of tRNAs

The tRNA sequences were downloaded from the joint genome institute portal (https://genome.jgi.doe.gov/) and National Center for Biotechnology Information (NCBI). A total of 3161 tRNA genes were found from 61 species (Supplementary Data 1, Supplementary Table 1 and Supplementary Table 1) (Mohanta et al., 2017). All the identified tRNA sequences were analyzed in tRNAscan-SE software using following statistical parameters; sequence source, bacterial; search mode, infernal first pass > infernal, covariance model, tRNAinf-euk.cm, infernal first pass and cutoff score 10 (Lowe and Eddy, 1997). The nucleotide positions of all the tRNAs were recorded individually.

3. Construction of phylogenetic tree of cyanobacterial tRNAs

All the tRNAs sequences were aligned to construct a clustal file using MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/) multiple sequence alignment approach. A MEGA file was created from the clustal file using MEGA6 software (Tamura et al., 2013). A model selection was conducted to find a suitable model to construct the phylogenetic tree. The model selection was carried out using the neighbor-joining tree and maximum likelihood statistical method. The study resulted a list of models that can be used to make the phylogenetic tree. Based on the lowest BIC (Bayesian information criterion) score (180413.955), we constructed the phylogenetic tree using maximum likelihood approach with 1000 bootstrap replicates. The model/method used was general real time reversible and rates pattern was Gamma distributed (G).

4. Results and discussion

4.1. Dynamics position of pseudouridine (Ψ) in cyanobacterial tRNAs

Pseudouridine (Ψ) is an universal structural unit of various RNAs including tRNA and mRNA (Charette and Gray, 2000). Pseudouridine is found universally in all tRNAs at the Ψ55 position in the TΨC stem-loop. It has been reported that, Ψ is also universally present in the D-stem (Ψ13), the anti-codon stem (Ψ39), and the anti-codon loop (Ψ38) in the prokaryotes, eukaryotes, eubacteria, and archaebacteria including mitochondrial and plastidal tRNAs as well (Charette and Gray, 2000). In our study we found that Ψ13 was conserved in the D-stem, whereas Ψ38 & Ψ39 were conserved in the anti-codon loop and anti-codon stem, respectively while Ψ55 was conserved in the Ψ loop (Charette and Gray, 2000). In addition to the presence of Ψ13, it was also present at the Ψ12 position in the D-arm. Conserved Ψ38 in the anti-codon loop forms a strong base pair with A32 (Davis and Poulter, 1991) and Ψ39 in the anti-codon stem forms a strong base pairing with residue 31 in the anti-codon stem (Charette and Gray, 2000). In our study, we found Ψ55 at the conserved position in all the studied tRNA genes (Fig. 1, Table 1). In cyanobacteria, not all of the tRNA had a Ψ13 residue in the D-arm, whereas instead, some of the tRNA had Ψ12 ($tRNA^{Ala}$, $tRNA^{Ile}$, $tRNA^{Phe}$, $tRNA^{Asn}$, and $tRNA^{Asp}$) residue (Table 1). One of the Ψ in $tRNA^{Trp}$ was conserved at the

Table 1
Variation of pseudouridine position in the cyanobacterial tRNA. Previous studies have reported that pseudouridine are present at 13, 38, 39, and 55 position in the D-arm, anti-codon loop, anti-codon arm and pseudouridine loop respectively. The pseudouridine residue at the pseudouridine loop is conserved at the 55th position, while variation was found in other parts of the cyanobacterial tRNAs. The right tick (√) represents conservation of pseudouridine and x represents the absence of conservation whereas A, G, or C are possible substituted nucleotide of Ψ.

