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Translation and mRNA Stability Control

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Abstract

Messenger RNA (mRNA) stability and translational efficiency are two crucial aspects of the post-transcriptional process that profoundly impact protein production in a cell. While it is widely known that ribosomes produce proteins, studies during the past decade have surprisingly revealed that ribosomes also control mRNA stability in a codon-dependent manner, a process referred to as codon optimality. Therefore, codons, the three-nucleotide words read by the ribosome, have a potent effect on mRNA stability and provide cis-regulatory information that extends beyond the amino acids they encode. While the codon optimality molecular mechanism is still unclear, the translation elongation rate appears to trigger mRNA decay. Thus, transfer RNAs emerge as potential master gene regulators affecting mRNA stability. Furthermore, while few factors related to codon optimality have been identified in yeast, the orthologous genes in vertebrates do not necessary share the same functions. Here, we discuss codon optimality findings and gene regulation layers related to codon composition in different eukaryotic species.

Contents

1. INTRODUCTION

Ribosomes translate the nucleotide sequence encoded in messenger RNA (mRNA) into amino acids by reading the coding sequence in three-nucleotide words called codons. While widely known for their primary function in protein synthesis (**Figure 1*a***), ribosomes are becoming increasingly recognized as important regulatory players. For example, ribosome and proteomic profiling analyses have revealed a large number of small (10 to 100 codons), translated open reading frames (ORFs) within previously designated untranslated regions (UTRs) and long noncoding RNAs (1–6). Although some of the peptides derived from small ORFs serve physiological functions (7), translation of small ORFs in 5′ UTRs (i.e., upstream ORFs) or 3′ UTRs (downstream ORFs) has been shown to have negative or positive regulatory effects, respectively, on the translation of adjacent main ORFs (**Figure 1*b*,*c***) (2, 8–10). In addition, ribosomes drive mRNA quality-control mechanisms by triggering degradation of abnormal mRNAs (**Figure 1*d***) (11). There are three translation-mediated mRNA decay routes that mitigate the deleterious effects of problematic mRNAs: nonsense-mediated mRNA decay (NMD) through recognition of premature stop codons, nonstop decay (NSD) through recognition of mRNAs lacking stop codons, and no-go decay (NGD) triggered by translational stalling (**Figure 1*d***). More recently, the ribosome has been shown to have prominent regulatory roles beyond translation of small ORFs or quality control. In particular, emerging evidence suggests that the act of translation itself appears to be coupled to mRNA stability to regulate gene expression on a global scale in eukaryotes through a mechanism referred to as codon optimality (**Figure 1*e***) (12).

2. CODON OPTIMALITY

Over the past decade, a growing body of evidence has emerged illustrating that codon composition can have a potent impact on mRNA stability and that information within the coding sequence extends beyond the amino acids it encodes (12–17). Hence, ribosome translation strongly influences mRNA stability in a codon-dependent manner, a process referred to as codon optimality (**Figure 2*a***) (12). Certain codons are beneficial for gene expression (optimal codons), leading

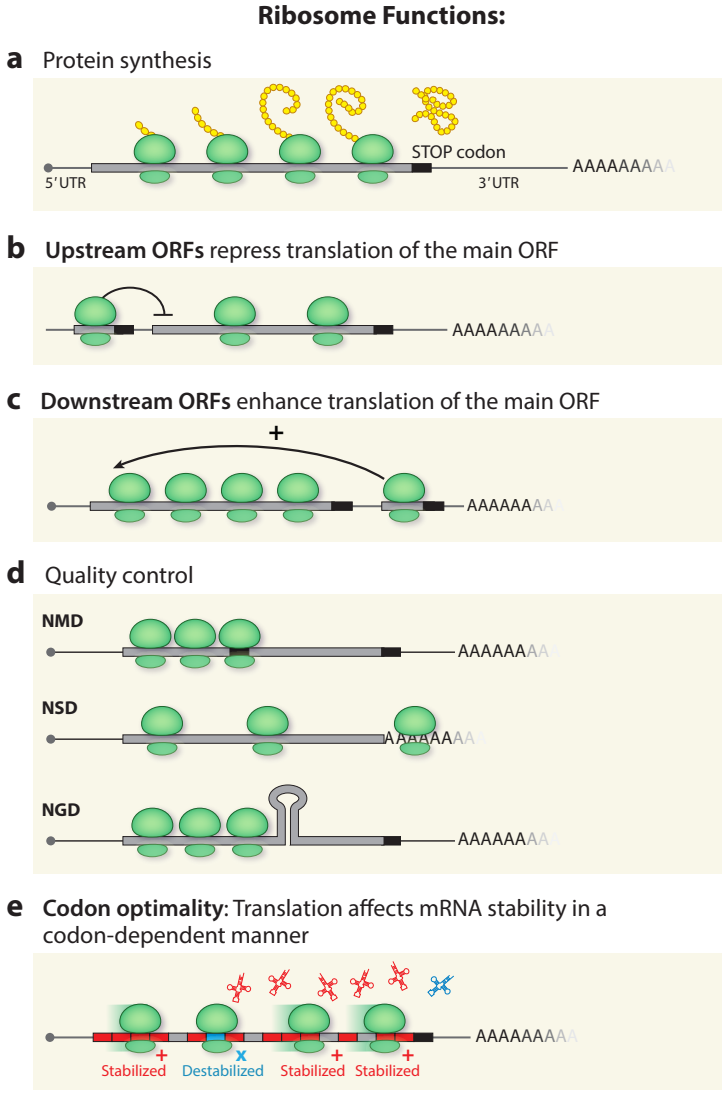

Figure 1

Function of ribosomes. (*a*) Ribosomes are the protein-production factories. (*b*) Translation of upstream ORFs in the 5′ UTR represses translation of the canonical ORF. (*c*) Translation of downstream ORFs in the 3′ UTR enhances translation of the canonical ORF. (*d*) Ribosome quality-control pathways for abnormal RNA: NMD, NSD, and NGD. (*e*) Translation affects normally processed mRNA stability in a codon-dependent manner, termed codon optimality. Abbreviations: NGD, no-go decay; NMD, nonsense-mediated decay; NSD, nonstop decay; ORF, open reading frame; UTR, untranslated region.

to increased mRNA stability, increased mRNA and protein levels, greater translation efficiency, and longer poly(A)-tail length, while other codons have the opposite effect (nonoptimal codons) (**Figure 2*b*,*c***) (12–14, 17, 18). The codon-mediated impact on mRNA stability was first described in yeast (12, 18, 19) and then extended to multiple species such as *Escherichia coli* (20), *Danio rerio* (13, 17), *Xenopus* (13), *Trypanosoma brucei* (21, 22), *Drosophila melanogaster* (23), plants (24), mice (25), and human cells (**Figure 2*d***) (14, 26, 27).

