

REVIEW ARTICLE

Mechanisms leading to the loss of the AUG codon function as a translation initiator: A review

Thualfiqar Ghalib Turki^{1*} and Arif Abbas Ghayyib²¹Department of Laboratory Techniques, Faculty of Medical Technologies, Islamic University of Najaf, Najaf, Iraq²Department of Criminal Evidence Sciences, Faculty of Medical Sciences, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Kufa, Najaf, Iraq

Abstract

The AUG codon represents the canonical start site for translation initiation in eukaryotic cells; however, its accurate recognition is governed by a complex network of regulatory mechanisms. In this review, we comprehensively examined recent literature to identify key determinants that impair AUG-mediated initiation. These include suboptimal nucleotide contexts such as weak Kozak consensus sequences, RNA secondary structures proximal to the start codon, and upstream open reading frames that compete with the primary initiation site. In addition, RNA-binding proteins and microRNAs can restrict ribosomal access, while alterations in initiation factor availability or activity (including eukaryotic translation initiation factor 1, eukaryotic translation initiation factor 5, and associated cofactors) further modulate start codon selection fidelity. Collectively, these elements influence the assembly and stability of the 43S pre-initiation complex, thereby affecting the efficiency and accuracy of AUG recognition and ultimately shaping translational output. Beyond the canonical eukaryotic scanning mechanism, bacterial translation initiation relies on the Shine–Dalgarno sequence to position the ribosome at the correct start codon, highlighting fundamental mechanistic differences while underscoring a shared requirement for precise start site selection. Together, these regulatory layers contribute to translational plasticity, with implications for gene expression control, stress adaptation, development, and disease. This review also identifies current gaps in understanding and proposes future directions to further elucidate how start codon selection is coordinated under physiological and pathological conditions.

***Corresponding author:**Thualfiqar Ghalib Turki
(dhulfikar.ghalib@iunajaf.edu.iq)

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1. Introduction

Translation initiation constitutes a critical regulatory checkpoint in gene expression, governing both the efficiency of protein synthesis and the fidelity of start codon selection. In eukaryotic cells, this process typically begins at an AUG codon through a ribosomal scanning mechanism mediated by the 40S subunit and a coordinated set of initiation factors.¹ Despite its canonical description, initiation is increasingly recognized as a dynamic and context-dependent process influenced by multiple interacting determinants.

Rather than acting independently, features such as Kozak sequence context, RNA secondary structure, upstream open reading frames (uORFs), and initiation factor activity collectively shape ribosomal decision-making during start codon selection. Perturbations in these elements can shift initiation away from canonical AUG codons toward alternative sites, including non-AUG codons, thereby expanding proteomic diversity and enabling adaptive responses.²

Recent advances in ribosome profiling and structural biology have revealed a widespread use of non-canonical initiation events, particularly under stress and developmental transitions. However, many existing studies treat regulatory mechanisms in isolation, limiting a comprehensive understanding of their functional integration.^{2,3}

This review synthesizes current knowledge on the molecular determinants that impair canonical AUG-mediated initiation. We propose an integrated framework in which start codon recognition is viewed as the outcome of competing influences on ribosomal scanning, RNA architecture, and initiation factor dynamics, and we discuss how these interactions contribute to translational plasticity.³

2. Functional basis of translation and initiation fidelity

Translation is a highly coordinated process that determines proteome composition through the decoding of mRNA sequences into polypeptides. The ribosome facilitates this process via three transfer RNA (tRNA)-binding sites: the aminoacyl (A), peptidyl (P), and exit (E) sites.⁴ While these structural features are classically associated with elongation, their configuration during initiation is essential for establishing the correct reading frame.

The placement of the initiator methionyl-tRNA (met-tRNA^{iMet}) within the P-site defines the starting point of translation and represents a major checkpoint for fidelity. Errors at this stage can lead to aberrant initiation, producing proteins with altered N-terminal sequences and potentially distinct functions

Thus, translation initiation should be considered not merely as the first step of protein synthesis but as a regulatory decision point in which ribosomes integrate multiple signals to select an appropriate start codon.⁵

3. Molecular insights into AUG recognition during translation initiation

A start codon is a specific sequence of three nucleotides in messenger RNA (mRNA) that signals the beginning

of protein synthesis. The most common start codon is AUG, which codes for the amino acid methionine. This codon plays a critical role in the initiation stage of translation, where ribosomes recognize the start codon to begin assembling amino acids into a polypeptide chain.⁶ Accurate translation of mRNA critically relies on the ability to initiate at the correct AUG codon. In most eukaryotic mRNAs, this is achieved through a scanning mechanism, whereby the small (40S) ribosomal subunit binds to the 5' end of the mRNA and progressively examines the 5' untranslated region (UTR) for an AUG codon situated within an appropriate sequence context. This process is guided primarily by base-pairing interactions between the AUG codon and the anticodon of the met-tRNA^{iMet}.⁷ Over the past decade, significant advances in yeast genetics, reconstituted biochemical systems, and structural biology have collectively contributed to a deeper understanding of the molecular mechanisms underlying ribosomal scanning.⁸

4. An integrated framework for AUG recognition and its disruption

The efficiency of AUG recognition is governed by a network of interconnected mechanisms that converge on ribosomal scanning behavior and start codon selection. These mechanisms can be broadly categorized into: (i) mRNA-intrinsic features (Kozak context, RNA structure, uORFs), (ii) trans-acting factors (initiation factors, RNA-binding proteins), and (iii) context-dependent signals (stress, environmental cues).

