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The structural basis for specific decoding of AUA by isoleucine tRNA on the ribosome

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Summary

Decoding of the AUA isoleucine codon in bacteria and archaea requires modification of a cytidine in the anticodon wobble position of the isoleucine tRNA. Here, we report the crystal structure of the archaeal $tRNA_2^{Ile}$, which contains the novel modification agmatidine in its anticodon, in complex with the AUA codon on the 70S ribosome. The structure illustrates how agmatidine confers codon specificity for AUA over AUG.

The ribosome is the macromolecular enzyme that converts genetic information into protein using a messenger RNA (mRNA) template and transfer RNA (tRNA) substrates. Accurate protein synthesis depends on the ability of the ribosome to faithfully select cognate tRNA by the complementarity of its anticodon to the mRNA codon, while rejecting near and non-cognate tRNA. Interactions with the ribosome ensure strict Watson-Crick base pairing at the first and second positions of the codon-anticodon helix, but allow a variety of non-canonical interactions at the third, or wobble, position (tRNA residue 34)[1]. This 'wobble recognition' is essential to allow a single tRNA anticodon to bind the multiple codons that represent a single amino acid. As many as 40% of all codons are decoded using this type of wobble recognition [2], making it a fundamental principle for synthesis of all proteins across biology.

It is increasingly clear however, that tRNA sequence alone is insufficient for accurate and efficient decoding at the wobble position. Post-transcriptional modifications in and around the tRNA anticodon are ubiquitous in all organisms, and are essential for binding of the tRNA to the ribosome, for maintaining protein reading frame *in vivo*, and ensuring fidelity in protein synthesis (reviewed in [3,4]). The physiologic relevance of these modifications is evidenced by the fact that defects in modification of the wobble base in mitochondrial tRNAs are associated with human disease [5].

Modifications at residue 34 can both expand and restrict the ability of a tRNA to recognize multiple codons [6]. For example, inosine allows decoding of three codons (i.e. NNU, NNC, and NNA) by a single tRNA [7,8]. Conversely, certain modified uridines at the wobble position restrict tRNA recognition to codons ending in a purine residue (i.e. NNR) [9]. In the late 1980s, a third type of wobble modification called lysidine (k^2C), which consists of the amino acid lysine linked to C2 of cytidine, was identified in *E. coli* $tRNA_2^{Ile}$, a minor

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Author contributions DM and CK purified tRNA substrates; RV and CN grew crystals, collected data, built and refined the structure, and analyzed the model; RV, CK, ULR, and VR wrote and edited the manuscript.

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although essential $tRNA^{Ile}$ species [10]. Accurate decoding of the AUA Ile codon by this tRNA requires $tRNA_2^{Ile}$ to discriminate between two purines (AUA vs AUG) in the wobble position, a phenomenon observed in the decoding of only one other amino acid, Trp, for which $tRNA^{Trp}$ must discriminate between the UGG Trp codon and the UGA stop codon. UGA stop codons, however, have unusually high inherent rates of readthrough [11], and only subtle changes in the tRNA structure are required for their suppression [12], indicative of the inherent difficulty of accurate discrimination between purines at this position.

Genetically, $tRNA_2^{Ile}$ contains a CAU anticodon, which alone, is perfectly complementary to the AUG Met codon. However, modification of C34 to k^2C34 in bacteria switches both the amino acid and codon specificity of the tRNA; the k^2C34-modified tRNA is acylated by Ile-tRNA synthetase and decodes the minor AUA Ile codon, while simultaneously rejecting the AUG Met codon [13] (Fig. 1a,b). The enzymes responsible for introducing this modification were later shown to be highly conserved and essential in bacteria [14]. Recently, it was found that archaea use an analogous modification, known as agmatidine (agm^2C), in the wobble position of $tRNA_2^{Ile}$ (refs. [15-17]), suggesting that the requirement for a post-transcriptional modification to discriminate between AUA and AUG codons is conserved across all kingdoms of biology [18]. In order to understand how lysidine and agmatidine, which are derived from cytidine, can base pair specifically with A of the AUA Ile codon but not with G of the AUG Met codon is, we decided to study the interaction of $tRNA_2^{Ile}$ with its cognate codon on the 70S ribosome.

Here we report the crystal structure, solved to 3.2 Å resolution, of the archaeal $tRNA_2^{Ile}$, bound to an AUA codon in both the A and P site on the 70S ribosome (Supplementary Table 1). The structure illustrates how the modification allows binding to the cognate AUA codon, and suggests a mechanism for discrimination against the near-cognate AUG. Due to the chemical similarities between the agmatidine and lysidine modifications, these insights will likely apply more generally across both the bacterial and archaeal kingdoms.