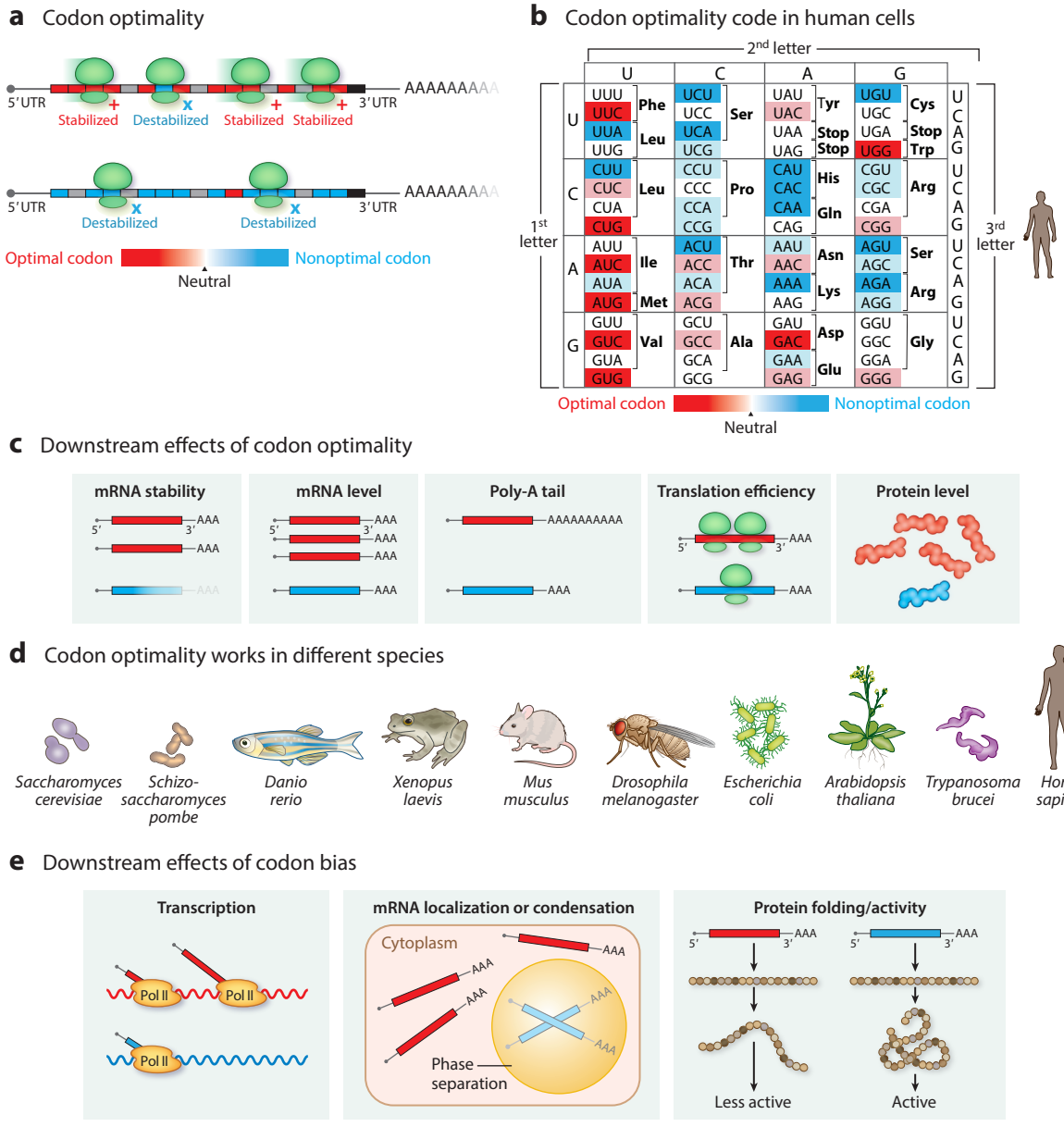

Figure 2

Codon optimality overview. (*a*) Translation of optimal codons (*red*) tends to stabilize mRNA, while translation of nonoptimal codons (*blue*) destabilizes mRNA. (*b*) Heatmap showing the codon optimality code underlying the genetic code in human cells. (*c*) Codon optimality can influence gene expression at multiple levels. Genes enriched in optimal codons exhibit higher RNA stability, higher RNA level, longer poly(A) tail, higher translation efficiency, and higher protein levels than genes enriched in nonoptimal codons. (*d*) Codon optimality effects on gene expression were observed in multiple species. (*e*) The codon composition can influence RNA transcription, mRNA localization, and condensation, as well as protein folding and function. Abbreviations: Pol II, polymerase II; UTR, untranslated region.

Besides influencing mRNA stability, codons can also influence gene expression at multiple stages. For example, codon optimality affects gene expression at the translation efficiency level (**Figure 2*b***) (13, 28). Genes enriched in optimal codons displayed higher translation efficiency based on ribosome profiling in different species (13). Differences between reporters enriched in optimal or nonoptimal codons tend to be greater at the protein level than at the RNA level, supporting translation efficiency differences (12, 13, 29). It was also recently proposed that enrichment of nonoptimal codons might repress translation initiation (30).

