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Mini-review

The importance of codon–anticodon interactions in translation elongation

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ABSTRACT

Translation is the process by which genetic information is turned into amino acid sequence, following the instructions of the genetic code. The formation of a correct codon–anticodon pair is essential to ensure efficiency and fidelity during translation. Here we review the influence that codon–anticodon interactions play over the elongation phase of translation; including the role of this interaction in cognate tRNA selection by ribosomes, the importance of relative codon frequencies in the cell, and the roles of tRNA modifications in the process of codon–anticodon recognition.

1. Introduction

The translation of genetic information into protein sequence by ribosomes occurs in three phases, namely initiation, elongation and termination. The initiation phase requires the assembly of mRNA, initiator tRNA and the small and large ribosome subunits into an initiator complex, whose composition and regulation vary from kingdom to kingdom and are not the focus of this review (see review [1–3]). Elongation can be defined as the phase of translation in which ribosomal activity generates a polypeptide chain based on the codon sequence of the mRNA. Under normal circumstances termination of polypeptide synthesis happens when a stop codon is identified causing the disassembly of the translation machinery complex.

The efficiency, fidelity, and regulation of elongation during protein synthesis depend upon a large number of factors (see review [4]), among which the pairing between codons and anticodons is central. All steps of translation are regulated through the interplay between each involved factor. Here we will review the current knowledge on the influence that codon–anticodon pairing has over the elongation phase of translation. First we will discuss the conformational changes that, driven by this interaction, regulate the assembly between the ribosome, elongation factors, and tRNAs. We will then review the importance of relative codon–anticodon frequencies in the cell, and how these parameters affect translation efficiency and fidelity. Finally, we will discuss current knowledge on the role of base modifications in the stability of codon–anticodon interaction and decoding. Our current understanding of the roles that fluctuations in the levels of post-transcriptional modifications of tRNA play in the control of protein synthesis will also be discussed.

2. Influence of codon–anticodon recognition during elongation

When elongation of a messenger RNA starts, the P (for Peptidyl) site of the ribosome, the central position of tRNA in the ribosome core, is occupied by fMet-tRNA, and the A (for Acceptor) site is empty and ready to receive a new tRNA that will decode the second codon of the open reading frame (ORF) being translated. This incoming aminoacyl-tRNA has to be selected among a pool of available aminoacylated tRNAs bound to the elongation factor (EF-Tu in bacteria, eEF1A in eukaryotes). The recruitment of a new aminoacyl-tRNA on the A site occurs rapidly, with a rate of up to ~20 amino acids per second in bacteria [5], and ensures the faithful transmission of genetic information to protein sequence with an overall estimated error rate of 10^{-3} to 10^{-5} [6–9]. A fine balance

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between incorporation speed and proper substrate selection is required to ensure translational fidelity and efficiency.

Fidelity during elongation requires the ability to differentiate between cognate and near-cognate tRNAs. To discriminate between near-cognate and cognate tRNAs, a structural dialogue between the decoding center of the small ribosomal subunit and the elongation factor is established. When a candidate aminoacyl-tRNA reaches the decoding center of the ribosome a conformational change occurs in the surrounding ribosomal RNA [10–14]. This change, called 'domain closure', involves interactions between the conserved ribosomal RNA residues A1492, A1493 and G530 and the minor groove of the codon–anticodon helix [13]. The establishment of these interactions depends strictly upon the formation of Watson-Crick base pairs between the two first bases of the codon and the second and third bases of the anticodon. The pairing at the third position of the codon (the wobble position) can accommodate non-Watson-Crick base pairs to adapt the system to the degeneracy of the code (e.g., G:U, I:A, I:U, I:C) [13]. The discrimination between near-cognate and cognate tRNAs also depends on domains of the large ribosomal subunit, whose 23S rRNA component was recently shown to participate in this process through base A1913 [15,16].

The formation of a correct codon–anticodon pair is transmitted through the tRNA in the form of a conformational change. Indeed, recent high-resolution structures of the bacterial ribosome bound to EF-Tu and aminoacyl-tRNA revealed distortions at the anticodon stem and at the junction between the acceptor and D stems (see review [4]). These distortions play a role in the fidelity of translation, as was shown in the analysis of mutations in the D stem of $tRNA^{Trp}$ which allow read-through of UGA stop codons [17]. Eventually, all the conformational information generated by codon–anticodon recognition in the ribosomes, the tRNA, and the elongation factors, influence the GTPase activity that allows the release of the tRNA from EF-Tu/eEF1A in the A site of the ribosome. Non-cognate and near-cognate codon–anticodon pairings that cannot induce the correct structural changes in their environment will prevent GTP hydrolysis, and result in a non-productive complex and the rejection of the aminoacyl-tRNA.