Importantly, these elements function in a coordinated manner rather than as isolated regulators. Their interplay determines whether a ribosome initiates at a canonical AUG codon, bypasses it, or utilizes an alternative start site.⁹⁻¹¹

4.1. Kozak context and probabilistic start codon recognition

The nucleotide environment surrounding the AUG codon plays a decisive role in initiation efficiency. The Kozak consensus sequence enhances ribosomal recognition, whereas deviations reduce the likelihood of initiation.¹²

One of the primary factors contributing to the reduced recognition of AUG as a translation initiation codon is the absence of an optimal Kozak sequence context. The canonical consensus sequence, (GCC)GCC(A/G)CCAUGG, plays a crucial role in guiding ribosomal recognition of the start codon. In particular, the presence of a purine at position -3 and a guanine at position +4, relative to the adenine of AUG, is essential for efficient initiation. Mutations at these sites, such as the substitution

of adenine with uracil at position -3, markedly diminish the recognition efficiency of AUG. Consequently, ribosomes may bypass this site and continue scanning until they encounter a downstream AUG within a stronger sequence context.¹²

Experimental studies have confirmed the critical role of these nucleotides. For instance, mutations at positions -3 and +4 in β -globin mRNA were shown to reduce translation initiation efficiency by more than 90%, underscoring the importance of the Kozak context in accurate start codon recognition.¹²

4.2. Leaky scanning as a consequence of contextual weakness

Leaky scanning arises when ribosomes bypass an AUG codon due to suboptimal recognition conditions. This mechanism is not independent but emerges directly from reduced initiation probability at weak Kozak contexts. Instead of engaging at the primary start site, the ribosome continues scanning along the mRNA until it encounters a downstream AUG or, in some cases, a non-AUG codon. This process enables the production of multiple protein isoforms from a single transcript, thereby expanding the coding potential of the mRNA.¹³ The frequency of leaky scanning is not fixed but can be modulated by the activity of translation initiation factors such as eukaryotic translation initiation factor (eIF) 1 and eIF1A, which influence start codon selection and fidelity. A well-characterized example of this mechanism is found in the CCAAT/enhancer-binding protein β .¹⁴ Through leaky scanning, this transcript gives rise to distinct isoforms, including liver-enriched activating protein, liver-enriched activating protein*, and liver-enriched inhibitory protein, each of which plays a different role in transcriptional regulation.¹⁵ The differential expression of these isoforms highlights how leaky scanning contributes to functional diversity at the protein level, thereby exerting significant regulatory control in cellular processes.

4.3. Regulation by upstream open reading frames

Upstream open reading frames act as regulatory elements that intercept scanning ribosomes before they reach the main coding sequence. Their impact depends on reinitiation efficiency, which is tightly linked to initiation factor availability. Oxidative stress, specific kinases including protein kinase R-like endoplasmic reticulum kinase, general control nonderepressible 2, and protein kinase R phosphorylate the α subunit of eIF2. Once phosphorylated, eIF2 α sequesters its guanine nucleotide exchange factor, eIF2B, thereby inhibiting the conversion of eIF2-guanosine diphosphate to eIF2-guanosine triphosphate (GTP).¹⁶ This event leads to a global reduction

in translation initiation from canonical AUG codons, effectively conserving cellular resources under stress. Despite this repression, a subset of transcripts can bypass the block by utilizing alternative translation initiation mechanisms, ensuring the synthesis of stress-responsive proteins.¹⁷

A well-studied example of this regulatory mechanism is the activating transcription factor 4 mRNA, which contains multiple uORFs. Under normal conditions, these uORFs act as barriers that prevent efficient translation of the main coding sequence. However, upon eIF2 α phosphorylation, ribosomes are able to bypass the inhibitory uORFs, enabling initiation at the downstream main ORF and allowing selective expression of activating transcription factor 4 during stress.¹⁷

Upstream ORFs are short coding sequences located in the 5' UTR that exert strong regulatory effects on downstream translation. By engaging ribosomes before they reach the main coding sequence, uORFs can trap ribosomes or modify their scanning behavior, often resulting in reduced translation of the primary ORF.¹⁸ Following the translation of a uORF, ribosomes may either dissociate from the mRNA or reinitiate translation at downstream sites, a process that depends on the availability of reinitiating factors. Importantly, this form of regulation is highly sensitive to cellular stress signals, particularly those involving eIF2 α phosphorylation, which alters the balance between ribosome dissociation and reinitiation.¹⁹ A well-studied example of uORF-mediated regulation is the CHOP mRNA. Under normal growth conditions, its uORFs inhibit the translation of the main coding sequence. However, during stress responses, eIF2 α phosphorylation promotes reinitiation, thereby allowing translation of the pro-apoptotic CHOP protein.^{20,21}

4.4. Initiation factor modulation under stress

Phosphorylation of eIF2 α represents a central mechanism by which cells globally repress translation while permitting selective gene expression. By limiting the availability of the ternary complex, this modification alters ribosomal initiation dynamics. Stable secondary structures within the 5' UTR of mRNAs, such as stem-loops or G-quadruplexes, can interfere with ribosomal scanning and hinder the recognition of the AUG start codon.²² The inhibitory effect is particularly pronounced when these structures exhibit high thermodynamic stability ($\Delta G < -30$ kcal/mol) and are located near the 5' cap or in proximity to the AUG codon.²³ To overcome these barriers, RNA helicases such as eIF4A are often required to unwind the secondary structures and facilitate efficient scanning by the ribosome.²⁴

A classic example of translational regulation is ferritin mRNA, which contains an iron-responsive element (IRE) within its 5' untranslated region. The IRE adopts a stem-loop secondary structure that can be recognized by iron regulatory proteins (IRPs). Under low-iron conditions, IRP binding inhibits translation initiation, thereby suppressing ferritin synthesis. This regulatory mechanism enables cells to modulate ferritin expression according to intracellular iron availability and maintain iron homeostasis.²⁵

4.5. RNA secondary structure as a physical barrier

Secondary structures within the 5' untranslated region (5' UTR) can impede ribosomal scanning, particularly when positioned near the 5' cap or start codon. The inhibitory effect of these structures depends largely on their thermodynamic stability and accessibility, often requiring RNA helicase activity for efficient unwinding. In addition, microRNAs (miRNAs) and RNA-binding proteins may suppress translation initiation by binding near or directly overlapping the AUG start codon, thereby obstructing ribosome recruitment and scanning.²⁶ miRNAs frequently exert this effect by recruiting the RNA-induced silencing complex, which blocks initiation and promotes repression of translation.²⁷ RNA-binding proteins, such as T cell intracellular antigen 1 and human antigen R, can alter RNA conformation through remodeling or directly compete with components of the translation machinery for access to the initiation site.²⁸

In hepatocytes, miR-122 plays a central role in regulating gene expression by binding to the 5' UTRs of target transcripts. This interaction suppresses translation from canonical AUG start sites, thereby fine-tuning protein output in a cell-type-specific manner.²⁹

4.6. RNA-binding proteins and microRNA-mediated control

RNA-binding proteins and miRNAs regulate translation initiation by altering mRNA accessibility or competing with ribosomal components. These interactions introduce an additional regulatory layer that modulates initiation efficiency in a transcript-specific manner. In certain contexts, translation can be initiated at non-canonical start codons such as CUG, GUG, or UUG.³⁰ These events typically rely on alternative initiation factors, including eIF2A or eIF2D, rather than the canonical eIF2, and often result in the synthesis of protein isoforms with N-terminal extensions or truncations. Such isoforms can exhibit distinct biochemical properties, including altered localization, stability, or regulatory capacity.³¹