Original work in Neurospora revealed that codon composition impacts transcription, triggering chromatin modification changes (e.g., H3K9me3 modification) (31) and early transcriptional termination for mRNAs containing rare codons (32); interestingly, these effects are promoter dependent (33) (**Figure 2*e***). In addition, RNA splicing and mRNA localization can also be influenced by nucleotide bias and thus codon content (**Figure 2*e***) (34–36). Codon composition can also affect cotranslational protein folding (**Figure 2*e***) (37), which influences protein conformation, stability, and function (38–40).

In sum, ribosomes interpret two codes within mRNA: the genetic code, which specifies the amino acid sequence, and a regulatory code, which shapes mRNA stability, RNA level, and protein activity across multiple organisms (**Figure 2*b***) (41). In this review, we focus on codon optimality and how translation affects mRNA stability in a codon-dependent manner in eukaryotes.

2.1. Codon Optimality Versus Codon Usage

Quite often, the term codon optimality is confused with codon usage. Codon optimality refers to the ability of a given codon to affect mRNA stability in a translation-dependent manner, while codon usage refers to the frequency of each codon in a given transcriptome. In vertebrates, the correlation between codon optimality and codon usage is weak (14). In human cells for example, GAA is a nonoptimal codon but also one of the most frequently used codons, while ACG is a rare yet optimal codon (14, 26, 27). Therefore, it is important to maintain clear definitions for codon usage and optimality.

Besides the codon optimality scores (see Section 2.2), other parameters have been used to study the relationship between codon usage and gene expression. For example, the codon adaptation index (CAI) attempts to assess synonymous codon bias based on highly expressed genes like ribosomal proteins (42). As such, the CAI is a measure of codon usage rather than codon optimality. The tRNA adaptation index is a metric that considers tRNA copy number to indicate tRNA abundance in translating each codon (43). In yeast, some of these metrics correlate with the codon optimality scores (12), but in higher organisms, they are not strong predictors of mRNA stability, which is likely due to highly abundant tRNA copy number and much more complex tRNA regulation (39). Depending on the species, there is a significant correlation between GC content and codon optimality, especially for codons containing either G or C at the third base position (GC3) (44). Together, these methods aim to uncover the relationship between codon usage and mRNA stability. Although correlations have been observed, it is essential to validate genomic observations with large classes or types of reporters.

2.2. Optimal Versus Nonoptimal Codons

The codon optimality code, or the assessment of the regulatory impact of all 61 coding codons, has been measured by assessing the relationship between codon composition and mRNA stability profiles, either of endogenous transcripts or of massive parallel reporter libraries in the presence or absence of translation (12–14, 26, 27). One of the most common parameters to determine codon optimality is the codon stabilization coefficient (CSC), the Pearson correlation coefficient between

mRNA half-life and codon frequency (12). Optimal codons have a positive CSC because they tend to appear in stable mRNAs, while nonoptimal codons have a negative CSC since they are typically enriched in unstable mRNAs. The CSC has previously been calculated using endogenous mRNA decay profiles after blocking transcription using genetic tools (e.g., rpb1–1 transcriptional shut-off experiments in yeast) (12) and/or drugs (e.g., α-amanitin in zebrafish and *Xenopus* embryos or actinomycin D in human cells) (13, 14). However, since blocking transcription can have pleotropic consequences, other techniques have been developed that incorporate modified nucleotides [e.g., SLAM-seq (thiol(SH)-linked alkylation for the metabolic sequencing of RNA) (45), TimeLapse-seq (46) or BRIC-seq (5′-bromo-uridine immunoprecipitation chase-deep sequencing analysis) (47)] and are used to calculate the codon optimality scores (14).

A challenge lies in differentiating the codon effects from other mechanisms that impact mRNA stability, such as those driven by cis-regulatory elements that lie outside of coding sequences (e.g., microRNA in the 3′ UTR). To parse, libraries have been used in which different coding sequences (e.g., ∼17,000 human coding sequences) are fused to common 5′ and 3′ UTR regions (48) to determine mRNA stability (13, 14, 26). Attempts to define codon optimality parameters have also involved the injection of massive mRNA reporter libraries (>1.5 million unique coding sequences) into zebrafish and *Xenopus* embryos at the single-cell stage (13, 29, 49, 50). This allows the relationship between codon composition and mRNA stability to be assessed without blocking endogenous transcription. Furthermore, the dependence on active translation can be assessed through the use of a single inhibitory morpholino that binds a common 5′ UTR or translation initiation site found within all introduced mRNA reporters (13, 49, 50).

These approaches have revealed a complex regulatory syntax underlying codon optimality. The regulatory effects of the ribosome on mRNA stability appear to be influenced not only by codon composition (e.g., optimal:nonoptimal codon ratio) but also by the positioning of codons across the coding sequence. Both optimal and nonoptimal codons within the 3′ end of the coding sequence tend to influence stability more heavily than similar codons located at the 5′ end (18, 50). Further, there appear to be neighbor effects, whereby specific dicodons (sequences of two codons) have distinct regulatory effects (51).

2.3. Codon Optimality as a Major Determinant of Gene Expression

The term mRNA expression level is usually associated with transcription; however, the amount of any transcript in a cell depends on both its transcription and its stability. For decades, the canonical view of mRNA stability in higher organisms has focused on the 3′ UTR, where the most-studied cis-regulatory elements are located (**Figure 3*a***). For example, microRNAs are small RNA molecules that repress translation and/or destabilize target mRNAs through the recognition of regulatory elements located mainly in the 3′ UTR (52). Several other cis-regulatory sequences or structural motifs, and/or RNA modifications such as methylation (m^6A) are also primarily found in the 3′ UTR (53, 54). However, the 3′ UTRs in most vertebrates represent just 30% of the overall sequences comprising the transcriptome. Further, since 3′ UTR regulatory pathways influence only those mRNAs that possess the corresponding target sequences, their impact is neither universal nor genome wide.

