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Change of tRNA identity leads to a divergent orthogonal histidyl-tRNA synthetase/tRNA^{His} pair

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ABSTRACT

Mature tRNA^{His} has at its 5′-terminus an extra guanylate, designated as G^{-1}. This is the major recognition element for histidyl-tRNA synthetase (HisRS) to permit acylation of tRNA^{His} with histidine. However, it was reported that tRNA^{His} of a subgroup of α-proteobacteria, including *Caulobacter crescentus*, lacks the critical G^{-1} residue. Here we show that recombinant *C. crescentus* HisRS allowed complete histidylation of a *C. crescentus* tRNA^{His} transcript (lacking G^{-1}). The addition of G^{-1} did not improve aminoacylation by *C. crescentus* HisRS. However, mutations in the tRNA^{His} anticodon caused a drastic loss of *in vitro* histidylation, and mutations of bases A73 and U72 also reduced charging. Thus, the major recognition elements in *C. crescentus* tRNA^{His} are the anticodon, the discriminator base and U72, which are recognized by the divergent (based on sequence similarity) *C. crescentus* HisRS. Transplantation of these recognition elements into an *Escherichia coli* tRNA^{His} template, together with addition of base U20a, created a competent substrate for *C. crescentus* HisRS. These results illustrate how a conserved tRNA recognition pattern changed during evolution. The data also uncovered a divergent orthogonal HisRS/tRNA^{His} pair.

INTRODUCTION

Transfer RNAs (tRNAs) fold into a highly conserved and compact common structure, yet the necessity for accurate protein biosynthesis requires tRNA species-restricted recognition by their cognate aminoacyl-tRNA synthetases. This amino acid identity is largely determined by a set of tRNA features termed recognition elements. In the 1980's, the recognition elements for each single aminoacyl-tRNA synthetase class were unravelled; they showed a conservation of the major recognition elements for a particular amino acid among organisms in all three domains of life (1,2). Histidine tRNA (tRNA^{His}) has an added guanylate at its 5′ terminus and this was shown to be the major recognition element (3–5).

Maturation of the 5′ terminus of precursor tRNA involves endonucleolytic cleavage by the ribonucleoprotein RNase P (6,7), which yields a tRNA molecule starting at position 1 [according to standard tRNA nomenclature (8)]. The nearly universal exception to this rule is the tRNA^{His} that starts at the −1 position (9). The additional 5′-terminal nucleotide, usually a guanosine residue (G^{-1}), is required for HisRS to specifically recognize and aminoacylate its cognate tRNA (3–5). The extra base was shown to properly position the tRNA's 5′-monophosphate within an arginine cluster in the active site of *Escherichia coli* HisRS (histidyl-tRNA synthetase) (10–12). The absence of G^{-1} causes a more than 100-fold reduction in the catalytic efficiency of both yeast and *E. coli* HisRS (3,13–15). Other recognition elements include C73 in *E. coli* tRNA^{His}, as well as A73 and—to a lesser degree—the anticodon in yeast tRNA^{His} (16).

Two pathways are known to guarantee that the G^{-1} residue is present in mature tRNA^{His}. In the first pathway, which is found in many bacteria, RNase P exhibits altered cleavage specificity for the tRNA^{His} precursor that maintains the genome encoded G^{-1} residue even though it cleaves the +1 position of all other tRNA precursors (9,17). It has been shown that the structure of the acceptor stem and primarily the pairing between the G^{-1} base and the C73 discriminator base are responsible for this unusual cleavage specificity (18–20). The second pathway is found in Eukaryotes (21,22) and some Archaea (23,24), which often do not encode a G^{-1} residue or remove it during RNase P cleavage. Rather, the G^{-1} is added post-transcriptionally by a tRNA^{His} guanylyltransferase (Thg1), which catalyzes the addition of a guanylate to the 5′-end of tRNA^{His} as an unusual 3′-5′ nucleic acid polymerase (25,26).

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A few years ago, an exception to the assumed universal occurrence of G^{-1} in $tRNA^{His}$ was observed in some α-proteobacteria including members of the Caulobacterales, Parvularculales, Rhizobiales and Rhodobacterales (27,28). Surprisingly, these organisms are viable with a shortened $tRNA^{His}$ (starting at position 1). Computational analyses suggested the co-evolution of a special HisRS containing several unique insertions with these $tRNA^{His}$ species lacking a G^{-1} 5′-terminus (27,28). Additionally, several conserved bases in the acceptor stem of $tRNA^{His}$ were found to be altered in these organisms.