One well-characterized case is the CCAAT/enhancer-binding protein α , whose translation can initiate from

a CUG codon. Similarly, several *Hox* genes have been shown to undergo non-AUG initiation, producing protein variants with unique cellular distributions and functional properties.³²

4.7. Non-AUG initiation as a regulated alternative

Non-AUG initiation events, involving codons such as CUG or GUG, represent regulated alternatives rather than translational errors. These events often depend on alternative initiation factors, including eIF2A and eIF2D, and can generate protein isoforms with distinct functional properties.³³ For instance, during cellular stress or specific developmental windows, ribosomes can be guided to these near-cognate start sites, often producing N-terminally extended or truncated variants. A well-known example is the oncogene *c-myc*, where a CUG-initiated isoform acts differently from the canonical AUG-initiated one. What makes this system interesting is its tunability: slight changes in factor availability or mRNA structure can shift the balance between isoforms without needing new transcription. This adds a layer of post-transcriptional control that's fast, reversible, and often tissue-specific. So far, non-AUG initiation has been linked to neurodevelopment, immune responses, and even viral gene expression. Ignoring it in genomic analyses may therefore miss functionally relevant protein diversity.³⁴

4.8. Environmental influences on AUG codon function

Environmental factors, including temperature fluctuations, oxidative stress, hypoxia, viral infection, nutrient deprivation, and exposure to toxins or heavy metals, profoundly influence the molecular machinery responsible for AUG codon recognition. These stressors frequently activate signaling pathways that result in the phosphorylation or inactivation of key translation initiation factors, such as eIF2 α and eIF4E.³⁵

Several transcripts exemplify the environmental regulation of translation initiation. Heat shock proteins (HSPs) are selectively upregulated through alternative initiation pathways when canonical AUG recognition is compromised under conditions of temperature stress.³⁶ Similarly, hypoxia-inducible factors rely on cap-independent internal ribosome entry site (IRES)-mediated initiation when AUG-dependent scanning is inefficient under hypoxic conditions.³⁷

By modulating start codon recognition, environmental signals contribute to adaptive patterns of gene expression across diverse biological contexts, including cancer, aging, development, and immune regulation. This mode of control is also critical in virus–host interactions, as

many viruses reprogram host translational machinery to favor non-AUG start sites, thereby ensuring efficient viral protein synthesis.³⁸

5. Context-dependent translation initiation

5.1. Natural translational initiation

Translation initiation via IRES represents an alternative mechanism that circumvents several canonical eukaryotic initiation pathways. Notably, IRES-mediated initiation does not necessarily require the conventional methionine start codon; instead, translation can commence at non-AUG codons such as GCU (alanine) or CAA (glutamine).³⁹

Moreover, in mammalian cells, translation initiation can occur from the codon CUG, which is decoded by a specialized leucyl-tRNA, allowing for the incorporation of leucine as the initial amino acid. This non-canonical initiation mechanism operates independently of the eukaryotic initiation factor eIF2 and does not require the presence of an IRES-like secondary RNA structure. Instead, it relies on ribosomal scanning, with initiation efficiency enhanced by a favorable Kozak consensus sequence.⁴⁰

5.2. Engineered start codons

Engineered initiator tRNAs have been employed to direct translation initiation at the amber stop codon UAG in *Escherichia coli*. These synthetic tRNAs not only facilitate the incorporation of the canonical formylmethionine but also allow the insertion of formylglutamine. This is due to the ability of glutamyl-tRNA synthetase to recognize and charge the engineered tRNA with glutamine. It is important to recall that the bacterial initiation system primarily recognizes the formyl modification on the initiator tRNA rather than the identity of the amino acid itself.⁴¹

Notably, a study has demonstrated that these amber initiator tRNAs do not support detectable levels of translation initiation from native genomic UAG codons. Instead, measurable initiation was observed exclusively in plasmid-based reporter constructs containing robust upstream Shine–Dalgarno sequences.⁴²

5.3. Ribosome specialization and functional diversity

Emerging evidence indicates that ribosomes themselves may exhibit functional specialization. For example, alternative initiation of mitochondrial ribosomal protein L18 (MRPL18) produces ribosomes with altered composition, enhancing translation of specific stress-responsive transcripts. This concept introduces an additional layer of regulation in which ribosome

heterogeneity contributes to selective translation.⁴³

6. Integration with bacterial initiation mechanisms

Although this review focuses primarily on eukaryotic systems, bacterial translation initiation provides a contrasting model. In bacteria, start site selection is guided by the Shine–Dalgarno sequence, which base-pairs with ribosomal RNA to position the start codon.⁴⁴

Unlike the scanning mechanism in eukaryotes, this system relies on direct recruitment, highlighting fundamental differences in initiation strategies while underscoring shared principles of start codon recognition.⁴⁴

When translation is initiated from a non-AUG start codon, the identity of the aminoacyl-tRNA utilized depends on both the nature of the start codon and the specific initiation mechanism involved. In the canonical eukaryotic initiation pathway—employed by the majority of protein-coding genes—eIF2 is responsible for delivering met-tRNA_i^{Met} to the ribosomal P-site. Given the high specificity of eIF2 for met-tRNA_i^{Met} and its minimal affinity for alternative tRNAs, initiation through this pathway is presumed to involve met-tRNA_i^{Met} exclusively.²⁶

This assumption is supported by early *in vitro* experiments using [³⁵S]-labeled met-tRNA_i^{Met}, which revealed that reporter mRNAs containing non-AUG start codons such as CUG, GUG, ACG, UUG, AGG, AAG, AUA, AUC, or AUU still yielded full-length proteins incorporating radiolabeled methionine.⁴⁵ These findings strongly suggest that met-tRNA_i^{Met} can be utilized for initiation at these non-canonical start sites. More recently, mass spectrometry analyses have further corroborated the use of met-tRNA_i^{Met} at alternative start codons such as ACG and AUU.⁴⁶