tRNA	D-arm (U/Ψ-12)	D-Arm (Ψ-13)	Anti-codon loop (Ψ-38)	Anti-codon Arm (Ψ-39)	Ψ-loop (Ψ-55)
Alanine	√	x	Ψ/A/C	Ψ/G/C	√
Arginine	Ψ/G/A	x	Ψ/A/C	Ψ/G/C/A	√
Asparagine	√	x	x	C/ Ψ/G	√
Aspartate	√	x	x	x	√
Cysteine	x	x	x	A/G/ Ψ	√
Glutamate	x	Ψ/A/G/C	x	G/A/ Ψ	√
Glutamine	x	x/ Ψ	Ψ/A	C/A/ Ψ	√
Glycine	Ψ/G/A	Ψ/C	Ψ/A/G	G/C/Ψ/A	√
Histidine	x	x	Ψ/A/C	Ψ/A/G	√
Isoleucine	√	x	x	x	√
Leucine	x	x	Ψ/A/G	Ψ/G/A/C	√
Lysine	Ψ/G/A	x	x	C/ Ψ/G	√
Methionine	Ψ/G/C/A	x	x	G/ Ψ/C	√
Phenylalanine	√	x	x	Ψ/A	√
Proline	x	x	Ψ/A	x	√
Serine	A/ Ψ/C	x	x	Ψ/A/G	√
Threonine	Ψ/A/G	x	x	Ψ/G/A	√
Tryptophan	Ψ/G	x	x	x	√
Tyrosine	x	x	x	Ψ/A	√
Valine	Ψ/A	x	Ψ/A/C	x	√

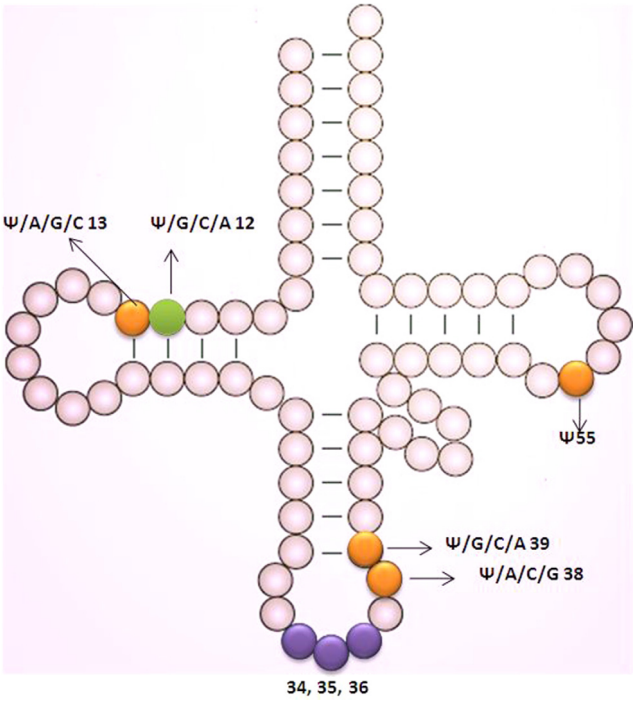

Fig. 1. Occurrence of pseudo-uridine nucleotide in the cyanobacterial tRNAs. The pseudo-uridine in cyanobacterial tRNAs are very dynamic at Ψ12 and Ψ13 position in the D-arm, Ψ39 position in the anti-codon loop and Ψ40 position in the anti-codon arm. The pseudo-uridine at position Ψ55 in Ψ-loop was conserved.

