Codon recognition by tRNA

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Summary

Accurate codon recognition by tRNAs is necessary for correct translation of mRNA nucleotide sequence into the protein sequence. Here, different factors contributing to the correct codon reading by tRNAs are reviewed. In particular, the monitoring of codon-anticodon helix geometry by 16S rRNA bases, and the role of tRNA sequence elements and posttranscriptional modifications for modulating codon-anticodon interactions are discussed.

Key words:
tRNA, anticodon, codon recognition, ribosome, translation.

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1. Introduction

Accurate codon recognition by tRNAs is necessary for proper mRNA decoding and faithful translation of mRNA nucleotide sequence into protein sequence. The role of tRNAs in delivering the aminoacids for protein synthesis was first envisioned by Francis Crick in his adaptor hypothesis (1). It was early recognized that the study of the mechanism of codon recognition by tRNA is crucial to understand how the accuracy of translation is achieved. First insights into this mechanism were obtained from the studies on the binding of tRNAs with complementary anticodons (2,3), as well as the binding of tRNAs to complementary oligonucleotides in the absence of the ribosomes (4). Our understanding of the decoding process was greatly increased by the kinetic studies of translation with the use of fluorescently labelled tRNAs (5). However, the detailed mechanism of the codon recognition by tRNA could only be revealed after X-ray structures

of 70S ribosomes (6) and 30S ribosomal subunits (7) complexed with mRNA and tRNAs became available. After more than four decades of tRNA research it is clear that tRNAs are not merely passive adaptors but they play an active role in translation (8).

2. tRNAs, mRNA, rRNAs

Early insights into the mechanism of codon-anticodon recognition were gained from the studies on interactions of tRNAs with complementary oligonucleotides or tRNAs containing complementary anticodons (2-4). The temperature jump method was used to study the stability of tRNA-tRNA interactions and it was shown that the binding of two tRNAs with complementary anticodons was 10^6-times tighter than that expected for two complementary trinucleotides in solution (2). It was further suggested that the correct codon recognition on the ribosome relies on the differences of the thermodynamic stability between correctly matched and mismatched codon-anticodon pairs (3). Also these studies indicated that the structure of the anticodon loop, the identity of the nucleotides adjacent to the codon-anticodon helix, as well as postranscriptional tRNA modifications all affect the stability of the codon-anticodon interaction (2). However, the major disadvantage of these studies, which were performed in the absence of the ribosome, was that the thermodynamic stability differences observed between the complementary codon-anticodon pairs and those mismatched at a single position were too small to explain the very high accuracy of translation.

