Trends in Genetics

CellPress

Review

Synonymous Codons: Choose Wisely for Expression

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The genetic code, which defines the amino acid sequence of a protein, also contains information that influences the rate and efficiency of translation. Neither the mechanisms nor functions of codon-mediated regulation were well understood. The prevailing model was that the slow translation of codons decoded by rare tRNAs reduces efficiency. Recent genome-wide analyses have clarified several issues. Specific codons and codon combinations modulate ribosome speed and facilitate protein folding. However, tRNA availability is not the sole determinant of rate; rather, interactions between adjacent codons and wobble base pairing are key. One mechanism linking translation efficiency and codon use is that slower decoding is coupled to reduced mRNA stability. Changes in tRNA supply mediate biological regulation for instance, changes in tRNA amounts facilitate cancer metastasis.

Trends

Ribosome speed during elongation is modulated by codon choice, tRNA abundance, and wobble decoding.

Codon pairs act as discrete signals that reduce expression and slow translation.

Codon usage modulates mRNA decay and Dhh1 preferentially targets mRNAs of low codon optimality for degradation.

Codon-mediated effects on translation rates facilitate co-translational protein folding.

Genome-wide and high-throughput analyses of codon use and tRNAs have facilitated recent discoveries.

Changes in tRNA supply facilitate changes in cell state.

Synonymous Codon Choice Affects Translation

Translation elongation is a major determinant of the composition of the proteome, affecting the amount of each protein [1–3], the errors within each protein [4–10], and protein folding [11–16]. During translation elongation, each triplet nucleotide **codon** (see Glossary) in mRNA is decoded in the A-site of the ribosome by interactions with the **anticodon** of its **cognate tRNA**, resulting in the insertion of an amino acid, followed by a precise three-base translocation of the mRNA (and tRNA) to maintain the reading frame (Figure 1). As elaborated below, translation elongation is influenced by the choice of **synonymous codons**, which specify the insertion of the same amino acid, but differ in their decoding properties.

There is a compelling case that synonymous codon choice modulates translation elongation and translation efficiency [17–24]. Synonymous codons differ from each other in their relative use in the genome, in the abundance of tRNAs to decode them, and in the requirement of some codons for **wobble** interactions (non-Watson–Crick base pairing) between the third base of the codon and the first base of the tRNA anticodon [17,24–27]. A subset of codons, called optimal codons, is decoded by abundant tRNAs, efficiently translated, and used nearly exclusively in many highly expressed genes in yeast and *Escherichia coli* [17,18,21,23,28]. Moreover, the influence of codon choice on translation efficiency is underscored by genome-wide correlations between codon use and translation efficiency [29–34], and by numerous examples in which recoding genes with optimal or suboptimal codons changes expression as predicted [32,35–41].

However, despite more than 30 years of analysis, the mechanism(s) underpinning codon-mediated effects on translation are not well understood. The principal ideas have been that the rate of translation elongation at different codons depends upon the supply of tRNA [18,21,34,41–46], and that slower translation elongation rates reduce translation efficiency. Thus, it is generally thought that reduced translation rates and yield are due to an accumulation of small effects from individual codons decoded slowly by rare tRNAs. There were numerous

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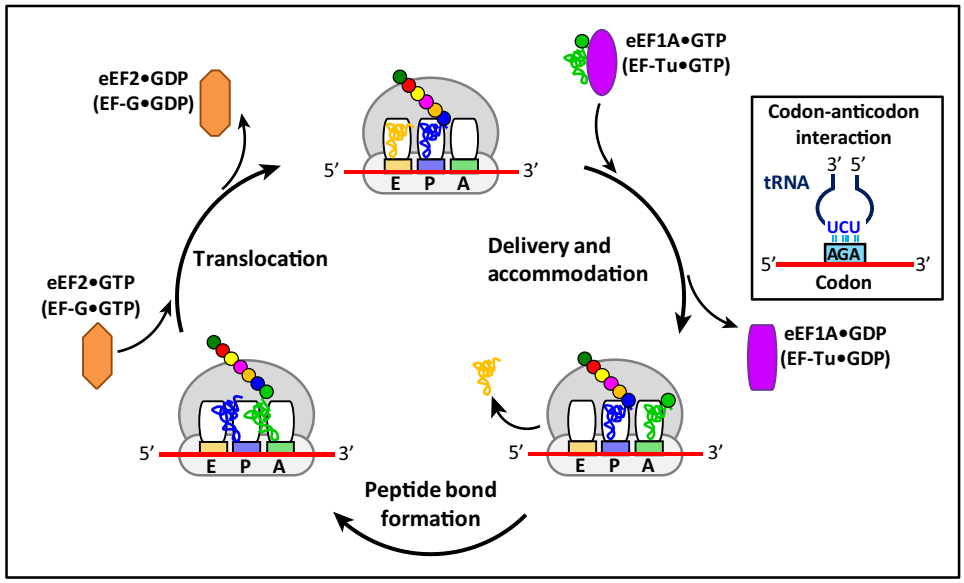