The coding sequence accounts for more than 60% of the transcriptome in most vertebrates, and codon composition has been shown to be a major determinant of mRNA stability from yeast to humans (**Figure 3*b***) (12, 50). Unlike other regulatory elements that simply target mRNAs with related seeds or binding sites, codon optimality is a mechanism that potentially influences every translated mRNA. Interestingly, genes within the same pathway tend to share similar codon optimality characteristics, providing a way to synchronize regulation inside the cell (12, 27, 29).

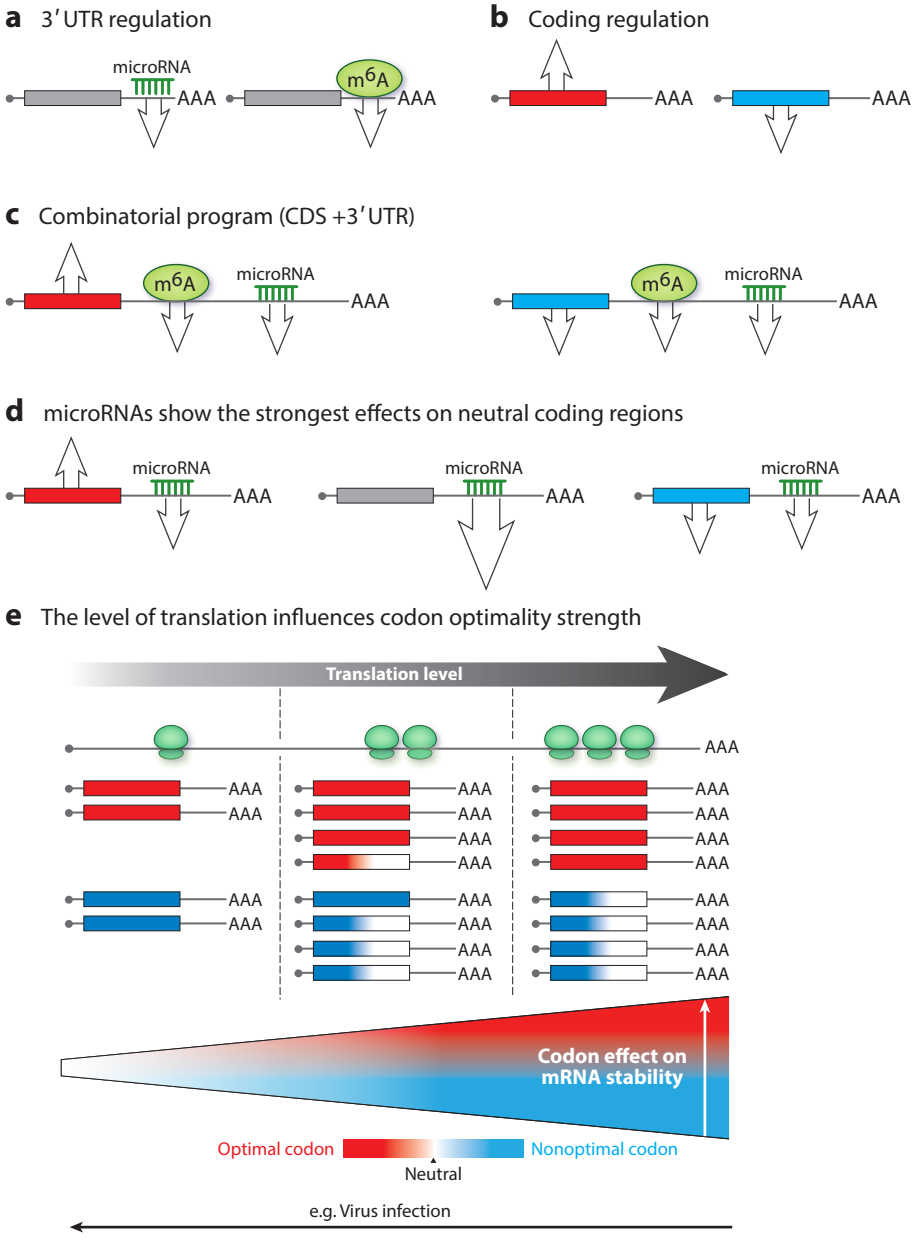

Figure 3

Decay pathways overlap. (*a*) Cis-regulatory elements in the 3′ UTR like microRNA and m^{6}A can decrease mRNA stability. (*b*) The coding region can also increase or decrease mRNA stability based on codon optimality. (*c*) mRNA stability depends on both coding region and UTR regulatory sequences. (*d*) microRNA decreases mRNA stability regardless of the optimality within the coding region, but microRNA shows the strongest effect with neutral coding regions. (*e*) The level of translation influences the effect of codon optimality on gene expression. High levels of translation increase codon optimality and low levels dilute it (e.g., during stress conditions like viral infection). Abbreviations: CDS, coding sequence; m^{6}A, methylation; UTR, untranslated region.

Synonymous codon substitutions based on codon optimality predictions can lead to >30-fold differences in mRNA half-life, with concomitant changes in RNA and protein levels (12, 29). Therefore, knowledge of the regulatory properties of codons (optimal and nonoptimal) can be used to predict and design coding sequences that provide the desired level of expression and have biomedical (e.g., mRNA vaccines) and research applications (29, 55, 56).

2.4. Toward a Unified Model for Explaining mRNA Half-Life

mRNA stability is dictated by the combinatorial effects of codon composition (codon optimality) and cis-regulatory elements located in the UTR. For example, within mRNAs that are known to be regulated by microRNAs or particular RNA modifications (e.g., m^6A), those enriched in nonoptimal codons were still less stable in human and mouse cells, as well as in zebrafish and *Xenopus* embryos (**Figure 3*c***) (50). In addition, the action of a microRNA (miR-430) during zebrafish embryogenesis (57, 58) was diminished for target mRNAs containing coding sequences with highly enriched optimal or nonoptimal codons compared to mRNAs with neutral codon optimality (**Figure 3*d***) (50). Therefore, the microRNA target efficacy can be affected by the codon composition (59).

