Biosynthesis and function of tRNA wobble modifications

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Abstract

Post-transcriptional modifications at the first (wobble) position of the tRNA anticodon participate in the precise decoding of the genetic code that is mediated by the codon-anticodon interaction. However, the biosynthesis and functions of many wobble modifications remain unknown. We describe, here, a reverse genetic approach that we used to explore the uncharacterized genes of *Escherichia coli* and yeast that are responsible for the wobble modifications (the Ribonucleome analysis). By combining this method with a comparative genomics approach, we identified an essential gene (*tilS*) that is responsible for the biosynthesis of lysidine at the wobble position of the bacterial $tRNA^{Ile}$ that is specific for the AUA codon. Lysidine is an essential wobble modification that is required for the identity of the tRNA and its AUA codon specificity. *In vitro* reconstitution of the wobble modification revealed the detailed mechanism by which lysidine is synthesized.

Accurate maintenance of wobble modifications is, thus, required for various biological functions. We also show that the subcellular localization of tRNAs in *Leishmania tarentolae* is controlled by different wobble modifications. Moreover, we describe our recent studies that have revealed that the lack of wobble modification of mitochondrial tRNAs leads to translational defects that are associated with mitochondrial diseases, which suggests that disordered RNA modification may be a causative factor of human diseases.

1 Introduction

1.1 The wobble rule and the role of RNA modification in decoding

The genetic code is deciphered by the anticodon of transfer RNAs (tRNAs), which are the adaptor molecules that bind amino acids at their 3' ends and then attach to specific codons in messenger RNAs (mRNAs), thereby, transferring their amino acid to the growing peptide chain. The anticodon (positions 34, 35, and 36) of the tRNA base-pair with a specific codon (positions 1, 2, and 3) in the mRNA strand by hydrogen bonding on the ribosomal A site (Fig. 1). In this interaction, the 2nd and 3rd letters (positions 35 and 36) of the anticodon base-pair with the 2nd and 1st letter of the codon, respectively, by employing Watson-Crick-type pairing rules. A recent study of the crystal structure of the 30S ribosomal subunit revealed

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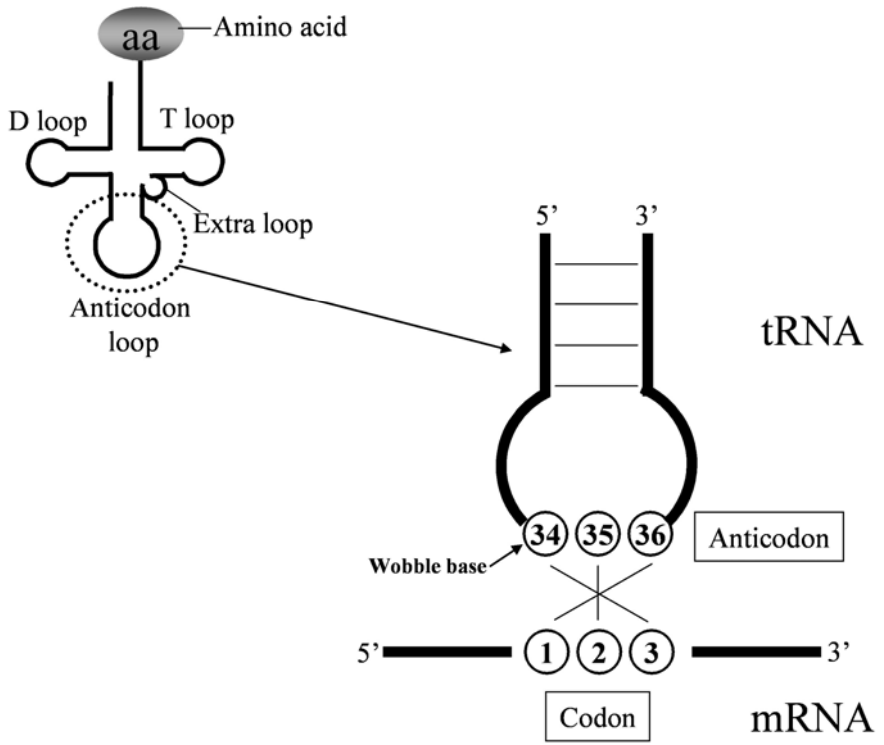

Fig. 1. Base pairing between a transfer RNA (tRNA) anticodon and a messenger RNA (mRNA) codon. Base pairing between the nucleoside at position 34 of the tRNA (wobble nucleoside) and that at position 3 of a codon does not always conform to the Watson–Crick base-pairing rule.

that the conserved bases A1492, A1493, and G530 in the decoding center of 16S rRNA specifically monitor these two Watson-Crick base-pairings by A-minor interactions (Ogle et al. 2002). These interactions induce a large conformational rearrangement in the 30S subunit that may be involved in codon selection and, therefore, the fidelity of the decoding. In contrast, the base-pairing between the 1st letter of the anticodon (position 34) and the 3rd letter of the codon does not always conform to the above rules since G34 pairs with U3 as well as with C3, so that all codons ending with a pyrimidine (e.g. UUU and UUC) are translated to the same amino acid by a single anticodon (Phe for UUU/C translated by $tRNA^{Phe}$ harboring an anticodon GAA). Such irregular pairing is called 'wobble' pairing. The system by which wobble pairing occurs, which was first proposed by Crick (1966), and is known as the 'wobble' rule. Wobble pairing is a well-developed sophisticated system by which 61 sense codons are deciphered by a limited species of tRNAs. There is enough room on the A site of the 30S ribosome to accept wobble pairing (Ogle et al. 2002), which indicates that the wobble pair is not strictly recognized by 16S rRNA during decoding, thus, allowing a number of modified