position eleven (Ψ11) in the D-arm. However, tRNAs with four nucleotides in the D-arm lacked the Ψ13 residue. Instead, the majority of them were contained C (cytosine) at the 13th position. In $tRNA^{Arg}$, $tRNA^{Gly}$, $tRNA^{Lys}$ and $tRNA^{Thr}$ genes, the Ψ/U was found to be substituted by either a G or an A nucleotide, whereas in the $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{Ile}$, and $tRNA^{Phe}$ had a conserved Ψ/U at the 12th position (Table 1). The $tRNA^{Gln}$, $tRNA^{His}$, $tRNA^{Cys}$, $tRNA^{Glu}$, $tRNA^{Leu}$, $tRNA^{Pro}$, and $tRNA^{Tyr}$ had no conserved Ψ/U nucleotide at the 12th position (Table 1). Besides $tRNA^{Glu}$ (Ψ/A/G/C), $tRNA^{Gln}$ (x/Ψ) and $tRNA^{Gly}$ (Ψ/C), all other tRNAs Ψ13 was found to be absent. At position 39th of the anti-codon arm, the U/Ψ was absent in the $tRNA^{Pro}$, $tRNA^{Trp}$, $tRNA^{Ile}$, $tRNA^{Asp}$, and $tRNA^{Val}$ whereas $tRNA^{Cys}$, $tRNA^{Glu}$, $tRNA^{Gln}$, $tRNA^{Gly}$, $tRNA^{Ala}$, $tRNA^{Arg}$, $tRNA^{Asn}$, $tRNA^{His}$, $tRNA^{Met}$, $tRNA^{Phe}$, $tRNA^{Ser}$, $tRNA^{Thr}$, $tRNA^{Leu}$, $tRNA^{Lys}$, and $tRNA^{Tyr}$, were found to possess Ψ/A/G/C nucleotides. At the 38th position in the anti-codon loop, $tRNA^{Gln}$ and $tRNA^{Pro}$ were contained conserved Ψ/A nucleotides, whereas $tRNA^{Gly}$ and $tRNA^{Leu}$ were contained conserved Ψ/A/G nucleotides and $tRNA^{Ala}$, $tRNA^{Arg}$, and $tRNA^{Val}$ were found to contain Ψ/A/C nucleotides (Table 1). The Ψ nucleotide at 39th position in the anti-codon arm was very dynamic and substituted by either A, G, or C nucleotides (Table 1). $tRNA^{Asp}$, $tRNA^{Ile}$, $tRNA^{Pro}$, $tRNA^{Trp}$, and $tRNA^{Val}$ did not have any Ψ/U nucleotide at the 39th position, while the rest of the tRNA genes were contained Ψ/A/G/C nucleotides (Table 1).

Genetic mutants those don't contain Ψ-residue in the tRNA exhibit problem in translation and exhibit a slower rate of growth and failed to strive compared to the wild type strains (Charette and Gray, 2000; Raychaudhuri et al., 1999). The Ψ has taxa specific variation and might be associated with RNA-RNA or RNA-protein interactions or tRNA-rRNA/mRNA interactions (Charette and Gray, 2000). However, Ψ in the tRNA do not control comprehensive three dimensional structure of tRNA, cell viability or aminoacylation (Arnez and Steitz, 1994; Davis and Poulter, 1991; Harrington et al., 1993). However, it does appear to affect the local structure depending on where it resides. If Ψ is found in the anti-codon loop, it plays a crucial role in "alternative codon usage". For example, the eichinoderm, mitochondrial $tRNA^{Asn}$ GΨC anti-codon translates the AAA lysine codon into asparagine (Tomita et al., 1999). The *hisT* gene encodes pseudouridine synthase that mediates the formation of Ψ at 38th, 39th, and 40th position (Charette and Gray, 2000). The tRNA that lacks Ψ at positions 38, 39, and 40 have a defect in aminoacyl tRNA during translation which might be due to the absence of the stabilizing effects at Ψ38/39/40 position (Yarian et al., 1999).

4.2. Cyanobacterial tRNA contains diverse m^5C methylation sites

tRNA undergoes post-transcriptional modification to produce the mature tRNA. The post-transcriptional modifications includes addition of a methyl group to different nucleotides within the tRNA (Holmes et al., 1992; Hori, 2014; Papatriantafyllou, 2012). Although all form of RNAs undergoes methylation, this mechanism was found to be the most prominent and widespread (Burgess et al., 2015; Nau, 1976; Papatriantafyllou, 2012). Although adenine, guanine, cytosine, and uracil undergo methylation, the modified nucleotide pseudouridine (Ψ), and inosine (I) also undergo methylation (Hori, 2014; Tidwell and Howard, 1972). There are fourteen possible methylation sites in tRNA (Swinehart and Jackman, 2015). They includes Gm, Am, Um, Cm (methylation at ribose oxygen), m^5C and m^5U (methylation at C5 carbon), m^1A, m^1G, m^2G, m^3U, m^3C, m^1 Ψ, m^6A and m^7G (methylation at endocyclic nitrogen) (Swinehart and Jackman, 2015). m^3C and $m^1Ψ$ methylation is absent in Archaea and Bacteria. The m^1G_{37} methylation occurs in all tRNAs that encode the G nucleotide at the 37th position (Christian et al., 2010; Takeda et al., 2006). Our study showed $tRNA^{Pro}$ contained conserved G_{37} nucleotide, suggesting it's