The X-ray studies of *T. thermophilus* 70S ribosomes complexed with mRNA and tRNAs (7) as well as 30S subunits complexed with mRNA and ASL^{Phe} (6) revealed the structure of the decoding site and suggested an important role of 16S rRNA nucleotides in the mechanism of codon recognition (Fig. 1A). The importance of the nucleotides at positions A1492 and A1493 for codon recognition by tRNA was previously indicated in experiments using mutant ribosomes, which showed that mutations of these 16S rRNA nucleotides affect tRNA binding to the A site in the presence of mRNA (9). These observations were explained by the X-ray structures of the decoding site of 30S subunits complexed with the U$_6$ message and ASL^{Phe} (6). It was shown that the geometry of the codon-anticodon helix is closely monitored by residues A1492, A1493 and G530 of 16S rRNA, which form hydrogen bonds to the minor groove of the codon-anticodon helix. The first base pair of the codon-anticodon helix is monitored by nucleotide A1493 which forms hydrogen bonds with both nucleotides of this pair (Fig. 1B). The 2' OH of the A1493 forms a hydrogen bond with 2' OH of uridine in the codon, and possibly also with the O2 of the uridine base. The N1 of A1493 hydrogen bonds with 2' OH of adenosine 36 of the anticodon. The second base pair of the codon-anticodon helix is monitored by both A1492 and G530, which bind in the minor groove of this pair (Fig. 1C). The 2' OH of the codon forms a hydrogen bond to the N3 and the 2' OH of A1492, while the 2' OH of adenosine
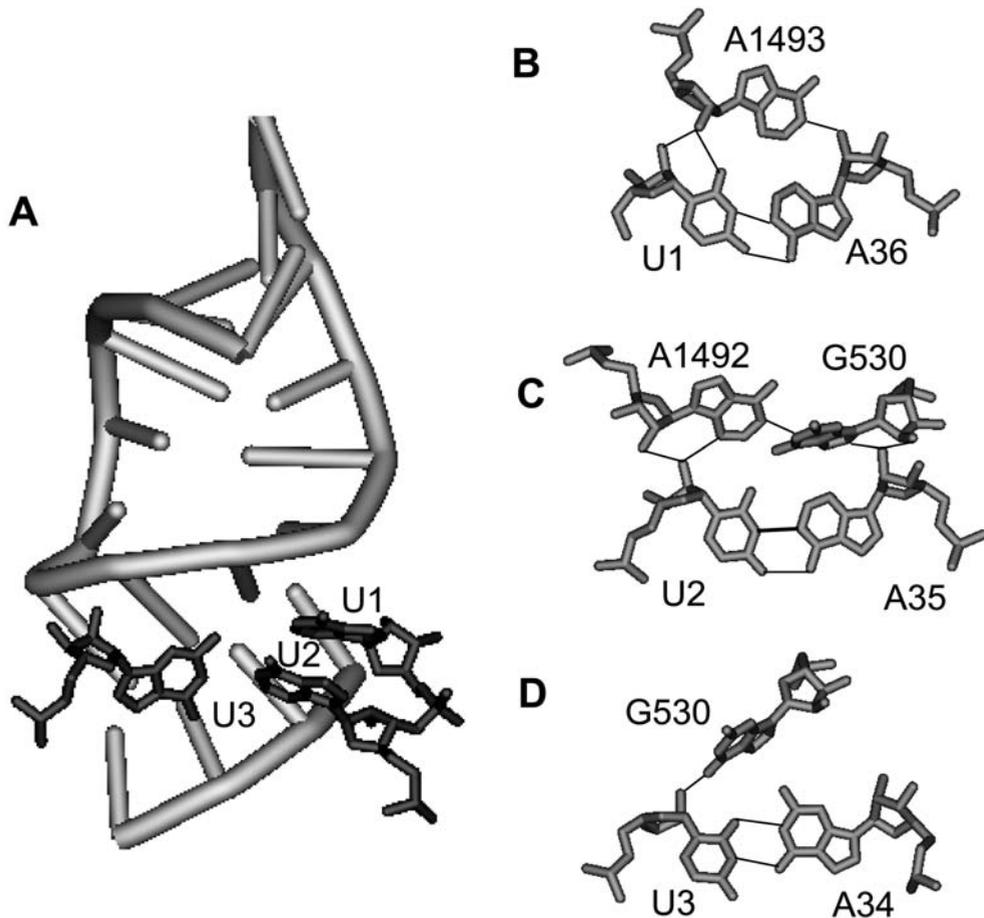
Fig. 1. The anticodon stem-loop of E.coli tRNA[Phe] bound to poly(U) in the X-ray structure of *T. thermophilus* 30S ribosomes (pdb file 1IBL) (6). A general view of the codon-anticodon helix (in grey) monitored by 16S rRNA bases (in black) is shown in (A). On the right, the hydrogen bonding patterns for the first (B), second (C) and third (D) codon-anticodon pairs are presented.

35 hydrogen bonds to the N3 and 2' OH of G530. In the third position only the 2'OH of the codon uridine forms a hydrogen bond with the O6 of G530, and G34 is not monitored by the 16S rRNA bases (Fig. 1D). In summary, the first two base pairs of the codon-anticodon helix are closely monitored by the ribosome allowing to discriminate base pairs with the correct Watson-Crick geometry from incorrect ones, while the third position allows for variations from classic Watson-Crick geometry, which explains wobble decoding.

The X-ray structure data on the role of 16S rRNA bases in monitoring the geometry of the codon-anticodon helix agree very well with available biochemical studies (10-12). In a recent study the roles of individual 2'-hydroxyl groups of the codon-an-

ticodon helix have been evaluated by introducing single 2'-deoxynucleotides into each position of the helix and testing their effects on the tRNA affinity to the ribosomal A site (10). The 2-deoxy substitutions affected tRNA affinity only when they were introduced instead of those 2' hydroxyl groups, which have been shown by X-ray structures of 30S ribosomes (6) to form hydrogen bonds with 16S rRNA nucleotides. The 2'-deoxy substitution at tRNA position 36 was the most detrimental as the affinity of tRNA to ribosomes decreased as much as 70-fold. The effect of substitutions at all codon positions and the position 35 of anticodon were moderate with effects in the range of 3 to 7-fold. However, the substitution of 2'-OH at the position 34 of the anticodon, which does not form hydrogen bonds with 16S rRNA bases, did not have any effect. Interestingly, 2'deoxy substitutions in the codon-anticodon helix are also detrimental for tRNA function in translocation assays (9) and in *in vitro* translation assays (10). All of these data confirm the importance of 2'-OH contacts with 16S rRNA that are observed in X-ray structure for the codon-anticodon recognition and tRNA function on the ribosome.