The codon-mediated effect on mRNA stability also depends on the level of translation (**Figure 3*e***). Specifically, reducing the global level of translation by treating with drugs (e.g., cycloheximide) or causing stress [e.g., with herpes simplex virus 1 (HSV-1) infection] (60) dilutes the codon-meditated effect on gene expression (14). Moreover, differences in translational efficiency (due to different UTR strengths) affect the degree to which codon optimality effects are observed. Specifically, a stronger 5′ UTR exacerbates the codon-mediated effect and a weaker 5′ UTR dilutes the differences between paired codon reporters (14). Therefore, the translation strength of the UTR(s) can modulate the overall impact of codon optimality.

Taken together, the combinatorial effects of regulatory information in the coding region and cis-regulatory elements within UTRs warrant a paradigm shift in how we view mRNA stability. To fully understand the complex regulation of mRNA stability observed across the transcriptome, we must consider resident regulatory information found throughout mRNA sequences, including information within coding sequences (**Figure 3**).

3. MECHANISM

The translation of mRNA can be divided into four steps: scanning, initiation, elongation, and termination. Briefly, the small (40S) subunit of the ribosome scans from the 5′ end of an mRNA strand until it recognizes the translation start site (most likely AUG). Then, translation initiates when the large (60S) subunit is recruited (for more details, please see 61). Initiation is followed by translation elongation, whereby the ribosome (80S), translocating three nucleotides at a time across the ORF, translates codon information into a nascent polypeptide (for more details, please see 62). Termination of translation occurs when the ribosome recognizes a stop codon (UAA, UAG, or UGA) (for more details, please see 63). Finally, the ribosome dissociates from the mRNA, and separated 40S and 60S subunits are recycled for subsequent rounds. While these four steps are well defined, they are not independent of each other. For example, translation elongation speed can influence the initiation rate (64), and ribosome recycling has also been associated with some initiation factors for the next round of mRNA translation (63).

There is growing evidence that codon optimality effects on mRNA stability are influenced by the rate of translation elongation (**Figure 4*a***). For example, ribosome runoff experiments in yeast indicate that ribosome elongation is slower for mRNAs enriched in nonoptimal codons and faster for optimal codon-enriched mRNAs (12). Similar trends have been observed with in vitro

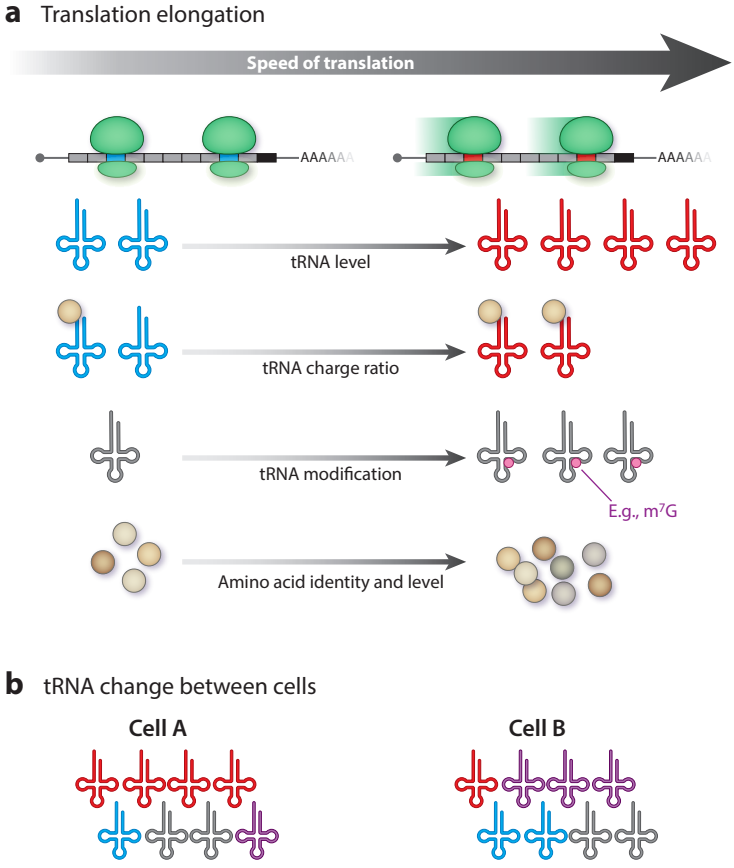

Figure 4

What determines translation elongation speed? (*a*) Codon optimality depends on translation elongation speed; nonoptimal codons have slow elongation while optimal codons have fast elongation. The translation elongation speed is determined by tRNA level, tRNA charged ratio, tRNA modifications, and amino acid identity and level. (*b*) The tRNA pool can vary between different cell types or conditions. Abbreviations: m^7G, N7-methylguanosine; tRNA, transfer RNA.

cell lysates (37), as well as through single-molecule microscopy in human cells (65). Therefore, the speed at which ribosomes translate coding sequences may act as a sensor that affects mRNA stability (39, 41). The phenomenon that the kinetics of a complex can affect follow up gene expression has been previously observed at the transcription level. For example, RNA splicing can be influenced by the speed of RNA polymerase II by skipping exons for splicing (66). Together, these results point to elongation kinetics as the main determinant of mRNA stability via the codon optimality mechanism (41), raising several questions: What dictates the rate of translation elongation? What are the cellular factors that sense the elongation rate? What are the factors that trigger mRNA decay? And what decay machineries and mechanisms are recruited?

3.1. How Does Codon Composition Influence Translation Elongation?

The decoding of codon information by the ribosome involves recruitment of cognate tRNAs. Very briefly, tRNAs are short noncoding RNA molecules used by the ribosome to decode the

information contained in mRNA into proteins. The tRNA copy number is very different in different species, with yeast, humans, and zebrafish containing 275, 400, and 8,676 tRNA genes, respectively (67). tRNAs are transcribed by RNA Polymerase III and then processed through multiple steps, including cleavage, splicing, and RNA modification (68–70). tRNAs are then charged with their respective amino acids by aminoacyl-tRNA synthetases to generate ready-to-go tRNAs. Ultimately, the rate of translation depends on the accessibility of each ready-to-go tRNA, which is thought to be related to its overall availability and abundance (71).