Here, we describe the biochemical characterization of this divergent $tRNA^{His}$ recognition by *Caulobacter crescentus* HisRS and analyze the orthogonality of this unusual $tRNA^{His}$/HisRS pair.

MATERIALS AND METHODS

Materials and reagents

Oligonucleotide synthesis and DNA sequencing were performed by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University. [^{14}C]Histidine (163 mCi/mmol) was obtained from Amersham Pharmacia Biosciences and *E. coli* MRE600 total tRNA was obtained from Roche.

Cloning, expression and purification of recombinant *C. crescentus* HisRS

The HisRS gene was PCR amplified from *C. crescentus* genomic DNA (gift from Dr Christine Jacobs-Wagner) and cloned into a pET20b vector. The resulting vector was then transformed into the *E. coli* BL21 Cd+ strain for recombinant *C. crescentus* HisRS-His_6 expression and purification. Transformed *E. coli* cells were grown in LB medium to A_{600} = 0.8 at 37°C and protein production was induced by the addition of 1 mM isopropyl β-d-thiogalactoside (IPTG). The incubation was continued for 4–6 h at 37°C. The cells were then harvested and resuspended in lysis buffer (50 mM Tris–HCl pH 8.0, 300 mM NaCl, 10 mM imidazole, 7 mM β-mercaptoethanol and protease inhibitor mix; Roche). Cell lysate was generated after sonication and centrifugation, and applied to a Ni-NTA column (Qiagen). The column was washed and the His_6-tagged *C. crescentus* HisRS was eluted according to the manufacturer's instructions. The recombinant HisRS protein was further purified with gel filtration column in buffer containing 50 mM HEPES (pH 7.5), 30 mM KCl, 3 mM $MgCl_2$ and 7 mM β-mercaptoethanol. The purified protein was concentrated and stored in the same buffer with 50% glycerol at −20°C. The *C. crescentus* HisRS was >99% pure, as judged by Coomassie brilliant blue stained SDS–PAGE.

Cloning, *in vitro* transcription and purification of tRNA substrates

Caulobacter crescentus $tRNA^{His}$ and *E. coli* $tRNA^{His}$ variants were synthesized by *in vitro* T7 RNA polymerase run-off transcription as described (29). Two complementary oligonucleotides containing the respective tRNA gene downstream of a T7 RNA polymerase promoter were annealed and cloned into the vector pUC18. To generate tRNA transcripts with the proper 3′-CCA end, a BstNI restriction site was utilized at the 3′-end of each tRNA gene sequence. The *in vitro* transcription reaction was performed at 37°C for 3–5 h in a buffer containing 40 mM Tris–HCl (pH 8.1), 22 mM $MgCl_2$, 5 mM DTT, 1 mM spermidine, 16 mM guanosine monophosphate, 4 mM of each nucleoside triphosphate, BstNI-digested vector containing the template DNA (0.1 μg/μl) and 1 mM T7 RNAP. The tRNA transcripts were purified by electrophoresis on denaturing polyacrylamide gels with 8 M Urea. Full-length tRNAs were eluted and desalted on Sephadex G25 Microspin columns (Amersham).

Histidinylation of tRNA

The histidinylation reaction was carried out with 3 nM purified recombinant *C. crescentus* HisRS at 30°C in buffer containing 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 20 μM ^{14}C-histidine, 5 mM DTT, 2.5 mM ATP and 0.1 mg/ml BSA. The concentrations of tRNA substrates range from 0.2 to 196 μM and the active fractions were estimated with reactions containing saturating amounts of enzyme. To calculate kinetic parameters, initial velocities of histidinylation reactions were obtained by monitoring ^{14}C-His-$tRNA^{His}$ formation. In details, aliquots from the reaction mixtures were removed periodically and spotted on Whatman 3MM paper filter disks (Whatman). After washing in 10% trichloroacetic acid three times, the radioactivity was measured by liquid scintillation counter. Kinetic parameters for histidinylation of the tRNAs were obtained using KaleidaGraph to plot initial velocity versus tRNA substrate concentration followed by Michaelis–Menten curve fitting.