While eukaryotic translation initiation typically relies on eIF2 to deliver met-tRNA_i^{Met} to the ribosomal P-site, accumulating evidence indicates that at least two additional initiation factors—eIF2A and eIF2D—are capable of mediating initiation at non-AUG start codons (Figure 1). Notably, eIF2A (distinct from the eIF2α subunit) can initiate translation in a GTP-independent manner and demonstrates a relaxed specificity toward tRNA binding. *In vitro* studies have shown that eIF2A binds both charged and uncharged forms of initiator met-tRNA_i^{Met} with comparable affinity.^{26,47}

Moreover, eIF2A may facilitate initiation at non-canonical codons such as CUG and UUG by delivering leu-tRNA^{Leu}, although the precise affinity of eIF2A for aminoacylated leu-tRNA remains unquantified.⁴⁸ It

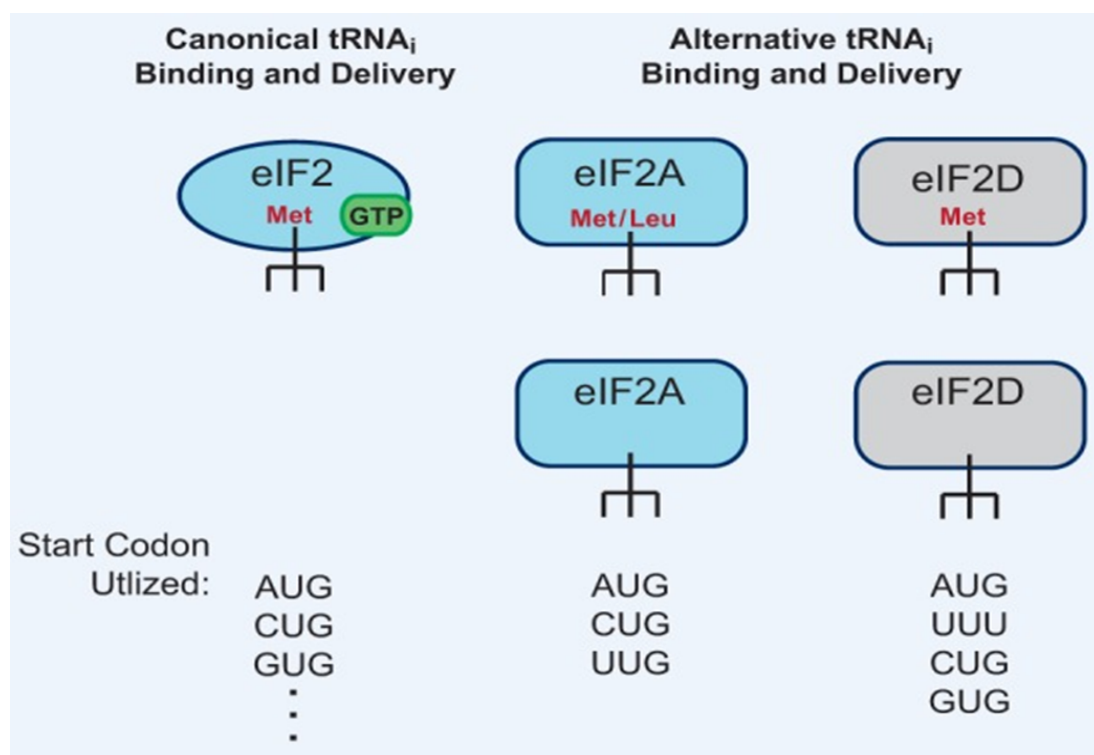


Figure 1. Comparison of canonical and alternative translation initiation pathways. This figure illustrates the molecular mechanisms responsible for initiator tRNA delivery to the 40S ribosomal subunit. In the canonical pathway, initiation is mediated by eIF2 in a GTP-dependent manner through the formation of a ternary complex with met-tRNA^{Met}, enabling efficient recognition of the AUG start codon. In contrast, alternative pathways mediated by eIF2A and eIF2D operate independently of GTP and can function under stress conditions where eIF2 activity is inhibited. These factors exhibit broader tRNA-binding flexibility and support initiation at non-AUG codons, thereby expanding translational diversity. Adapted from Kearse *et al.*²⁶ Abbreviations: eIF: Eukaryotic translational initiation factor; GTP: Guanosine triphosphate; tRNA: Transfer RNA.

remains possible that eIF2A does not directly deliver leu-tRNA but instead promotes its recruitment to the 40S ribosomal subunit. using an ELISA-based reporter system, demonstrated that translation initiation at a CUG codon can occur up to ~40% more efficiently when initiated with leu-tRNA_{CUG} than with met-tRNA^{Met}, implicating eIF2A in this process. Depletion of eIF2A specifically impaired initiation at the CUG codon without affecting AUG-initiated translation.⁴⁵

Despite these specialized roles, eIF2A appears to be non-essential under standard physiological conditions, as evidenced by the lack of overt phenotype in eIF2A knockout mice.⁴⁹ However, its expression is upregulated under stress conditions, and it has been implicated in IRES-mediated translation. These findings suggest that eIF2A may become particularly important in contexts where canonical eIF2-dependent translation is suppressed, thereby allowing non-AUG initiation to play a more prominent role in the cellular translational landscape.⁵⁰

Similar to eIF2A, the initiation factor eIF2D—also

known as ligatin—has been demonstrated to mediate the delivery of met-tRNA^{Met}, including its uncharged form, to the P-site of the 40S ribosomal subunit in a GTP-independent manner. Although eIF2D is primarily recognized for its essential role in ribosome recycling and reinitiation *in vivo*, it can also support translation initiation at non-AUG codons *in vitro*, albeit with selectivity depending on the RNA context.⁵¹

For instance, eIF2D has been shown to initiate translation at a GUG codon on the 26S mRNA of Sindbis virus via the delivery of val-tRNA_{GUG}, but this activity was not observed on the hepatitis C virus IRES.^{52,53} This selectivity suggests that eIF2D's ability to promote non-canonical initiation may be context-dependent and possibly influenced by the structural features of the 5' UTR of the mRNA.⁵⁴

Intriguingly, eIF2D has been identified as an autoantigen in patients with hepatocellular carcinoma, implying a potential role for this factor in oncogenesis or tumor-associated translational control mechanisms.⁵⁵

Moreover, the malignant T cell-amplified sequence 1 (MCT-1)/density-regulated protein (DENR) heterodimer—which is structurally homologous to the N-terminal and C-terminal domains of eIF2D, respectively—has also been implicated in facilitating translation reinitiation and ribosome recycling.^{56,57} *In vitro* studies indicate that MCT-1/DENR can promote the eIF2-independent recruitment of met-tRNA_i^{Met} to the ribosomal P-site on select viral mRNAs. However, whether MCT-1/DENR can recruit other aminoacyl-tRNAs or initiate translation at non-AUG start codons remains an open question requiring further investigation.^{57,58}