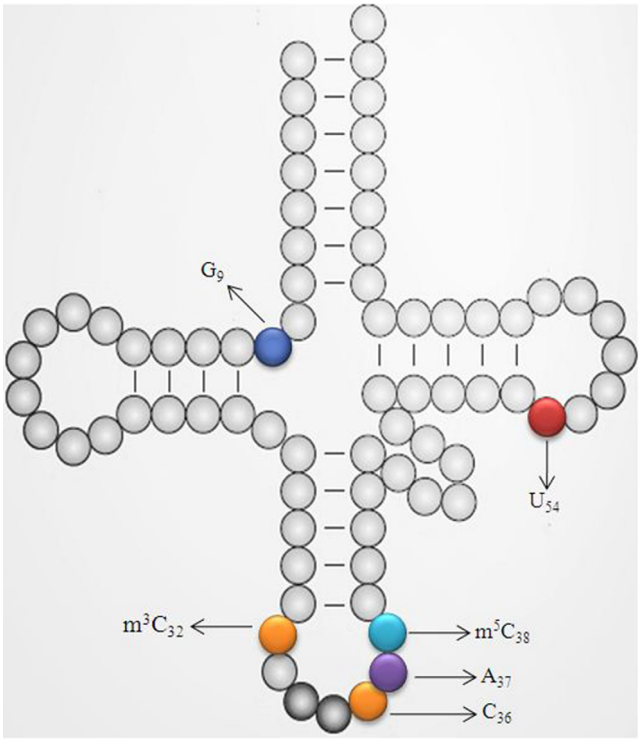

Fig. 2. Methylation site in cyanobacterial tRNAs. Cyanobacterial tRNAs show the presence of conserved methylation site at G_9 in the acceptor arm, m^3C_{32}, C_{36}, A_{37}, and m^3C_{38} in the anti-codon loop, and U_{54} in the Ψ loop. The conserved methylation sites of cyanobacterial tRNAs are sometimes tRNA family (isotype) specific.