As canonical Watson-Crick interactions are present at both the first and second position of the codon-anticodon helix, the ribosome is, as expected, in its ‘closed conformation’ and the conserved interactions between A1492, A1493, and G530 to monitor the geometry at these positions are observed as previously reported [1,19]. In the wobble position, the A3•agm^2C34 adopts a non-standard geometry that would allow a single hydrogen bond to form between the exocyclic amine of agm^2C34 and N1 of A3 (Fig. 1c). This is unexpected, as two of the three predicted tautomeric forms of lysidine and agmatidine [13,17] could theoretically form two hydrogen bonds with adenosine. The long chain modification at C2 appears to sterically prevent adoption of the canonical Watson-Crick geometry that would be required for this more stable interaction.

Instead, the configuration at the wobble position is more similar to the A3•C34 mismatch observed for binding of the $tRNA^{Trp}$-derived Hirsh suppressor tRNA to the UGA stop codon than to a canonical wobble or Watson-Crick interaction [12] (Fig. 2a). Interestingly, a similar geometry of the A3•agm^2C34 base pair is maintained in the P site of the ribosome as well. Formation of this interaction requires a shift in both the mRNA and tRNA, which is in contrast to previous studies where the mRNA remained stationary while interacting with several modified cognate anticodons [20]. The Watson-Crick interactions at the first and second positions of the codon-anticodon helix appear unaffected by this distorted geometry in the wobble position.

The presence of only a single hydrogen bond between A3 and agm^2C34 in the wobble position raises the question of how and why this is thermodynamically sufficient to stabilize productive binding of $tRNA_2^{Ile}$ to the ribosome. Indeed an unmodified C34•A3 mismatch,

which can also form a single hydrogen bond at the wobble position, is normally disallowed. Based on the structure, it appears that the long agmatine side chain interacts with the backbone of a downstream mRNA residue, as the terminal amine of the agmatine side chain in the A site is within hydrogen bonding distance of an mRNA ribose (O4′) (Fig. 2b). An interaction of this terminal amine with a crystallographic water molecule may also be possible. A similar interaction may also be maintained in the P site, as the terminal amine of the agmatine side chain is within hydrogen bonding distance of the phosphate oxygen of A3 of the P-site codon. Similar hydrogen bonding would also be chemically possible with the terminal amine of the lysidine modification, and therefore may represent a conserved mechanism for stabilizing binding of $tRNA_2^{Ile}$ to the AUA codon (Fig. 3a). These downstream interactions appear to be sufficient to compensate for the weaker interaction at the wobble position, consistent with the observation that only a small perturbation to the energetic balance of the Hirsh suppressor $tRNA^{Trp}$ is required to allow productive recognition of a C•A mismatch at the wobble position [12].

Finally, the structure also suggests a mechanism by which the agmatidine and lysidine modifications could prevent binding of $tRNA_2^{Ile}$ to the near-cognate AUG codon. Modeling a guanosine residue at the third position of the mRNA codon suggests that the agmatidine modification would clash with the exocyclic amine (N2) of the guanosine residue (Fig. 3b). This steric clash would prevent interaction of agm^2C or $k^2C$with G in a canonical Watson-Crick geometry. Furthermore, while it may be sterically possible for the agm^2C or k^2C•G3 pair to adopt a distorted geometry similar to that observed for agm^2C•A3 in this configuration no hydrogen bonding interactions at the wobble position would be possible (Fig. 3b). These combined effects would therefore lead to rejection of modified $tRNA_2^{Ile}$ at the near-cognate AUG codon, explaining how the modification specifically results in accurate decoding on the ribosome.

The role of the agmatidine and lysidine modifications in Ile decoding is a striking example of the essential function of modifications in tRNA and, more generally, RNA biology. It is increasingly evident that while the genetic code was first elucidated over forty years ago, we are just beginning to appreciate the sheer complexity of ensuring accuracy in protein synthesis on the ribosome.

Online Methods

Ribosomes, mRNA, and tRNAs

Thermus thermophilus HB8 70S ribosomes were purified as previously described [21] from cells grown at the Bioexpression and Fermentation Facility at the University of Georgia. mRNAs were purchased from Dharmacon (Thermo Scientific) with sequence: 5′GGCAAGGAGGUAAAA **AUA AUA** AAA 3′ ($tRNA_2^{Ile}$ codons in A and P sites are underlined).