RESULTS

Analysis of $tRNA^{His}$ nucleotide conservation

As the HisRS of nearly all organisms in all domains of life utilizes the unusual G^{-1} base to select its cognate tRNA, we asked how the unusual $tRNA^{His}$ without this extra base is recognized by the *C. crescentus* HisRS. Sequence alignments of $tRNA^{His}$ genes reveal the different conservation of certain bases within the two groups of $tRNA^{His}$ genes, particularly in the acceptor stem (27,28). The first group contains nearly all known $tRNA^{His}$ (G^{-1} $tRNA^{His}$ group) and displays a highly conserved discriminator base C73 as well as the acceptor stem bases G1-C72 in all proteobacteria (Figure 1A). In contrast, the much rarer second group of α-proteobacterial $tRNA^{His}$ without G^{-1} (ΔG^{-1} $tRNA^{His}$ group) has a conserved A73 discriminator base and acceptor stem bases G1-U72 and C3-G71 (Figure 1B). In addition, the D-loop size is always nine nucleotides with an extra base U20a, while the D-loop of the G^{-1} $tRNA^{His}$ group is in most cases only eight nucleotides long. These changes in conserved $tRNA^{His}$ positions were the starting point of analyzing the divergent tRNA identity elements for the *C. crescentus* HisRS.

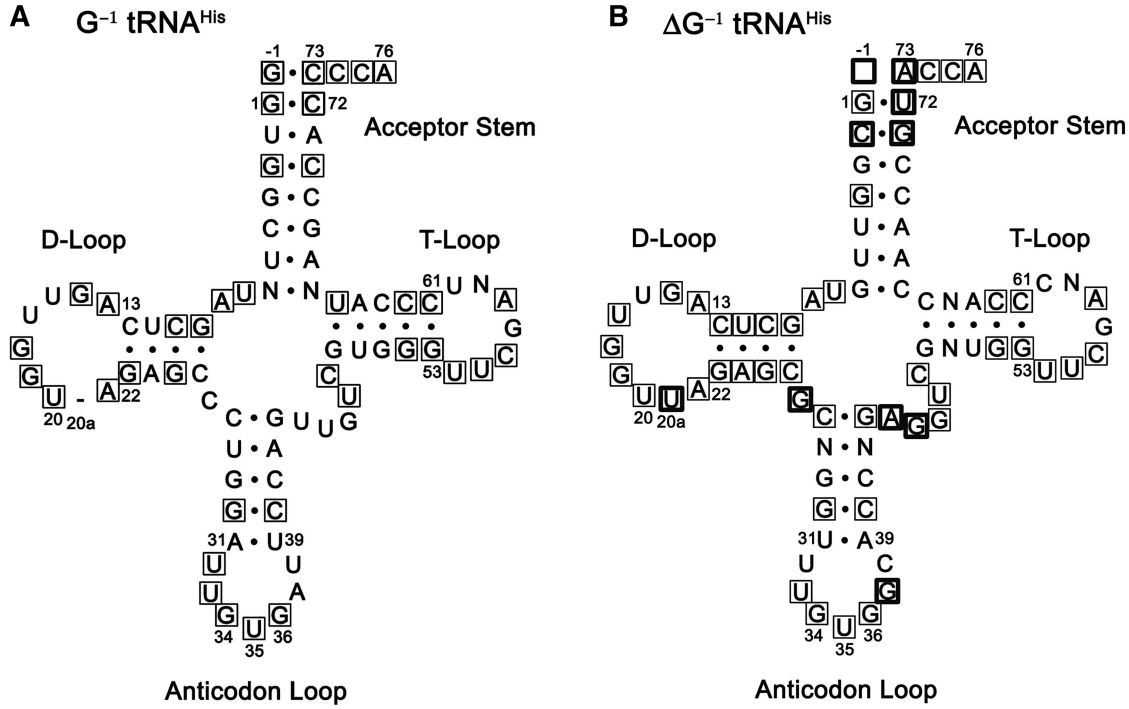

Figure 1. Nucleotide conservation of $tRNA^{His}$. Schematic representation of the dominant nucleotides within the two $tRNA^{His}$ groups. (**A**) The G^{-1} $tRNA^{His}$ group displays an alignment of all proteobacterial $tRNA^{His}$ genes. (**B**) The ΔG^{-1} $tRNA^{His}$ group displays an alignment of the α-proteobacterial $tRNA^{His}$ genes without base G^{-1} deposited in the tRNAdb database (8). N indicates the absence of a dominant base at the specific position. Boxed nucleotides are 100% conserved and thick boxes highlight the divergent conservation between these two groups.