participation in m^1G_{37} methylation. Remainder of the tRNAs contained conserved A nucleotide at 37th position. In previous study, it was reported that *yfiC* gene of *Escherichia coli* modifies A_{37} in $tRNA^{Val}$ (Golovina et al., 2009). The presence of conserved A_{37} nucleotides in the cyanobacterial tRNA shows that m^6A_{37} methylation might have been a predominant methylation site in the prokaryotic lineage as well (Fig. 2). tRNA methylation events are family specific too; for example, m^3C_{32} methylation is only found in $tRNA^{Thr}$ (Swinehart and Jackman, 2015). In the current study, cyanobacterial C_{32} was found to be conserved in few tRNAs including $tRNA^{Ile}$, $tRNA^{Met}$, $tRNA^{Trp}$, $tRNA^{Cys}$, $tRNA^{Asn}$, $tRNA^{Lys}$ and $tRNA^{Asp}$. This indicates the presence of a conserved m^3C_{32} methylation site in these tRNAs. In the eukaryotes, m^5C_{38} methylation has been found in $tRNA^{Asp}$, but cyanobacterial $tRNA^{Asp}$ lacks the conserved C_{38} nucleotide. Instead, they contained conserved C_{36} in $tRNA^{Asp}$, suggesting the presence of probable methylation site at C_{36} instead of C_{38} position. Apart from $tRNA^{Asp}$, other tRNA including $tRNA^{Gly}$, $tRNA^{Ala}$, $tRNA^{Val}$, and $tRNA^{Glu}$ also contained conserved C_{36} nucleotide. In *S. cerevisiae*, G_9 is methylated by the m^1G_9 methyltransferase (Trm10) (Swinehart and Jackman, 2015). In the current analysis of cyanobacteria, G_9 was found to be conserved in $tRNA^{Leu}$, $tRNA^{Tyr}$, and $tRNA^{Ser}$ whereas other tRNAs were contained either $A_9/G_9/U_9$ ($tRNA^{Met}$), A_9/G_9 ($tRNA^{Thr}$, $tRNA^{Lys}$, $tRNA^{Arg}$), $A_9/C_9/G_9$ ($tRNA^{Cys}$, $tRNA^{Gln}$), A_9/C_9 ($tRNA^{His}$), or $A_9/C_9/U_9$ ($tRNA^{Glu}$) nucleotides. The presence of the A_9/G_9 nucleotide suggest promiscuous substrate specificity (Swinehart and Jackman, 2015). However, the A_9/G_9-type specificity is only found in the mitochondrial methyl transferases which have broader substrate specificity and can catalyze G_9 and A_9 methylation. The presence of C_9 and U_9, rather than G_9 or A_9, in cyanobacterial tRNA is intriguing and more specifically those contain A_9/G_9, $A_9/G_9/U_9$, $A_9/C_9/G_9$, A_9/C_9, $A_9/C_9/U_9$ nucleotide are of particular interest. The dual specificity of methyl transferase can methylate two different nucleotides at the same position. However, it is difficult to predict the methylation outcome when the same position is occupied by a random nucleotide. Although the methylation event occurs at N1 position of the purine ring, the question arises about its specificity and mechanism of action. Because, only a single active site can able to catalyze the reaction for a particular substrate and how it could be the same for two different substrates/nucleotides. However, previous study has reported that, in bases other than m^1A versus m^1G, the methyl transferase can remove the proton from the N1 position in guanine and from the N6 position in adenosine (Swinehart and Jackman, 2015). The biochemical mechanism associated with the N1 proton before the rate-limiting step was reported in mitochondrial *Trm5* gene which might explain its dual specificity for the methylation event (Swinehart and Jackman, 2015). In the Ψ-loop, m^5U_{54} methylation is predominant and all of the cyanobacterial tRNAs contain conserved U_{54} nucleotide. The conserved U_{54} nucleotide plays a crucial role in the conformational conservation that cause refolding of tRNA targets and hence became easier for the specificity of its target enzymes (Alian et al., 2008; Leulliot and Grosjean, 2008). tRNA methylation is very important, and the loss of tRNA methylation has a significant effect on the stability of the tRNA and its function (Alexandrov et al., 2006; Motorin and Helm, 2010; Swinehart and Jackman, 2015; Voigts-Hoffmann et al., 2007). However, the complex and intriguing features of tRNA methylation and its sites are organism specific. Each organism contain its own unique tRNA modification profile (Swinehart and Jackman, 2015). Consequently, there are numerous modifications in tRNA methylation sites in the cyanobacterial species. It is possible that these modifications might also be organism specific as well (Machnicka et al., 2013; Sprinzl and Vassilenko, 2005; Swinehart and Jackman, 2015).

