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Modifications Modulate Anticodon Loop Dynamics and Codon Recognition of *E. coli* tRNA^{Arg1,2}

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Three of six arginine codons are read by two tRNA^{Arg} isoacceptors in *Escherichia coli*. The anticodon stem and loop of these isoacceptors (ASL^{Arg1,2}) differs only in that the position 32 cytidine of tRNA^{Arg1} is posttranscriptionally modified to 2-thiocytidine (s^2C_{32}). The tRNA^{Arg1,2} are also modified at positions 34 (inosine, I_{34}) and 37 (2-methyladenosine, m^2A_{37}). To investigate the roles of modifications in the structure and function, we analyzed six ASL^{Arg1,2} constructs differing in their array of modifications by spectroscopy and codon binding assays. Thermal denaturation and circular dichroism spectroscopy indicated that modifications contribute thermodynamic and base stacking properties, resulting in more order but less stability. NMR-derived structures of the ASL^{Arg1,2} showed that the solution structures of the ASLs were nearly identical. Surprisingly, none possessed the U-turn conformation required for effective codon binding on the ribosome. Yet, all ASL^{Arg1,2} constructs efficiently bound the cognate CGU codon. Three ASLs with I_{34} were able to decode CGC, whereas only the singly modified ASL$^{Arg1,2}{}_{ICG}$ with I_{34} was able to decode CGA. The dissociation constants for all codon bindings were physiologically relevant (0.4–1.4 μM). However, with the introduction of s^2C_{32} or m^2A_{37} to ASL$^{Arg1,2}{}_{ICG}$, the maximum amount of ASL bound to CGU and CGC was significantly reduced. These results suggest that, by allowing loop flexibility, the modifications modulate the conformation of the ASL^{Arg1,2}, which takes one structure free in solution and two others when bound to the cognate arginyl-tRNA synthetase or to codons on the ribosome where modifications reduce or restrict binding to specific codons.

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Abbreviations used: ASL, anticodon stem and loop; COSY, correlated spectroscopy; HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser enhancement spectroscopy; DQF, double quantum filtered.

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Introduction

tRNA molecules translate the genetic code by recognizing triplet codons on messenger RNA (mRNA) during protein synthesis. The ribosome-mediated interaction of the mRNA codons with the anticodon of the tRNA results in discrimination of synonymous *versus* non-synonymous codons. No less than 93 different naturally occurring posttranscriptional modifications are found in tRNAs, each with unique hydrophobic or hydrophilic properties.[1–3] These modifications play an important role in the accuracy and efficiency of protein synthesis. Modifications occurring in the anticodon stem and loop (ASL) domain of tRNAs are the most studied and the best understood. The majority of modifications and the most chemically complex occur at position 34 or at position 37, immediately 3′ adjacent to the anticodon triplet. These anticodon domain modifications alter conformation and thermal stability,[4–10] enhance decoding specificity,[10–14] enhance ribosomal binding,[4,6,15–17] promote proper translocation[17] and maintain the translational reading frame.[18–21] Anticodon domain modifications have been shown to be essential for prestructuring the ASL toward a canonical U-turn structure in solution for proper recognition in the ribosomal A-site.[2,10,18,22]

Escherichia coli has five $tRNA^{Arg}$ isoacceptors, four of which have the modification 2-thiocytidine at position 32, s^2C_{32} (Fig. 1a).[23] The only other *E. coli* tRNA having s^2C_{32} is $tRNA^{Ser2}_{GCU}$.[23] As such, the s^2C_{32} modification is highly interesting. It is one of the rarest of modifications[1] but found within a very common anticodon domain consensus sequence element, $C_{32}U_{33}NNNA_{37}A_{38}$. A C_{32} or s^2C_{32} appears in 56% of all *E. coli* sequences along with A_{38} that appears in 69% of the sequences. The only other known nucleoside thiolations are s^2U_{34} and its derivatives, s^4U_8, and four different 2-methylthio-derivatives of adenosine.[1] In contrast to what is known about these modifications, the contribution of s^2C_{32} to the structure and function of the $tRNA^{Arg}$ isoacceptors and their abilities to decode the six arginine codons has not been explored in detail.[24,25]