In several species, including humans, codon optimality scores are correlated with tRNA abundance (**Figure 4*a***) (12–14, 39). Specifically, optimal and nonoptimal codons are associated with high and low cognate tRNA abundances, respectively (12–14). Moreover, codon optimality scores and the ratio of charged tRNAs (loaded with amino acids) to total tRNAs are also positively correlated (14). Several studies have shown that changes in charged tRNA levels with respect to total tRNA levels (72, 73), the charged ratio (74), or even tRNA modifications (75) can cause codon-specific effects in their respective mRNAs (**Figure 4*a***). Mutations in tRNAs have been associated with human diseases, such as neurodegenerative diseases (76) and cancer (77). However, not all mutations and changes in tRNAs necessarily affect gene expression though codon optimality (78).

The above results suggest that tRNA abundance and availability is a limiting step for translational efficiency, with implications for codon-mediated stability dynamics. The tRNA repertoire (level, isomer, quality, etc.) can change in response to different stimuli (**Figure 4*b***) (70–73, 79–81). In addition, the regulatory properties of any given codon can potentially vary depending on condition- and tissue-specific differences in tRNA availability (72–74). Comprehensive interrogation toward understanding tRNA regulatory mechanisms requires the quantification of ready-to-go tRNAs. However, with high levels of modifications, high copy numbers, and complicated sequence analyses (73, 80, 82–85), no single method has emerged to quantify charged tRNAs in a simple, robust, and systematic manner.

Besides tRNAs, some amino acids can also influence elongation speed. Similar to the CSC, the amino acid stabilization coefficient can be calculated from the Pearson correlation between amino acid frequency and mRNA stability (13, 14, 26, 27). However, an amino acid should be defined as optimal or nonoptimal only if all the synonymous codons encoding that particular amino acid share similar optimality. Otherwise, the most common codons will dominate the calculation and cause artifacts. For example, in zebrafish and Xenopus embryos (13), as well as in human cells (14, 26, 27), histidine and serine appear to be encoded entirely by nonoptimal codons; therefore, those amino acids can be proposed to potentially affect mRNA stability (**Figure 4*a***) (14, 27).

3.2. Which Factors Sense the Elongation Rate?

The ribosome contains three sites or pockets: the A site, which accepts the incoming charged tRNA; the P site, in which the nascent peptide chain is held; and the E site, in which the non-charged tRNA resides before leaving the ribosome. Competition between regulatory factors and tRNAs for these ribosome sites has been hypothesized to be a potential method for monitoring elongation rates (**Figure 5*a***). If the ribosome slows down when encountering nonoptimal codons due to, for example, low levels of the cognate tRNA, then increased vacancy times at the E and/or A sites may enable binding of factors that would normally be outcompeted (**Figure 5*a***). Such interactions may serve to signal decreased elongation rates and/or trigger mRNA decay. However, the identification of competing factors has remained elusive. Recently, structural studies in yeast have proposed that Not5 (CNOT3 in humans) is involved in codon optimality sensing. Not5, a subunit of the CCR4–NOT complex, binds to the ribosome E site after tRNA departure (**Figure 5*b***) (86). It was suggested that the interaction of Not5 with the E site depends on an empty

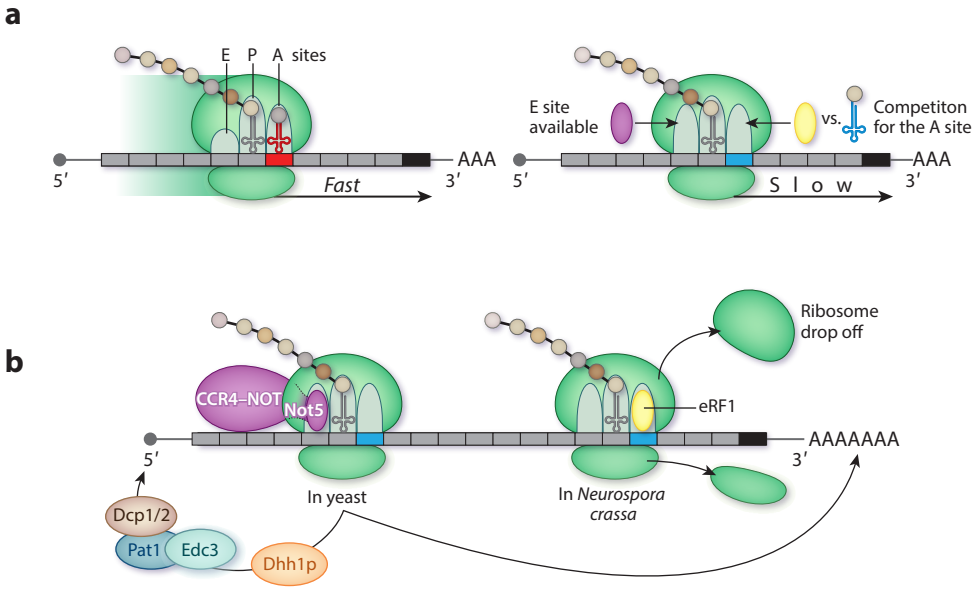

Figure 5

The molecular mechanism of codon optimality. (*a*) Due to different translation elongation speeds, ribosomes have different conformations for optimal and nonoptimal codons. For optimal codons, the A site is occupied by the associated tRNA, and the E site is reduced in size. For nonoptimal codons, the A site is empty, leaving the E site and/or A site available for binding of other factors. (*b*) In yeast, Not5 can bind to the ribosome E site when a nonoptimal codon is in the A site, which recruits the CCR4–NOT complex and decapping factors like Dhh1p to decay nonoptimal mRNAs. In *Neurospora crassa*, eRF1 can bind to the ribosome A site at nonoptimal codons and cause ribosome drop off and early ribosome translation termination.