In specific instances, such as those involving the IRESs of the cricket paralysis virus, Taura syndrome virus, and *Plautia stali* intestine virus, translation initiation proceeds via a mechanism that is fundamentally distinct from the canonical eukaryotic pathway. Notably, in these systems, neither an aminoacyl-tRNA positioned in the ribosomal P-site nor canonical eIFs are required.^{59,60}

Instead, these viral IRES elements adopt highly structured conformations that directly associate with the 40S ribosomal subunit and facilitate the formation of an active 80S-IRES initiation complex. Within this complex, a specific pseudoknot motif of the IRES mimics the positioning of initiator met-tRNA_i^{Met}, and the AUG start codon in the P-site of the 40S subunit. This structural mimicry enables the recruitment of a cognate aminoacyl-tRNA to the ribosomal A site, thus initiating polypeptide synthesis from the A site, rather than the P-site—a striking deviation from the canonical paradigm.⁶¹ To date, this unique initiation strategy has been exclusively described for a subset of viral IRESs. Nevertheless, these findings provide compelling evidence that translation initiation can be achieved in the complete absence of met-tRNA_i^{Met} and traditional initiation factors, thereby underscoring the remarkable versatility and adaptability of the translational machinery in response to distinct RNA architectures.⁶²

7. Usage of non-AUG initiation codons changes during development and upon stress

Ribosome profiling provides a snapshot of active translation, revealing how endogenous genes, including those with non-AUG start codons, are regulated in response to developmental or environmental signals. To investigate translational control during development, the researchers applied ribosome profiling to *Saccharomyces cerevisiae* as it transitioned from vegetative to meiotic growth.⁶³ By generating and analyzing a large dataset of over 25 time-resolved libraries, they observed not only the expected broad changes in gene expression but also

a fundamental shift in translation initiation patterns. Specifically, translation events originating from antisense RNAs, intergenic regions, and uORFs were found to be highly enriched in meiotic cells. This was quantified by mapping the location of ribosome footprints: while only 5% of footprints were located outside of noted ORFs in vegetative cells, this proportion increased sixfold to about 30% in meiotic cells, leading to a widespread use of non-canonical start sites.⁶³ These findings identify a shift away from standard protein synthesis pathways in meiotic cells. While this is a significant discovery, the functions of most of these newly identified protein-coding regions remain a mystery. Many of these regions are a specific type of element called a uORF, which often begins with a non-AUG start codon. This is surprising because the established role of a uORF is to act as a barrier, competitively blocking the ribosome from reaching and translating the main protein downstream.^{63,64} The discovery that non-AUG uORFs positively regulate downstream translation in meiosis has opened new questions about the underlying mechanisms. This finding is in direct contrast to the canonical repressive role observed for about half of the AUG-initiated uORFs in the same study.⁶³ While the precise mechanism is unknown, two compelling hypotheses are being considered. One hypothesis suggests a cis-acting effect, whereby the process of translating a non-AUG uORF might alter the scanning dynamics of the ribosome, thereby “priming” it for more efficient initiation at the downstream start codon. Alternatively, the short polypeptides encoded by these uORFs may be functional, acting in trans to regulate translation or other cellular processes, thus indirectly leading to the observed increase.²⁹ In vegetatively growing yeast, the synthesis of ribosomal proteins is a major transcriptional activity, accounting for nearly 50% of all transcription by RNA polymerase II.⁶⁵ Therefore, a plausible hypothesis is that the drastic changes in translation patterns observed in meiotic cells are driven by alterations in the stoichiometry of ribosomal proteins and eIFs, or by their post-translational modifications. This idea is consistent with recent findings demonstrating that ribosome composition and the relative abundance of ribosomal proteins can indeed influence the translation of specific classes of mRNAs.⁶⁶

In mammalian cells, heat shock induces a switch in translation initiation on the *MRPL18* transcript from the annotated AUG start codon to a downstream CUG codon (Figure 2). This alternative initiation event generates an N-terminally truncated MRPL18 protein that lacks the mitochondrial targeting signal. Consequently, this isoform is not imported into the mitochondria but is instead incorporated into cytoplasmic ribosomes. The resulting “hybrid” ribosomes are functionally active and