$tRNA_2^{Ile}$ from *Haloarcula marismortui* was purified in several batches by hybrid selection with a biotinylated DNA oligonucleotide bound to streptavidin sepharose as described previously [17,22]. The $tRNA_2^{Ile}$ retained on the column was eluted and further purified by electrophoresis on an 8% native polyacrylamide gel. The yield of purified $tRNA_2^{Ile}$ from 60 L of culture of *H. marismortui* was 11.8 A_{260} units. The purity of the tRNA used in this study was confirmed by *in vitro* aminoacylation with isoleucine and RNA sequencing. The codon specificity of tRNA binding to *H. marismortui* ribosomes has been described previously [17]; the purified tRNA was also shown to bind well to AUA on bacterial ribosomes.

Complex formation and crystallization

Complexes were formed as previously described [21] in buffer G (50 mM KCl, 10 mM NH_4Cl, 10 mM $MgOAc_2$, 5 mM K-HEPES (pH 7.5), 6 mM β-mercaptoethanol). 70S ribosomes, at a concentration of 4.4 μM, were incubated with 3-fold excess mRNA for 6 minutes, followed by a 4-fold excess $tRNA_2^{Ile}$ for 30 min at 55°C. Paromomycin was added to a final concentration of 100 μM and complexes were incubated at room temperature. After addition of Deoxy Big Chaps (Hampton) to a concentration of 2.8 mM, crystals were grown via vapor diffusion in sitting drop trays using reservoir solutions containing 0.1 M Tris-acetate pH 7, 0.2 M KSCN, 3.5-5.5% (w/v) PEG 20K, and 3.5-5.5% (w/v) PEG 550 monomethyl ether (PEG 550 MME). Crystals were cryoprotected stepwise to a final solution containing 30% (w/v) PEG 550 MME and buffer G. Crystals were harvested and frozen by plunging into liquid nitrogen, and data were collected at 100 K.

Data collection and refinement

Data was collected at beamline ID 14-4 of the European Synchrotron Light Source [23], and processed using XDS [24]. Iterative rounds of model building and refinement were carried out in coot [25] and CNS [26]. All figures were produced in Pymol [27].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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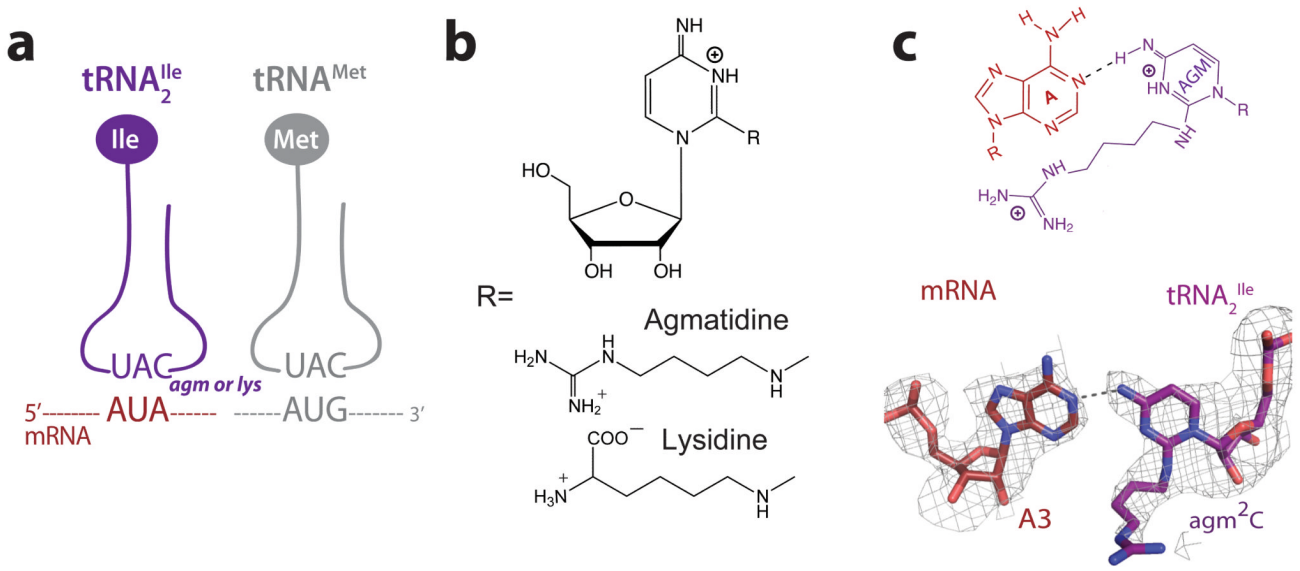

Figure 1.

Decoding of the Ile AUA codon in prokaryotes. A) Post-transcriptional modification of C34 with either lysine or agmatine switches the amino acid and codon specificity of $tRNA_2^{Ile}$ from Met to Ile. B) Chemically, the bacterial and archaeal agmatidine and lysidine modifications are very similar, suggesting they play similar roles in decoding of the AUA codon. C) The crystal structure of the archaeal $tRNA_2^{Ile}$ bound to its cognate AUA codon on the ribosome, demonstrates that a single hydrogen bonding interaction between A3 (red) and agm^2C (purple) forms in the wobble position.