Posttranscriptional modifications of the anticodon loop nucleotides are essential for the correct codon reading by several tRNAs. Modified nucleotides are present in anticodon loops of most tRNAs (11) and the removal or introduction of individual modifications have been shown to affect codon recognition (14-16). For example, the modification N6-threonylcarbamoyladenosine at position 37 (t(6)A37) of tRNA[Lys] enables it to bind AAA codon in the A site of the ribosomal 30S subunit. However this tRNA needs both t(6)A37 and an additional modification of either 5-methylaminomethyluridine or 2-thiouridine at the position 34 of its anticodon to be able to recognize both AAA and AAG lysine codons (16). Interestingly, removing all the posttranscriptional modifications from aminoacylated tRNAs weakens the binding of some tRNAs to the ribosomal A or P sites but has no effect in case of others (17). Hence, modifications can affect different tRNAs binding to their cognate codons to different extents. The mechanisms according to which some of the modifications affect codon recognition have been revealed. X-ray studies showed that t(6)A37 modification of tRNALys increases its affinity to AAA codon by stacking on the first base of the codon. On the other hand, 2-thiouridine in position 34 was shown to affect both stacking on U35 and hydrogen bonding to guanosine in the third position of the codon, thus allowing for AAG codon binding (16). Moreover, nuclear magnetic resonance spectroscopy studies of unmodified tRNA[Lys] anticodon stem-loops showed that instead of a canonical anticodon loop structure they form a triloop which is not able to bind to its cognate codon (18). The available data show that modifications in different ways affect codon binding by different tRNAs, which suggests that they evolved to tune codon-anticodon interactions to ensure accurate codon reading.

tRNA ability to recognize mRNA codons can also be affected by sequence elements in the anticodon loop or elsewhere in a tRNA body. Proper functioning of tRNA in translation requires a canonical three-dimensional anticodon loop structu-

re, which includes a U-turn motif defined by the formation of the hydrogen bond between N3-H of U33 and an oxygen of the phosphate group of the nucleotide 36 (19). Other conserved elements of anticodon stem-loop include a purine at position 37 and a non-Watson Crick isosteric basepair at positions 32 and 38 (20). This pair has been shown to affect the binding of $tRNA_2^{Ala}$ to its cognate codon. It was proposed that the identity of 32-38 pairs have been selected in evolution to compensate for inherently different codon-anticodon strengths and different contributions of tRNA sequence and modifications to the stability of codon binding by tRNA (21). In case of $tRNA^{Gly}$, it has also been shown that the identity of 32-38 pair can affect tRNA's ability to recognize cognate from near-cognate codons (22,23). However, codon recognition can also be affected by sequence elements positioned far away from the anticodon stem loop. The G24A mutation in $tRNA^{Trp}$ allows it to read not only its cognate UGG codon but also UGA stop codon (24). Recently, the detailed kinetic analysis of this tRNA's performance in translation showed that this mutation affects codon recognition by accelerating the rates of GTPase activation and tRNA accommodation into the A site (8). Clearly, the role of tRNA sequence in the codon recognition by tRNA in not limited to the anticodon trinucleotide but it also includes other sequence elements both in the anticodon stem loop and elsewhere in the tRNA body.

3. Conclusion

In summary, the data reviewed here show that the correct codon recognition by tRNAs results from an intricate interplay of contributions from both the ribosome and tRNA. The codon-anticodon helix geometry is monitored by 16S rRNA bases and is modulated by sequence elements in the tRNA body as well as posttranscriptional modifications. Revealing the mechanism of the codon recognition by tRNAs was crucial to understand the basic mechanism of life, the process of mRNA translation into protein sequence, and should also be important for industrial or pharmaceutical applications by providing hints on the incorporation of non-natural aminoacids into proteins.

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