Release of non-cognate aminoacyl-tRNAs can also take place after GTP hydrolysis [18,19] (Fig. 1) if the appropriate structural conformations in the decoding center are not generated. The recently obtained crystal structure of the ribosome-eEF1A2-GDP complex has revealed that the first step of eEF1A-GDP dissociation from the 80S ribosome involves the rotation of the nucleotide-binding domain in eEF1A-GDP upon GTP hydrolysis. This conformational change resembles the dissociation of EF-Tu from the 70S ribosome [20]. In contrast to the bacterial mechanism, however, eukaryotic eEF1A-GDP release does not require Mg^{2+} in the GDP/GTP exchange process [20]. This indicates that eukaryotic GDP/GTP exchange may be a spontaneous reaction that does not require guanine nucleotide exchange factors [20].

Following the release of EF-Tu/eEF1A, the aminoacyl-tRNA is held on the ribosome almost entirely via interactions with the decoding center. The tRNA conformation relaxes, and the acceptor stem is repositioned to promote peptide bond formation. This process is called accommodation. The nascent polypeptide is linked to the A site tRNA and the now deacylated-tRNA in the P site shifts to the E (for Exit) site during the translocation step, with the help of the translocase activity of the GTP-binding protein EF-G (eEF2 in eukaryotes).

The stable interaction of the codon–anticodon duplex with the decoding center is required to overcome the energy threshold needed for translocation after the peptidyl transfer step. The passage of the deacylated- and the peptidyl-tRNAs to the E and P sites respectively depends upon a ratchet-like motion of the small ribosomal subunit against the large subunit. This motion is induced by EF-G/eEF2, and has been recently shown to require the highly conserved loops I and II of the domain IV [21].

Due to the codon–anticodon base pairing, the mRNA moves relative to the ribosome during the translocation step, leaving the A site open to decode the next codon and start a new elongation step. In the E site, the continued codon–anticodon base paring is postulated to prevent potentially serious frame-shift errors. To avoid such errors, the deacylated-tRNA in the E site is released only after the binding of the correct aminoacyl-tRNA in the A site, which causes a decreased of affinity for tRNA in the E site. The P site has also a role in preventing frame-shifts. It was demonstrated that the C-terminal tail of the S9 ribosomal protein prevents both the −1 and +1 frame-shifts [22]. Similarly, the specific contacts that the tip of domain IV of EF-G makes with tRNA and mRNA prevent frame-shifting during the translocation from the A to P site [23].

3. Codon usage and its role in translation efficiency and fidelity

The variation of codon composition is commonly called codon usage bias. There are 61 different codons encoding for 20 different translated amino acids. This codon redundancy, coupled to differences in individual tRNA concentrations, plays an important role in the efficiency and fidelity of translation, and offers the opportunity to individually tune the efficiency and the accuracy of transcript translation. In addition, variations in codon composition induce changes in mRNA secondary structure and stability [24–26].

Codon bias is often measured with the codon adaptation index (CAI) [27]. The CAI was designed to provide a normalized estimate that can be used across genes and species, ranging from 0 to 1. The boundary values refer to the cases in which only the most frequent codons (CAI = 1) or only the least frequent codons (CAI = 0) are used within a gene. A second parameter that is commonly used, the tRNA adaptation index (tAI), gauges the availability of tRNAs at each codon along any given gene [28].

The biology of codon usage has been strongly debated, but it is widely accepted that codon usage is driven by the adaptation of codon usage to tRNA abundance or *vice versa* [29–33]. Indeed, translation elongation rates of specific codons positively correlate with the corresponding tRNA abundances [31,34–36]. As an approximation, the amount of tRNAs in cells is commonly deduced from the number of tRNA-coding genes in the genome [32–34,37–41]. This approximation holds true for some unicellular organisms [37,38] but measurements of the tRNA pool in different tissues and cellular conditions showed significant levels of variability [42,43], indicating that the same gene may be translated differently depending on cellular context.

Codon usage plays an important role in translation elongation rates. Generally speaking, translation elongation rates are thought to be faster along mRNAs with higher codon adaptation to tRNA pools, and *vice versa*. Thus, codon usage potentially allows for a global regulation of translation elongation rates. On the other hand, rare codons may contribute to the accuracy of translation at the expense of speed. Indeed, the ability of unfrequent codons to slow down elongation rates is exploited in regulatory genes, or in cases where excessive protein production may be harmful for the cell [44–46].

A recent report by Gingold et al. [47] has described that two distinct genetic programs, serving proliferating or differentiating cells respectively, present specific codon signatures that are coupled to variations in tRNA populations, suggesting the existence of transcriptional programs that coordinate codon usage and tRNA abundance. In contrast to this observation, a recent study suggests that preferentially used codons are not translated faster than non-preferred ones. Indeed, genome-wide ribosome profiling data has

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