A site, as the E site changes conformation once a tRNA is incorporated into the A site (86). Further work in yeast showed that both Not5 and Not4 can affect mRNA translation elongation through A site occupancy in a codon-dependent manner (87). Interestingly, in *Neurospora crassa*, the translation termination factor eRF1 can also bind to vacant A sites at nonoptimal codons (**Figure 5*b***) (88). Together, these findings point to cellular factors that bind to ribosomal E or A sites to potentially monitor and/or antagonize translational elongation. Further work will determine whether Not5 and eRF1 impact codon optimality in other species and whether there are additional factors that are potential sensors of elongation speed.

3.3. How Does Codon Optimality Trigger mRNA Decay?

There are several potential scenarios leading to the clearance of mRNA due to codon composition. First, after sensing that the speed of translation elongation has decreased due to a particular codon, the factors involved in sensing codon-mediated changes in elongation speed, through the E or A sites, may directly recruit decay machineries. Consistent with this, the recruitment of the CCR4–NOT complex may be facilitated by Not5 in yeast, triggering deadenylation of the of poly(A) tails (see Section 3.4) (**Figure 5*b***) (86).

Alternatively, distinct ribosome conformations may directly recruit RNA decay factors. Ribosomes have a different status or conformation during translation elongation (89) or under problematic situations that can affect the elongation rate (90). For example, ribosome collision occurs when two ribosomes crash into each other (90). The mechanism driving mRNA degradation

after ribosome collision acts through the recognition of specific surfaces formed by a disome (two ribosomes) (90, 91). Previous studies in yeast and zebrafish embryos have shown that the collision factors are not involved in codon optimality regulation (49, 92), suggesting that codon optimality does not depend on collision factors and disome surface conformations. However, other factors might recognize a potential different conformation of slow ribosomes and trigger mRNA decay.

When mRNAs are degraded cotranslationally, the ribosome needs to be split for recycling (93), and the nascent peptide needs to be released from the ribosome and degraded. Due to the fast kinetics of each step, the order of the events—RNA decay, ribosome recycling, and nascent peptide degradation—remain unclear, and all steps may facilitate one another. For example, in *Neurospora crassa*, eRF1 recognizes empty A sites at rare codons, which can mediate early translation termination with nascent peptide drop off (**Figure 5*b***) (88); this may be an early indicator signal to trigger RNA decay. However, in vertebrates, the relationship between ribosome drop off and codon optimally is unknown. Thus, it can be proposed that translation of nonoptimal codons might trigger ribosome drop off and mRNA decay.

Therefore, to define how codon optimality triggers mRNA decay, it is important to continue identifying the molecular factors affecting gene regulation in a codon-dependent manner in multiple species and their relationships with other RNA decay pathways.

3.4. What Decay Machineries Are Involved?

The canonical mRNA decay pathway often starts with deadenylation, the shortening of poly(A) tails by the CCR4–NOT complex or Pan2–Pan3 complex (94), followed by either decapping (by Dcp1–Dcp2) and 5′-to-3′ decay with Xrn1 or 3′-to-5′ decay via the exosome (95).

The CCR4–NOT complex has been shown to be important in the decay of mRNAs enriched in nonoptimal codons in yeast (87). Caf1, the deadenylase component of the CCR4–NOT complex, preferentially reduces the poly(A)-tail lengths of mRNAs enriched in nonoptimal codons in yeast (**Figure 5*b***) (96). The CCR4–NOT complex can also interact with Dhh1p in yeast, a major decapping factor for RNA decay (86, 97). In fact, the first downstream factor identified in the fast decay of mRNAs enriched in nonoptimal codons in yeast was Dhh1p (**Figure 5*b***) (18). Taken together, these findings suggest that mRNAs enriched in nonoptimal codons are degraded by deadenylation and decapping decay pathways in yeast (18, 86).

However, in vertebrates the mechanism appears to be different. For example, DDX6, the Dhh1p homolog in higher organisms, is not associated with codon-mediated effects on mRNA stability (39, 98). Moreover, during zebrafish embryogenesis, inhibiting Dcp2 (the decapping factor) and Cnot7 (deadenylation factor) evidenced that the clearance of the maternal mRNA by these general pathways is not uniform (99). The sensitivity of mRNA stability depends on multiple mRNA features such as length of the 5′ UTR, coding sequence, or poly(A) tail (99). This is very important, because after perturbation (e.g., knockdown) of an mRNA decay factor, it is expected that mRNAs enriched in nonoptimal codon might be more significantly impacted than stable mRNAs (e.g., enriched in optimal codons).

In some cases, mRNA decay starts with endocleavage (100), a common pathway for RNA degradation during mRNA quality control. For example, SMG6 promotes mRNA endocleavage in NMD in metazoans (101, 102). Cue2 can endocleave mRNAs during NGD (103). Since codon optimality mechanisms occur cotranslationally in a similar manner to quality-control mechanisms (e.g., NMD), it is possible that endocleavage is also involved. Recent work with 5′-P sequencing suggests there are more degradation-associated ribosome stalling events at nonoptimal codons (87). Future work will assess whether endocleavage at nonoptimal codons underlies RNA decay.

There are additional factors that have been associated with codon optimality, like ILF2 and ILF3, but the mechanism is still unclear (44). Once factors are associated with codon optimality, it is important to determine at which step they operate—whether it is the upstream factor that determines the codon identity (tRNA-related genes, for example), a factor to detect translation elongation speed, a factor to trigger RNA decay, or a more general RNA decay or translation repression pathway. Since codon optimality depends on translation to decay the nonoptimal mRNAs, those general steps also influence the effect of codon optimality. For example, codon optimality regulation has been associated with the poly(A) tail due to strong correlation between poly(A)-tail length and codon composition from yeast to humans (13, 14, 17, 96). However, the poly(A) tail itself is important for translation and RNA decay, and therefore, the strong correlation between poly(A)-tail length and codon composition is not a proof of causality.