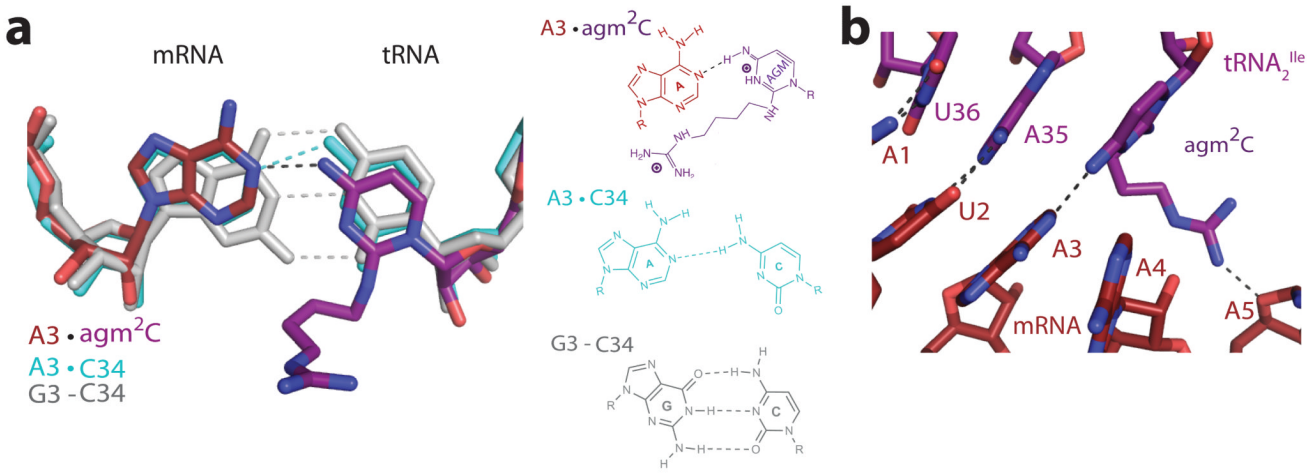

Figure 2.

The role of the agmatidine modification in decoding. A) Comparison of the A3•agm^{2}C wobble pair with a canonical G3-C34 Watson-Crick base pair (grey) and an A3•C34 mismatch (cyan), both observed in the wobble position [12]. B) Interaction of the terminal amine of the agmatidine modification on the A-site tRNA with the backbone of a downstream mRNA residue important for stabilizing the codon-anticodon interaction.

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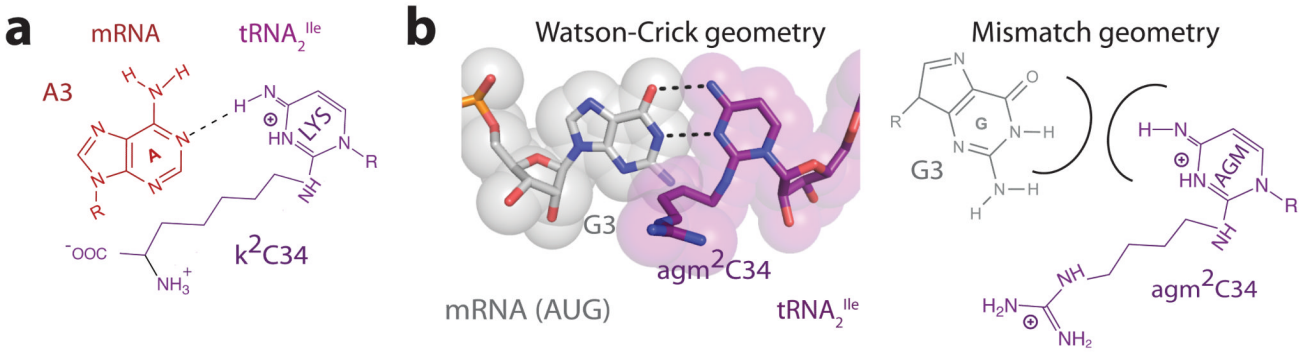

Figure 3.

Predicted implications of this structure. A) Model of how the lysidine modification could allow a similar interaction with A3 as observed for agmatidine. B) Model of how the agmatidine modification could lead to discrimination against the near-cognate AUG codon either by a steric clash with the exocyclic amine of G3 if it were to adopt a Watson-Crick geometry[12], or by its inability to form hydrogen bonding interactions in the mismatch geometry observed in this structure.

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