The unmodified, primary nucleotide sequence of the ASL of the $tRNA^{Arg1}$ isoacceptor and that of $tRNA^{Arg2}$ are identical.[26,27] Therefore, they are denoted $ASL^{Arg1,2}$ for the purposes of this study to avoid confusion (Fig. 1a). The two isoacceptors are derived from a common gene and only differ at two positions in the entire tRNA sequence. $tRNA^{Arg1}$ has the rare modification s^2C_{32}, whereas $tRNA^{Arg2}$ has an unmodified C_{32} (Fig. 1a). In addition, $tRNA^{Arg1}$ lacks A_{20} of the dihydrouridine loop. A_{20} is invariant in the other four isoacceptors[23,26,27] and has been shown to be an important identity determinant for aminoacylation.[28] Indeed, mutant $tRNA^{Arg2}$ transcripts in which the A_{20} is either substituted or deleted result in a 370-fold decrease in aminoacylation activity, suggesting that $tRNA^{Arg1}_{ICG}$ is aminoacylated with a lower efficiency than $tRNA^{Arg2}$.[29] The anticodon domains of the two isoacceptors also have an inosine modification at the "wobble" position 34 (I_{34}) and a 2-methyladenosine at position

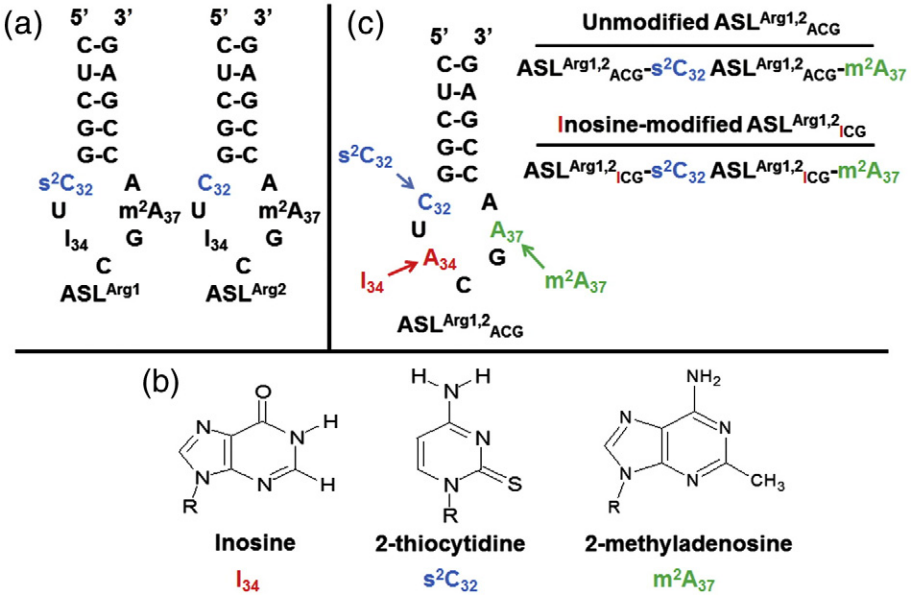

Fig. 1. ASL domains of *E. coli* $tRNA^{Arg}$ ($ASL^{Arg1,2}$) and their modified nucleosides. (a) The primary sequence and secondary structures of the ASLs of the *E. coli* $tRNA^{Arg1}$ and $tRNA^{Arg2}$ with the difference between the two isoacceptors noted as a blue s^2C residue at position 32 of ASL^{Arg1}. (b) The chemical structures of the three naturally occurring modified nucleosides, inosine (I_{34}), 2-thiocytidine (s^2C_{32}) and 2-methyladenosine (m^2A_{37}). (c) Primary sequence and secondary structure of the $ASL^{Arg1,2}$ showing the sites of modification that resulted in the variously modified six ASLs used in this study.