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Figure 1. Schematic of Translation Elongation. The top diagram shows an elongating ribosome, including the three sites for tRNA binding (A, P, and E) that span the large and small ribosomal subunits; the nascent polypeptide (which is attached to the P site tRNA and exits through the exit tunnel in the large subunit); and the mRNA (which enters and exits through the small subunit). Bacteria-specific components are shown in parentheses. First, addition of a new amino acid begins with the delivery, recognition, and accommodation of an **aminoacyl-tRNA** into the A site of the ribosome. In this step, GTP-bound elongation factor (eEF1A•GTP in eukaryotes or EF-Tu•GTP in bacteria) delivers an aminoacyl-tRNA to the A site of the ribosome, where base pairing interactions between the anticodon of the tRNA and the three bases of the codon in the mRNA trigger hydrolysis of GTP, release of the deacylated tRNA from the E site of the ribosome, release of the GDP-bound elongation factor, and accommodation of the tRNA (a second proofreading step that depends upon codon-anticodon base pairing). Second, the addition of the amino acid to the growing polypeptide requires peptide bond formation, which involves close approximation of the 3′ ends of the aminoacyl and the peptidyl site tRNAs in the **peptidyl transferase center** of the large subunit, and large-scale movement of the ribosomal subunits relative to each other to form a hybrid state. Third, to complete the process, translocation of the mRNA (with its associated tRNAs) involves another GTP-bound elongation factor (eEF2•GTP in eukaryotes or EF-G•GTP in bacteria), and large-scale ribosome movement to restore the classical state. This process is repeated until the ribosome encounters a termination codon

indications that this model did not fit the facts, including unpredictable effects of changing the coding sequence on expression [37]. The rate-limiting factors in translation elongation were unknown, due in part to a lack of information about specific codons or codon combinations that either slow translation or reduce translation efficiency. Furthermore, it was not understood how differences in rates of translation elongation bring about reduced protein expression or why suboptimal codon use is important for health of the organism. Recent work using newly developed high-throughput methods has shed light on these questions.

Codon-Dependent Differences in Elongation Rate Are due to Wobble, tRNA, and Gene Position

One impediment to understanding how codon choice affects translation has been the inability to distinguish the effects of individual codons on the rate of translation elongation, an essential piece of information to the deduction of rate-limiting factors. Differences in the rates of decoding were detected in some early studies in *E. coli* in a variety of assays [47–50]. For instance, a threefold difference in translation rates of Glu GAA and GAG codons, which are decoded by a single **isoacceptor tRNA**, was discerned by determining the *in vivo* rate of translation of lacZ with 30–60-codon inserts of GAA or GAG (using a pulse-chase with [^{35}S]methionine and examination of synthesis of radioactive β-galactosidase protein) [51]. However, these assays

Glossary

Aminoacyl-tRNA: a tRNA in which an amino acid is covalently attached to its 3′ end; also referred to as charged tRNA.
Anticodon: the three nucleotides in the anticodon loop of a tRNA that base pair with the codon in the mRNA. In the tRNA, these nucleotides are numbered 34, 35, and 36 in the 5′ to 3′ direction, with nucleotide 36 base pairing with nucleotide 1 of the codon. Thus, nucleotide 34 is responsible for wobble base pairing with nucleotide 3 of the codon.
Codon: three adjacent nucleotides in an mRNA that specify insertion of a particular amino acid to the growing polypeptide chain.
Cognate tRNA: the tRNA with an anticodon that forms complementary base pairs with the codon and is selected preferentially by the ribosome when decoding that codon.
Isoacceptor tRNAs: the set of tRNAs that insert the same amino acid; they may interact with different codons.
Near-cognate tRNA: a tRNA that contains a single base mismatch between its anticodon and the codon in question.
Peptidyl transferase center (PTC): the location within the large ribosomal subunit at which a new peptide bond is formed, linking the nascent polypeptide to the next amino acid.
Shine-Dalgarno: ribosomal binding site found in mRNAs in bacteria and Archaea that directs translation initiation.
Synonymous codons: different codons that specify insertion of the same amino acid to the growing polypeptide chain.
Wobble decoding: interactions in the ribosome in which the tRNA anticodon forms Watson–Crick base pairs with the first two bases in the codon, but forms a non-Watson–Crick base pair between N34 of the tRNA and the third base in the codon. The wobble hypothesis was initially proposed by Francis Crick in 1966.