3.5. Is the Poly(A) Tail Part of the Codon Optimality Mechanism?

As mentioned in Sections 2 and 3.4, the regulation of codon optimality has been associated with the poly(A) tail. Both endogenous and reporter mRNAs enriched in optimal codons tend to possess longer poly(A) tails than mRNAs enriched in nonoptimal codons in yeast, zebrafish embryos, and human cells (**Figure 2*c***) (13, 14, 17, 96). These results are consistent with CCR4–NOT complex recruitment and poly(A) shortening in yeast (**Figure 5*b***) (86). However, the causal relationship between poly(A) tails and codon optimality is unclear. For example, paired codon optimality reporters that contain a histone tail, a nonpolyadenylated 3′ end (104), or a poly(A) tail display similar codon-mediated effects on gene expression (14). Namely, independent of the 3′ end used [poly(A) tail or histone tail], reporters enriched in optimal codons displayed higher levels of expression (RNA and/or protein level) than counterparts enriched in nonoptimal codons in human cells and in zebrafish embryos (14). These results suggested that the modulation in poly(A)-tail length is not required for codon-mediated regulation, at least in human cells and zebrafish embryos. Therefore, the poly(A)-tail shortening in nonoptimal mRNAs might occur as a consequence of mRNA destabilization rather than as the primary cause. In the future, it might be interesting to use alternative 3′ ends [e.g., the 3′ end of MALAT1 (105)] in combination with a large set of reporters in different organisms to elucidate poly(A)-tail and codon optimality regulation.

3.6. The mRNA Localization or Fate Based on the Codon Composition

It is important to note that translation of mRNA can also be affected by mRNA localization, phase, and condensate state (106). For example, RNA granules like stress granules and P-bodies are associated with translational repression (107). In addition to the effects on mRNA stability, the codon composition may also impact mRNA localization and condensation (**Figure 2*e***). In human cells, mRNAs localized in P-bodies tend to contain low percentage of GC3 (36), and the GC3 content attempts to correlate with codon optimality properties. In yeast, genes enriched in nonoptimal codons are associated with insoluble fractions (87). Interestingly, protein families or orthologs associated with the codon optimality mechanism such as Not5 and DDX6 also can affect mRNA localization, RNA granule formation, and RNA condensation (87, 108). Further, Not1 and Not4, both components of the CCR4–NOT complex, inversely determine the solubility of mRNA in yeast (87). Specifically, knockdown of Not1 and Not4 differentially affect mRNA solubility, translation, and decay (109). DDX6 regulates mRNA translation and/or decay in different species, as well as assembly of P-bodies (108). All these results together incorporate one extra post-transcriptional regulatory layer: mRNA localization or solubility status related to the nucleotide sequence and codon composition. Therefore, the mRNA localization and the translational status of mRNAs must be considered to fully understand the codon optimality effects on mRNA stability.

4. SUMMARY AND PERSPECTIVES

Over the last decade, new research has revealed an intimate connection between mRNA translation and stability. Codon optimality is a molecular mechanism whereby translation influences mRNA stability in a codon-dependent manner (12). This process has been observed across a multitude of species, highlighting its essential role in impacting protein production. Given its universality, several questions arise concerning its evolutionary origins and maintenance within different lineages.

First, while the genetic code is universal, and codon identity for each amino acid is conserved, the regulatory properties of codons (the codon optimality code) can differ between species. Codons defined as optimal in one species can display the opposite behavior in another. Such differences in the codon optimality code tend to increase with evolutionary distance between species. Thus, it will be interesting to explore, from an evolutionary standpoint, how codon optimality evolved to maximize its advantage in different species (110). Such exploration will be particularly pertinent when considered within the context of tRNA availability. How are tRNAs regulated in different species, and how do those differences impact optimality characteristics? Furthermore, while yeast is an excellent model for studying many cellular processes, multicellular organisms may provide additional insights based on their cellular and tissue complexity. Codon optimality research has helped demonstrate that tRNAs are not passive regulatory players; in fact, we propose that tRNAs, through their global influence on mRNA stability due to the codon optimality mechanism, are master regulators of gene expression. In turn, recent research has uncovered complex mechanisms impacting tRNA activity, from their transcription to subsequent processing and amino acid charging, highlighting their roles in human disease, as well as their potential therapeutic targets.

As outlined in Section 3.4, we further emphasize that factors so far identified as part of the codon optimality mechanism in one species do not necessarily correspond to orthologous functions in other species. For example, dhh1p regulates codon optimality in yeast but not in *Neurospora crassa* or humans. This emphasizes the importance of studying codon optimality mechanisms in multiple species to avoid generalizations. Furthermore, exploring the molecular mechanisms in multiple species will allow a comparative approach to parse those pathways that are evolutionarily shared from more recent regulatory idiosyncrasies. Importantly, identifying the factors involved in codon optimality in humans will provide the ability to explore its connection to human diseases.

Beyond the factors discussed, human diseases can be associated with codon substitutions. As discussed in Section 2.3, synonymous codons are not silent from a regulatory point of view. Synonymous mutations can affect mRNA stability, translation efficiency, mRNA localization, mRNA splicing, mRNA structure, and protein folding (12, 14, 39, 41). Recently it was reported that synonymous mutations in certain genes were predominately nonneutral in yeast (111). And while there are examples of synonymous variants related to human diseases (112–114), we need to be cautious about the pathogenic implications of synonymous variants when comparing to nonsynonymous ones in vertebrate organisms (39, 115, 116).

Codon optimality is not only about mRNA decay; future research should aim to understand the interface between translation, mRNA decay, and other related processes operating in the cell. For example, while evidence suggests that nonoptimal codons reduce translation elongation speed, in some cases, the reduction may be related to nascent peptide drop off, priming mRNA for decay. Moreover, nonoptimal mRNAs triggered for decay have been associated with RNA condensation in P-bodies or stress granules. The condensation of RNA may be the step that reduces translation and/or signals for RNA decay. And while some regulatory pathways can be neatly dissected by the specific loss of function of a few factors, codon optimality may involve multiple or overlapping

pathways that compensate for each other; therefore, it is crucial to integrate and interrogate the function of multiple factors in different species.

DISCLOSURE STATEMENT

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