Caulobacter crescentus HisRS efficiently aminoacylates $tRNA^{His}$ transcripts

Caulobacter crescentus HisRS protein (52.3 kDa each monomer) was produced and purified to enable histidylation efficiency analyses of a member of the ΔG^{-1} type HisRS group. The optimal histidylation reaction conditions for *C. crescentus* $tRNA^{His}$ transcripts were determined by titrating the pH value and altering the temperature and salt concentrations. The enzyme showed optimal activity at pH 7.5, a reaction temperature of 30°C and in the absence of KCl. The optimal concentration of HisRS in the reaction was established as 3 nM, which ensures constant reaction velocity in the first 5 min during the histidylation of the wild-type $tRNA^{His}$. *Caulobacter crescentus* $tRNA^{His}$ transcripts showed a catalytic efficiency (Table 1) similar to histidylation of $tRNA^{His}$ in *E. coli* (3).

Caulobacter crescentus HisRS recognizes the anticodon and the acceptor stem of $tRNA^{His}$

Several *C. crescentus* $tRNA^{His}$ mutants were tested for their catalytic efficiency as HisRS substrates (Table 1, Figure 2A). The first set of mutants involved bases in the acceptor stem. It was determined that changing the discriminator base 73 to the standard C found in most bacterial $tRNA^{His}$ species results in a slight increase in K_m value. However, mutation of base 72 decreased the k_{cat}/K_m value approximately 100-fold. Also, the addition of a G^{-1} residue did not improve the catalytic efficiency of the tRNA substrate, but rather increased the K_m value nearly 6-fold. These results indicate that the acceptor stem is still recognized by the ΔG^{-1} type HisRS and substantiate that the absence of the G^{-1} in the *C. crescentus* cell does not negatively impact histidylation.

Next, we determined the location of new recognition elements ($tRNA^{His}$ identity elements) that might have replaced the G^{-1} recognition mechanism *Caulobacter crescentus* $tRNA^{His}$ variants with each of the three anticodon bases replaced with the remaining three bases were analyzed. All twelve possible anticodon mutants showed drastically reduced catalytic efficiency (Table 1) with losses of k_{cat}/K_m ranging between 91- and 1000-fold. Mutation of G37 only had a slight K_m effect on histidylation. In conclusion, the major new identity elements recognized by the ΔG^{-1} type HisRS are the three anticodon bases and base U72 in the acceptor stem, highlighting a drastic deviation from the $tRNA^{His}$ recognition mechanism of standard G^{-1} type HisRS.

Escherichia coli $tRNA^{His}$ becomes a substrate for *C. crescentus* HisRS after identity element transplantation

Next, we tested whether the conventional G^{-1} type $tRNA^{His}$ of *E. coli* can serve as a substrate for *C. crescentus* HisRS. Surprisingly, the catalytic efficiency was drastically reduced [over 250-fold loss of efficiency (Table 2)] even though this tRNA contains the same anticodon sequence (GUG) required for recognition by the ΔG^{-1} type HisRS. Consequently, we decided to analyze the *E. coli* $tRNA^{His}$ for the transplantation of *C. crescentus* recognition elements. Nevertheless, a transplant containing

Table 1. Aminoacylation of *C. crescentus* $tRNA^{His}$ variants by *C. crescentus* HisRS