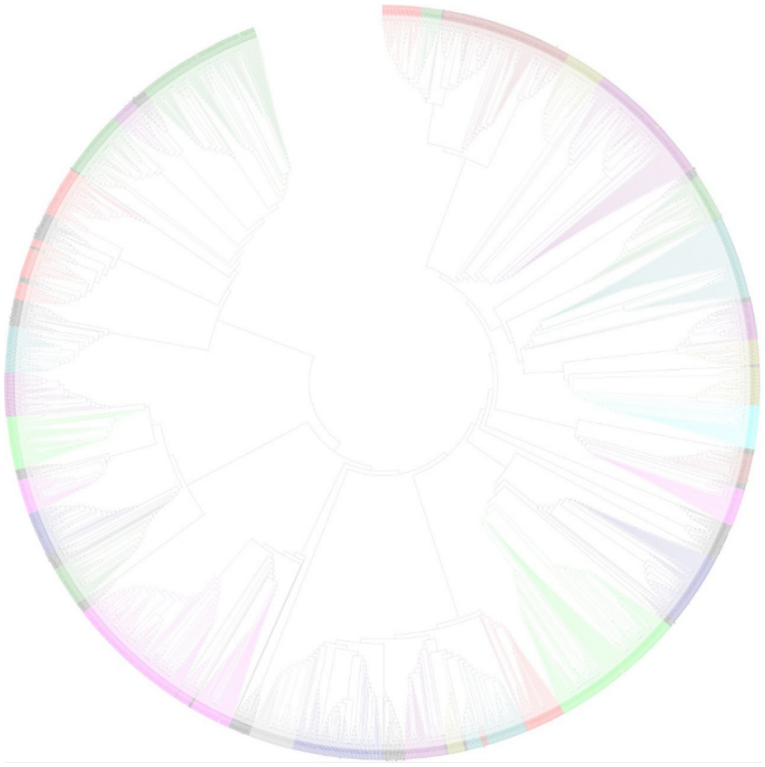

Fig. 3. Phylogenetic tree of anti-codons of cyanobacterial tRNAs. Phylogenetic tree reflects $tRNA^{Met}$ and $tRNA^{Ile}$ were evolved earlier than other tRNA Isoacceptors. Subsequent duplication and divergence of these tRNA Isoacceptors led to origin of other Isoacceptors of tRNAs. The phylogenetic tree was constructed using MEGA6 software with 500 bootstrap replicates (Tamura et al., 2013).

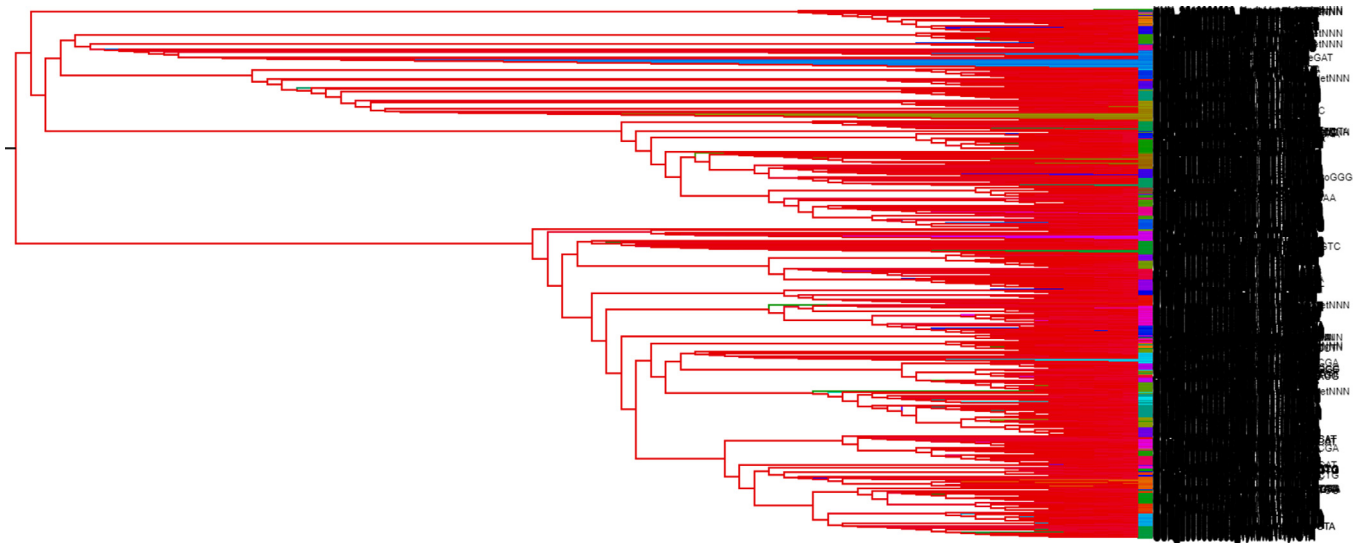

Fig. 4. Recombination events of cyanobacterial tRNAs. The line shading represents locus of recombination event. Different color represents their respective locus and their distribution in the tree. The recombination tree was deduced using IcyTree (Vaughan, 2017).