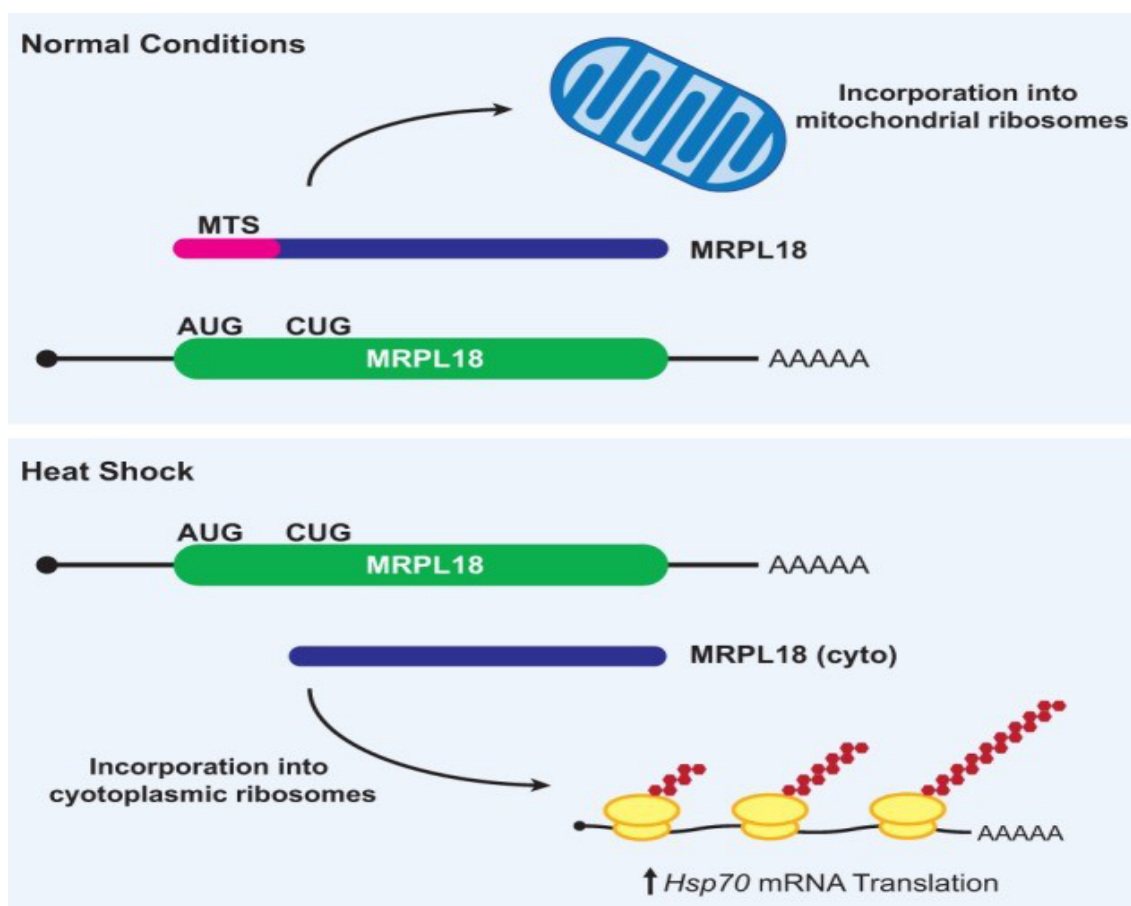


Figure 2. Stress-induced shift in start codon selection in mitochondrial ribosomal protein L18 (MRPL18). This figure illustrates how heat shock alters translation initiation on *MRPL18* mRNA. Under normal conditions, translation initiates at the canonical AUG codon, producing a full-length protein containing a mitochondrial targeting sequence that directs it to mitochondria. In contrast, under stress conditions, ribosomes bypass the upstream AUG and initiate at a downstream CUG codon, generating a truncated isoform lacking the targeting signal. This alternative isoform is incorporated into cytoplasmic ribosomes, contributing to stress-responsive translational regulation. Adapted from Kearse *et al.*²⁶

are essential for the enhanced translation of the Hsp70 chaperone protein during the heat shock response^{43,67}, a model of ribosome specialization, where the presence of the truncated MRPL18 protein confers translational specificity for *Hsp70* mRNA. This principle is consistent with the established precedent of the ribosomal protein Rpl38, which guides ribosomes to regulate the translation of *Hox* genes.^{43,68} This is perhaps because the presence of MRPL18 permits directed ribosome recruitment to *Hsp70* mRNA, similar to what has been reported previously for ribosomal protein L38 and the *Hox* genes.^{43,69} Regardless of the exact underlying mechanism, the *MRPL18* locus beautifully demonstrates how changes in the efficiency of non-AUG start codon usage can have a profound impact

on cell survival and homeostasis.⁴³

8. Conclusion

Translation initiation at AUG codons emerges as a highly regulated and context-dependent process shaped by the interplay of mRNA features, initiation factors, and cellular conditions. Rather than functioning independently, regulatory elements such as Kozak context, RNA structure, uORFs, and factor availability operate within an integrated network that determines ribosomal behavior.

Importantly, deviations from canonical AUG initiation are not merely aberrations but can represent adaptive responses that expand proteomic complexity. Advances

in genome-wide approaches have revealed a pervasive landscape of alternative initiation events, challenging traditional models of translational control.

Future research should focus on elucidating how these regulatory mechanisms are hierarchically coordinated and how ribosomes integrate competing signals during start codon selection. Such insights will be essential for understanding the role of translational regulation in development, stress adaptation, and disease.

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Author contributions

Conceptualization: Thualfiqr Ghalib Turki

Visualization: Arif Abbas Ghayyib

Writing—original draft: Thualfiqr Ghalib Turki

Writing—review & editing: Thualfiqr Ghalib Turki

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References

1. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell*. 2009;136(4):731–745.
doi: 10.1016/j.cell.2009.01.042
2. Irshad IU, Sharma AK. Understanding the regulation of protein synthesis under stress conditions. *Biophys J*. 2024;123(20):3627–3639.
doi: 10.1016/j.bpj.2024.09.014
3. Duc KD, Song YS. Correction: The impact of ribosomal interference, codon usage, and exit tunnel interactions on translation elongation rate variation. *PLoS Genet*. 2018;14(8): e1007620.
doi: 10.1371/journal.pgen.1007620
4. Subramanian S. Effect of genetic drift on determinants of protein evolution. *Biol Lett*. 2018;14(7):20180167.
doi: 10.1098/rsbl.2018.0167
5. Asano K. Why is start codon selection so precise in eukaryotes? *Translation*. 2014;2(1): e28387.
doi: 10.4161/trla.28387
6. Benitez-Cantos MS, Yordanova MM, O'Connor PBF, et al. Translation initiation downstream from annotated start codons in human mRNAs coevolves with the Kozak context. *Genome Res*. 2020;30(7):974–984.
doi: 10.1101/gr.257352.119
7. Brito Querido J, Díaz-López I, Ramakrishnan V. The molecular basis of translation initiation and its regulation in eukaryotes. *Nat Rev Mol Cell Biol*. 2024;25(3):168–186.
doi: 10.1038/s41580-023-00624-9
8. Hinnebusch AG. Structural insights into the mechanism of scanning and start codon recognition in eukaryotic translation initiation. *Trends Biochem Sci*. 2017;42(8):589–611.
doi: 10.1016/j.tibs.2017.03.004
9. Dever TE, Ivanov IP, Hinnebusch AG. Translational regulation by uORFs and start codon selection stringency. *Genes Dev*. 2023;37(11–12):474–489.
doi: 10.1101/gad.350752.123
10. Hershey JWB, Sonenberg N, Mathews MB, et al. Principles of translational control. *Cold Spring Harb Perspect Biol*. 2019;11(9):a032607.
doi: 10.1101/cshperspect.a032607
11. Moro SG, Hermans C, Ruiz-Orera J, et al. Impact of uORFs in mediating regulation of translation in stress conditions. *BMC Mol Cell Biol*. 2021;22(1).
doi: 10.1186/s12860-021-00363-9
12. Kozak M. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell*. 1986;44(2):283–292.
doi: 10.1016/0092-8674(86)90762-2
13. Andreev DE, Loughran G, Fedorova AD, Mikhaylova MS, Shatsky IN, Baranov PV. Non-AUG translation initiation in mammals. *Genome Biol*. 2022;23(1):111.
doi: 10.1186/s13059-022-02674-2
14. Weiss B, Dikstein R. Unraveling the landscapes and regulation of scanning, leaky scanning, and 48S initiation complex conformations. *Cell Rep*. 2024;43(5):114126.