tRNA	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m	fractional k_{cat}/K_m	Loss of efficiency (x-fold)
WT	3.25 ± 0.26	4.00 ± 0.11	1.231 ± 0.133	1	1
Δ20a	8.44 ± 0.99	4.21 ± 0.31	0.499 ± 0.096	0.405	2
G34U	Nd	Nd	0.014 ± 0.005	0.011	91
G34C	Nd	Nd	0.009 ± 0.003	0.007	143
G34A	Nd	Nd	0.014 ± 0.004	0.011	91
U35A	Nd	Nd	0.001 ± 0.001	0.001	1000
U35C	Nd	Nd	0.007 ± 0.001	0.006	167
U35G	Nd	Nd	0.010 ± 0.002	0.008	125
G36A	Nd	Nd	0.006 ± 0.001	0.005	200
G36C	Nd	Nd	0.005 ± 0.001	0.004	250
G36U	Nd	Nd	0.010 ± 0.002	0.008	125
G37A	8.86 ± 1.12	3.34 ± 0.41	0.377 ± 0.095	0.306	3
U72C	13.74 ± 1.04	0.19 ± 0.01	0.014 ± 0.002	0.011	91
A73C	9.93 ± 0.98	4.38 ± 0.16	0.441 ± 0.061	0.358	3
$+G^{-1}$	19.02 ± 2.99	4.71 ± 0.28	0.248 ± 0.055	0.201	5

Nd, Not determined. The rate of product formation was insufficient with respect to practical experimental time scales to allow determination of steady-state kinetic parameters. The k_{cat}/K_m value is estimated using subsaturating tRNA concentrations.

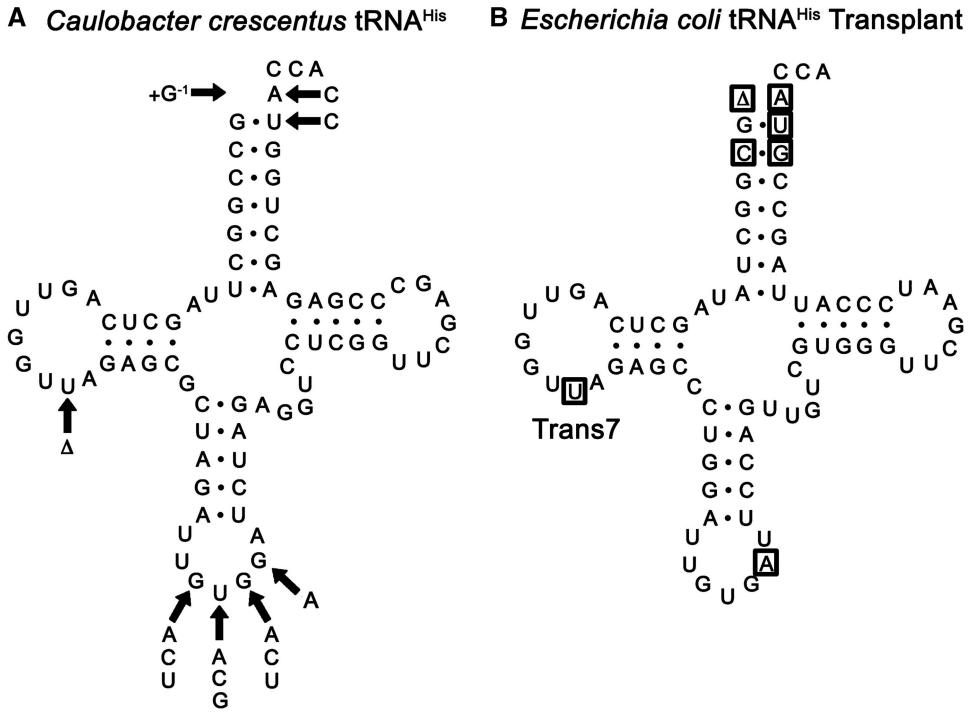

Figure 2. Recognition elements for *C. crescentus* HisRS. (**A**) The investigated mutants of the *C. crescentus* $tRNA^{His}$ transcripts are indicated by arrows. (**B**) Transplantation of the *C. crescentus* HisRS recognition elements into *E. coli* $tRNA^{His}$ (Trans7). Trans6 differs from Trans7 in the size of the D-loop due to the presence of base 20a.