4.3. tRNA^{Met} and tRNA^{Ile} evolved earlier than other tRNAs

Cyanobacteria is the primitive prokaryotic organism found in diverse habitat of the world and they are considered as the ancestral lineage of the plant kingdom (Bhattacharya and Medlin, 1998; Mulkidjanian et al., 2006). The evolution of cyanobacteria is date back to 2500 million years ago (Mohanta et al., 2016). However, according to the "molecular paleontology", life has been originated some 4000 million years ago. Therefore, the evolution of the molecular messenger tRNA can be traced back to at least 4000 million years ago and hence the anti-codon of tRNAs as well. However, a big question arises, whether all the 21 tRNA isotypes were evolved together or their evolution occurred one after another. To address this question, we conducted an anti-codon based evolutionary study of the cyanobacterial tRNA. We found that tRNA^{Met} and tRNA^{Ile} were evolved earlier than others (Fig. 3). Recombination study revealed, cyanobacterial tRNA undergone vivid recombination (Fig. 4). tRNA^{Ile} was found to be most intact and did not undergone recombination with other tRNA genes (Fig. 4). tRNA^{Met} is associated with transfer of the methionine (1st amino acid) amino acid during the protein translation events (initiation codon) and our study suggests, the anti-codon of tRNA^{Met} that work for initiation of protein translation is evolved earlier than others. The evolution of tRNA^{Met} was followed by the evolution of tRNA^{Ile} and subsequent duplication and deletion events led to the evolution of other tRNAs in the cyanobacteria. The prokaryotic tRNA^{Ile} contain four isodecoders i.e. AAU, GAU, CAU, and UAU whereas tRNA^{Met} contain only one isodecoder i.e. CAU. The CAU isodecoder of tRNA^{Ile} is same as of isodecoder of the tRNA^{Met}. Therefore, tRNAs with CAU isodecoder became an easy choice to encode for two different tRNAs to undergo diversification. Therefore, the evolution of tRNA^{Met} and tRNA^{Ile} occurred earlier than other tRNAs. The phylogenetic tree showed the presence of four distinct clusters that consisted of at least 30 groups (although there are only 20 tRNA isotypes) (Fig. 3). This suggests that the tRNAs and the new anticodons of cyanobacteria were evolved through mutation and gene duplication events. However, the methylation and pseudouridine sites remained conserved suggesting their conserved functional role in the evolution. Multiple studies have been completed regarding the evolution of tRNAs (Root-Bernstein et al., 2016; Sun and Caetano-Anollés, 2008). However, the evolutionary studies were particularly based on the nucleotide sequences of stems and loops of the tRNA molecule (Pak et al., 2017; Root-Bernstein et al., 2016; Sun and Caetano-Anollés, 2008) and we are the first to report about the evolution of anti-codons in tRNAs. Study reported that the upper half of the tRNA is older compared to the dihydrouridine and anti-codon arm (Sun and Caetano-Anollés, 2008). The evolution of tRNA arms and loops also reflect the molecular evolution of ribosomes and rRNA as well. However, the presence of tRNA before 4000 million years ago tell us the story that, the upper half of the tRNA might be older than 4000 million years and ribosomes and rRNA might also be date back to the similar time period of the evolutionary era.

5. Conclusion

Cyanobacterial tRNAs are possess diverse pseudouridine and methylation sites. The presence of conserved Ψ nucleotide is yet to decipher properly and the question remained partially answered. Given its abundant and universal existence, genome has invested enormous effort towards encoding Ψ into the tRNA. Laboratory based experimental study can help to understand the role of conserved Ψ nucleotide in the cyanobacterial tRNA. However, the consensus view is that, Ψ is served the structural role in the tRNA. If a certain amino acid in a protein can serve either a structural or catalytic role, similarly, all Ψ residues might not play the same role at all the positions. Some might be responsible for structural stability while others might be playing catalytic role. Unlike pseudouridine, methylation event can also directly impact the resistance of the tRNA to protect against the ribonuclease and hence play important role to maintain great metabolic stability to the tRNA. In addition, the presence of conserved site plays important role in the evolution to keep the function intact. However, the methylation event adjacent to the anti-codon position can be responsible for the interactions of tRNA with ribosome.

Authors contributions

TKM: Conceived the idea, performed the experiments, analyzed data, drafted and revised the manuscript; AM: Drafted and revised the manuscript, AH: drafted and revised the manuscript; SHQ: revised the manuscript; EFA: drafted and revised the manuscript; ALK: revised the manuscript; AH: revised the manuscript.

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