- doi: 10.1016/j.celrep.2024.114126
15. Hinnebusch AG. Molecular mechanism of scanning and start codon selection in eukaryotes. *Microbial Mol Biol Rev.* 2011;75(3):434-467.
doi: 10.1128/MMBR.00008-11
 16. Chen CW, Papadopoulos D, Szkop KJ, *et al.* Plasticity of the mammalian integrated stress response. *Nature.* 2025;641(8065):1319-1328.
doi: 10.1038/s41586-025-08794-6.
 17. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. *Mol Cell.* 2010;40(2):228-237.
doi: 10.1016/j.molcel.2010.09.028
 18. Iwakawa HO, Tomari Y. The Functions of MicroRNAs: mRNA Decay and Translational Repression. *Trends Cell Biol.* 2015;25(11):651-665.
doi: 10.1016/j.tcb.2015.07.011
 19. Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet.* 2015;16(7):421-433.
doi: 10.1038/nrg3965
 20. Anderson P, Kedersha N. Stress granules: the Tao of RNA triage. *Trends Biochem Sci.* 2008;33(3):141-150.
doi: 10.1016/j.tibs.2007.12.003
 21. Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol.* 2010;11(2):113-127.
doi: 10.1038/nrm2838
 22. Kears MG, Wilusz JE. Non-AUG translation: a new start for protein synthesis in eukaryotes. *Genes Dev.* 2017;31(17):1717-1731.
doi: 10.1101/gad.305250.117
 23. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009;136(2):215-233.
doi: 10.1016/j.cell.2009.01.002
 24. Calkhoven CF, Bouwman PR, Snippe L, Ab G. Translation start site multiplicity of the CCAAT/enhancer binding protein alpha mRNA is dictated by a small 5' open reading frame. *Nucleic Acids Res.* 1994;22(25):5540-5547.
doi: 10.1093/nar/22.25.5540
 25. Dever TE, Ivanov IP, Hinnebusch AG. Translational regulation by uORFs and start codon selection stringency. *Genes Dev.* 2023;37(11-12):474-489.
doi: 10.1101/gad.350752.123
 26. Wek RC. Role of eIF2 α Kinases in Translational Control and Adaptation to Cellular Stress. *Cold Spring Harb Perspect Biol.* 2018;10(7):a032870.
doi: 10.1101/cshperspect.a032870
 27. Peabody DS. Translation initiation at non-AUG triplets in mammalian cells. *J Biol Chem.* 1989;264(9):5031-5035.
doi: 10.1016/S0021-9258(18)83694-8
 28. Vattam KM, Wek RC. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc Natl Acad Sci USA.* 2004;101(31):11269-11274.
doi: 10.1073/pnas.0400541101
 29. Gordiyenko Y, Ll  cer JL, Ramakrishnan V. Structural basis for the inhibition of translation through eIF2 α phosphorylation. *Nat Commun.* 2019;10(1):2640.
doi: 10.1038/s41467-019-10606-1
 30. Babendure JR, Babendure JL, Ding JH, Tsien RY. Control of mammalian translation by mRNA structure near caps. *RNA.* 2006;12(5):851-861.
doi: 10.1261/rna.2309906
 31. Andreou AZ, Harms U, Klostermeier D. Single-stranded regions modulate conformational dynamics and ATPase activity of eIF4A to optimize 5'-UTR unwinding. *Nucleic Acids Res.* 2019;47(10):5260-5275.
doi: 10.1093/nar/gkz254
 32. Wilkinson N, Pantopoulos K. The IRE/IRE system in vivo: insights from mouse models. *Front Pharmacol.* 2014;5:176.
doi: 10.3389/fphar.2014.00176
 33. Touriol C, Bornes S, Bonnal S, *et al.* Generation of protein isoform diversity by alternative initiation of translation at non-AUG codons. *Biol Cell.* 2003;95(3-4):169-178.
doi: 10.1016/s0248-4900(03)00033-9
 34. Hann SR, Sloan-Brown K, Spotts GD. Translational activation of the non-AUG-initiated c-myc 1 protein at high cell densities due to methionine deprivation. *Genes Dev.* 1992;6(7):1229-1240.
doi: 10.1101/gad.6.7.1229
 35. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. *Mol Cell.* 2010;40(2):228-237.
doi: 10.1016/j.molcel.2010.09.028
 36. Ghadarian T, Iyer S, Lazzari L, Vera M. Selective Translation Under Heat Shock: Integrating HSP70 mRNA Regulation with Cellular Stress Responses in Yeast and Mammals. *Mol Biol Cell.* 2025;36(5):re2.
doi: 10.1091/mbc.E24-12-0564
 37. Purdy SC, Matlin K, Alderman C, *et al.* eIF3d and eIF3e mediate selective translational control of hypoxia that can be inhibited by novel small molecules. *bioRxiv.* 2025.
doi: 10.1101/2025.05.29.656739
 38. Zhong Z, Li Y, Sun Q, *et al.* Tiny but mighty: Diverse