the terminal acceptor stem bases and the anticodon loop base G37 improved the charging efficiency of this hybrid tRNA (Trans 6) only approximately 19-fold. Even the transplantation of the entire *C. crescentus* $tRNA^{His}$ acceptor stem or entire T-stem did not improve histidylation (data not shown). This suggested the presence of further negative determinants for *C. crescentus* HisRS in *E. coli* $tRNA^{His}$. A comparison of $tRNA^{His}$ sequences from both HisRS groups (Figure 1) indicated that the D-loop of the ΔG^{-1} type $tRNA^{His}$ always contains an extra residue, U20a, which is most often missing in other bacteria, including *E. coli*. The addition of this extra nucleotide to Trans 6 (Trans 7, Figure 2B) drastically increased histidylation efficiency, yielding a K_m value similar to the *C. crescentus* wild-type $tRNA^{His}$ transcript and only a slightly reduced k_{cat} value (Table 2). Thus, the size of $tRNA^{His}$ D-loop is important for the *C. crescentus* HisRS to eliminate a negative determinant in *E. coli* $tRNA^{His}$. A larger D-loop likely also influences the overall 3D structure of a tRNA molecule that forms

Table 2. Aminoacylation of *E. coli* $tRNA^{His}$ transplants by *C. crescentus* HisRS

tRNA	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m	fractional k_{cat}/K_m	Loss of efficiency (x-fold)
Cc WT	3.25 ± 0.26	4.00 ± 0.11	1.231 ± 0.133	1	1
Ec WT	Nd	Nd	0.005 ± 0.001	0.004	250
Trans6	32.21 ± 3.71	2.11 ± 0.24	0.066 ± 0.016	0.054	19
Trans7	3.51 ± 0.32	2.72 ± 0.15	0.775 ± 0.127	0.630	2

Nd, Not determined. The rate of product formation was insufficient with respect to practical experimental time scales to allow determination of steady-state kinetic parameters. The k_{cat}/K_m value is estimated using subsaturating tRNA concentrations.

tertiary interactions between the D- and T-loops. However, the reduction of the D-loop size in the *C. crescentus* $tRNA^{His}$ backbone has only a small effect on histidylation efficiency (Table 1).

Caulobacter crescentus HisRS and $tRNA^{His}$ form an orthogonal pair in *E. coli* background

Finally, we tested whether the *C. crescentus* HisRS is able to aminoacylate any *E. coli* tRNA or if the *C. crescentus* HisRS/$tRNA^{His}$ pair would constitute a different orthogonal enzyme/substrate pair for histidylation. Indeed, *C. crescentus* HisRS was not able to aminoacylate total *E. coli* tRNA to a significant level above background (Figure 3). On the other hand, *E. coli* HisRS reached a 30-fold higher plateau level of total tRNA (Figure 3) containing 3.79% of $tRNA^{His}$ (30).

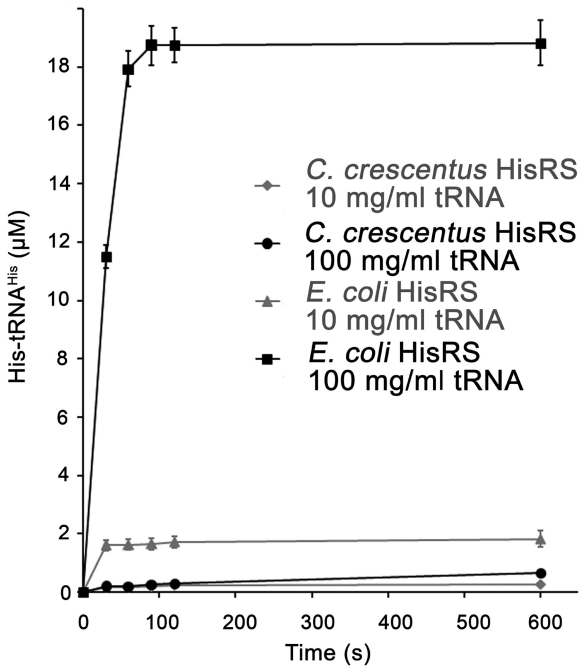

Figure 3. Orthogonality of the *C. crescentus* HisRS/$tRNA^{His}$ pair. HisRS (60 nM) from *C. crescentus* or *E. coli* were used to aminoacylate 10 and 100 mg/ml total *E. coli* MRE 600 tRNA with [^{14}C]histidine. The conditions were optimized for *C. crescentus* HisRS. Error bars represent the standard deviation of two separate experiments.

DISCUSSION

The divergence of $tRNA^{His}$ recognition elements

The recognition of the $tRNA^{His}$ acceptor stem and especially the presence of the G^{-1} base are believed to be necessitated by the low anticodon discrimination ability of HisRS (15,16). Studies focusing on the importance of the $tRNA^{His}$ anticodon have generated conflicting results that might be the consequence of species-specific differences. It was recently shown that yeast can survive without a G^{-1} containing $tRNA^{His}$ as long as both tRNA and HisRS are overexpressed (31). It is also established that the C-terminal domain of *E. coli* HisRS forms binding interactions with the $tRNA^{His}$ anticodon that are important for tRNA selection *in vivo* (15,18). Recent studies indicated that $tRNA^{His}$ anticodon primarily affects the thermodynamics of initial HisRS/$tRNA^{His}$ complex formation, while acceptor stem mutants force a specific kinetic block on aminoacyl transfer and decrease the tRNA-mediated kinetic control of amino acid activation (32). However, other studies concluded that the anticodon contribution for $tRNA^{His}$ recognition is largely negligible (14,33). The importance of the acceptor stem, on the other hand, is unquestioned and evidenced by the histidylation capacity of $tRNA^{His}$ suppressor tRNAs (34), $tRNA^{His}$ minihelices (lacking the complete anticodon arm) (4) and viral tRNA-like structures (14).

The importance of the G^{-1} residue is also underscored by the evolution of two different mechanisms to ensure its presence, namely the unusual RNase P cleavage in Bacteria and guanylyltransferase activity by the essential Thg1 enzyme in Eukaryotes and some Archaea. Therefore, it was initially puzzling to see that a few α-proteobacteria are able to survive with a single $tRNA^{His}$ species that does not contain a G^{-1} residue (27,28). It has been suggested that such $tRNA^{His}$ would be less distinctive among the set of tRNAs in the cell and that either the HisRS might compensate for the lost G^{-1} identity element or the organisms are able to tolerate a decreased $tRNA^{His}$ discrimination (28).

Our results highlight that these α-proteobacteria evolved a special HisRS, that emphasizes the recognition of the $tRNA^{His}$ anticodon, as opposed to the G^{-1} recognition that is found in most organisms. Universal rules for tRNA identity have been described with major determinants that were conserved in evolution (1). Therefore, the described deviation serves as a fascinating example of

drastically altered tRNA species recognition that diverged from the universal tRNA discrimination established in the last universal common ancestor. Other unusual tRNA species recognition mechanisms have been described before, for example for certain mammalian mitochondrial aminoacylation systems (35), but we believe the described change of the $tRNA^{His}$ recognition to be unique in its impact on RNase P, Thg1 and cytosolic HisRS evolution.

The loss of the standard identity elements G^{-1} and C73 appears to be compensated by co-evolution with a HisRS that contains several inserts. HisRS alignments and modeling of the *C. crescentus* HisRS-tRNA structure in comparison to the *E. coli* HisRS indicated one insert that is unique among all anticodon-binding domains of class IIa aminoacyl-tRNA synthetases (28) and is a likely candidate for the observed anticodon discrimination of the ΔG^{-1} type HisRS.

Where does this unusual bacterial $tRNA^{His}$ come from? One striking feature is the difference in the discriminator base in $tRNA^{His}$ as it is always an A73 in Eukaryotes and always a C73 in Bacteria with a G^{-1} type HisRS. It is interesting to note, that of all its tRNAs, only $tRNA^{His}$ has a C73 discriminator base in *E. coli*. The unusual presence of A73 in the tRNAs of Bacteria with a ΔG^{-1} type HisRS suggests that it might have been acquired by lateral transfer from a eukaryotic cell. However, recent phylogenetic analyses concluded that it diverged from an ancestral α-proteobacterial $tRNA^{His}$ gene (27).

It has also been reported, that the discriminator base C73 plays a role in altered RNase P processing in the context of a stable G^{-1}/C73 base pair (18). The presence of A73 in the ΔG^{-1} type $tRNA^{His}$ might decrease cleavage efficiency at the −1 position and might have encouraged evolution of the anticodon recognition mechanism.