- functions of uORFs that regulate gene expression. *Comput Struct Biotechnol J*. 2024; 23:3771-3779.
doi: 10.1016/j.csbj.2024.10.042
39. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell*. 2009;136(4):731-745.
doi: 10.1016/j.cell.2009.01.042
 40. RajBhandary UL. More surprises in translation: initiation without the initiator tRNA. *Proc Natl Acad Sci USA*. 2000;97(4):1325-1327.
doi: 10.1073/pnas.040579197
 41. Starck SR, Jiang V, Pavon-Eternod M, *et al.* Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. *Science*. 2012;336(6089):1719-1723.
doi: 10.1126/science.1220270
 42. Shetty S, Shah RA, Chembazhi UV, *et al.* Two highly conserved features of bacterial initiator tRNAs license them to pass through distinct checkpoints in translation initiation. *Nucleic Acids Res*. 2017;45(4):2040-2050.
doi: 10.1093/nar/gkw854
 43. Zhang X, Gao X, Coots RA, Conn CS, Liu B, Qian SB. Translational control of the cytosolic stress response by mitochondrial ribosomal protein L18. *Nat Struct Mol Biol*. 2015;22(5):404-410.
doi: 10.1038/nsmb.3010
 44. Kohl MP, Bahena-Ceron R, Chane-Woon-Ming B, *et al.* Extended Shine-Dalgarno motifs govern translation initiation in *Staphylococcus aureus*. *Nat Commun*. 2026;17(1):2678. Published 2026 Feb 12.
doi: 10.1038/s41467-026-69079-8
 45. Mehdi H, Ono E, Gupta KC. Initiation of translation at CUG, GUG, and ACG codons in mammalian cells. *Gene*. 1990;91(2):173-178.
doi: 10.1016/0378-1119(90)90085-6
 46. Vincent RM, Wright BW, Jaschke PR. Measuring amber initiator tRNA orthogonality in a genomically recoded organism. *ACS Synth Biol*. 2019;8(4):675-685.
doi: 10.1021/acssynbio.9b00021
 47. González-Almela E, Williams H, Sanz MA, Carrasco L. The Initiation Factors eIF2, eIF2A, eIF2D, eIF4A, and eIF4G Are Not Involved in Translation Driven by Hepatitis C Virus IRES in Human Cells. *Front Microbiol*. 2018;9:207.
doi: 10.3389/fmicb.2018.00207
 48. Roiuk M, Neff M, Teleman AA. Human eIF2A has a minimal role in translation initiation and in uORF-mediated translational control in HeLa cells. *eLife*. 2025;14:RP105311.
doi: 10.7554/eLife.105311
 49. Golovko A, Kojukhov A, Guan BJ, *et al.* The eIF2A knockout mouse. *Cell Cycle*. 2016;15(22):3115-3120.
doi: 10.1080/15384101.2016.1237324
 50. Komar AA, Merrick WC. A Retrospective on eIF2A-and Not the Alpha Subunit of eIF2. *Int J Mol Sci*. 2020;21(6):2054.
doi: 10.3390/ijms21062054
 51. Dmitriev SE, Terenin IM, Andreev DE, *et al.* GTP-independent tRNA delivery to the ribosomal P-site by a novel eukaryotic translation factor. *J Biol Chem*. 2010;285(35):26779-26787.
doi: 10.1074/jbc.M110.119693
 52. Sanz MA, Almela EG, García-Moreno M, Marina AI, Carrasco L. A viral RNA motif involved in signaling the initiation of translation on non-AUG codons. *RNA*. 2019;25(4):431-452.
doi: 10.1261/rna.068858.118
 53. Grove DJ, Russell PJ, Kearse MG. To initiate or not to initiate: A critical assessment of eIF2A, eIF2D, and MCT-1·DENR to deliver initiator tRNA to ribosomes. *Wiley Interdiscip Rev RNA*. 2024;15(2):e1833.
doi: 10.1002/wrna.1833
 54. Ichihara K, Matsumoto A, Nishida H, *et al.* Combinatorial analysis of translation dynamics reveals eIF2 dependence of translation initiation at near-cognate codons. *Nucleic Acids Res*. 2021;49(13):7298-7317.
doi: 10.1093/nar/gkab549
 55. Heo CK, Hwang HM, Lee HJ, *et al.* Serum anti-eIF3A autoantibody as a potential diagnostic marker for hepatocellular carcinoma. *Sci Rep*. 2019;9(1):11059.
doi: 10.1038/s41598-019-47365-4
 56. Schleich S, Strassburger K, Janiesch PC, *et al.* DENR-MCT-1 promotes translation re-initiation downstream of uORFs to control tissue growth. *Nature*. 2014;512(7513):208-212.
doi: 10.1038/nature13401
 57. Skabkin MA, Skabkina OV, Dhote V, *et al.* Activities of Ligatin and MCT-1/DENR in eukaryotic translation initiation and ribosomal recycling. *Genes Dev*. 2010;24(16):1787-1801.
doi: 10.1101/gad.1957510
 58. Lomakin IB, Stolboushkina EA, Vaidya AT, *et al.* Crystal Structure of the Human Ribosome in Complex with DENR-MCT-1. *Cell Rep*. 2017;20(3):521-528.
doi: 10.1016/j.celrep.2017.06.025
 59. Ou Z, Petrov A. Sordarin bound eEF2 unlocks spontaneous forward and reverse translocation on CrPV IRES. *Nucleic Acids Res*. 2023;51(13):6999-7013.
doi: 10.1093/nar/gkad476
 60. Pestova TV, Hellen CU. Translation elongation after

- assembly of ribosomes on the Cricket paralysis virus internal ribosomal entry site without initiation factors or initiator tRNA. *Genes Dev.* 2003;17(2):181-186.
doi: 10.1101/gad.1040803
61. Fernández IS, Bai XC, Murshudov G, *et al.* Initiation of translation by cricket paralysis virus IRES requires its translocation in the ribosome. *Cell.* 2014;157(4):823-831.
doi: 10.1016/j.cell.2014.04.015
62. Koh CS, Brilot AF, Grigorieff N, *et al.* Taura syndrome virus IRES initiates translation by binding its tRNA-mRNA-like structural element in the ribosomal decoding center. *Proc Natl Acad Sci USA.* 2014;111(25):9139-9144.
doi: 10.1073/pnas.1406335111
63. Brar GA, Yassour M, Friedman N, *et al.* High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science.* 2012;335(6068):552-557.
doi: 10.1126/science.1215110
64. Sachs MS, Geballe AP. Downstream control of upstream open reading frames. *Genes Dev.* 2006;20(8):915-921.
doi: 10.1101/gad.1427006
65. Warner JR. The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci.* 1999;24(11):437-440.
doi: 10.1016/S0968-0004(99)01460-7
66. Li D, Wang J. Ribosome heterogeneity in stem cells and development. *J Cell Biol.* 2020;219(6):e202001108.
doi: 10.1083/jcb.202001108
67. Zhang X, Ji Q, Qian SB. Reply to: Reevaluating the role of human mitochondrial uL18m in the cytosolic stress response. *Nat Struct Mol Biol.* 2021;28(6):476-477.
doi: 10.1038/s41594-021-00600-x
68. Xue S, Tian S, Fujii K, Kladwang W, Das R, Barna M. RNA regulons in Hox 5' UTRs confer ribosome specificity to gene regulation. *Nature.* 2015;517(7532):33-38.
doi: 10.1038/nature14010
69. Kondrashov N, Pusic A, Stumpf CR, *et al.* Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell.* 2011;145(3):383-397.
doi: 10.1016/j.cell.2011.03.028