It has been hypothesized that the G^{-1} might have a different important cellular function separate from its role in $tRNA^{His}$ aminoacylation and that the standard HisRS may then have evolved to recognize this unique feature (31). While it is difficult to pinpoint such an alternate role for the G^{-1} residue, it should be noted that Thg1 (which guarantees the presence of G^{-1} in eukaryotic $tRNA^{His}$) has been associated with cell cycle control in yeast (36) and DNA replication (37). If this alternate role was not essential or lost in a group of organisms, they would be free to evolve a $tRNA^{His}$ that does not require an elaborate G^{-1} addition mechanism such as either aberrant RNase P cleavage or Thg1 activity. Also, a $tRNA^{His}$ with a conventional acceptor stem might be beneficial during translation in the ribosome as the base pairing between G^{-1} and C73 may decrease the flexibility of the 3′-terminal CCA end tail.

Two divergent HisRS/$tRNA^{His}$ orthogonal pairs

The identification of the altered recognition elements of the ΔG^{-1} type HisRS reveals the presence of two different orthogonal HisRS/$tRNA^{His}$ pairs. Previous studies reported that conventional HisRS is not able to recognize a $tRNA^{His}$ without the acceptor stem identity elements that are missing from the *C. crescentus* $tRNA^{His}$ (Figure 4) (10,18). Similarly, we found that the *C. crescentus* HisRS is also not able to recognize the $tRNA^{His}$ of *E. coli* even though the necessary anticodon is present (Figure 4). Instead, the acceptor stem and especially the D-loop size are antideterminants in *E. coli* $tRNA^{His}$.

These observations indicate that it should be possible to create a cell that contains both of these two different orthogonal HisRS/$tRNA^{His}$ pairs. As the anticodon of the $tRNA^{His}$ with G^{-1} is not recognized by the G^{-1} type HisRS, its backbone can be utilized to construct an amber suppressor tRNA with an 8–20-fold K_m increase (15,34). Thus, a HisRS/$tRNA^{His}_{CUA}$ could be employed in a scenario where the normal histidine codons are accounted for by the ΔG^{-1} type HisRS/$tRNA^{His}$ system. This enables

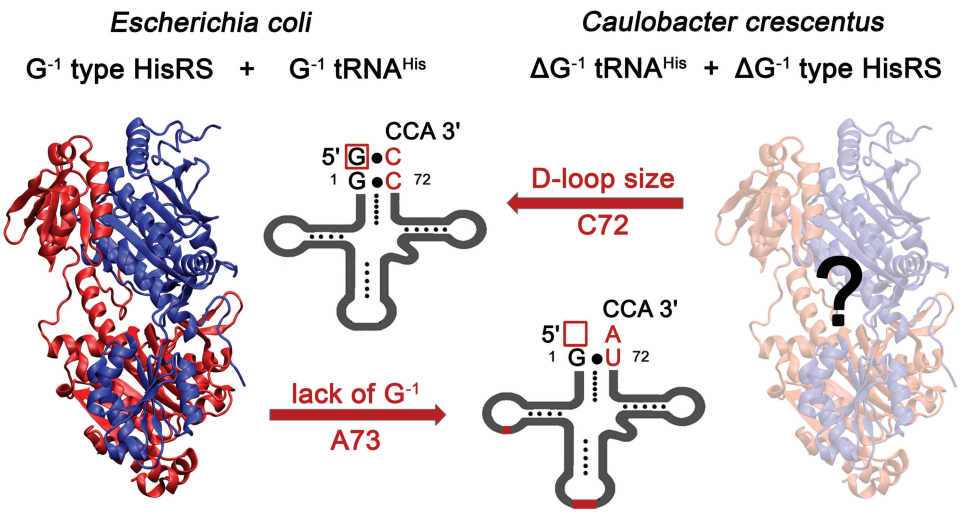

Figure 4. Two divergent HisRS/$tRNA^{His}$ pairs. Two different HisRS/$tRNA^{His}$ pairs are found in Bacteria, the G^{-1} type HisRS/$tRNA^{His}$ pair present in most bacteria (*E. coli* HisRS structure, pdb id 2EL9) and the ΔG^{-1} type HisRS/$tRNA^{His}$ pair found in a few α-proteobacteria. Recognition elements are indicated in red on the schematic tRNA depiction and the elements that prevent charging of the contrary $tRNA^{His}$ are indicated by red arrows. A ΔG^{-1} type HisRS crystal structure is not available.

the engineering of HisRS variants for the targeted incorporation of histidine analogs into proteins. Such analogs would be useful for various studies involving enzyme activity, protein engineering and metal binding site studies among others (38–40).

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