MINIREVIEW

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 291, NO. 33, pp. 16927–16935, August 12, 2016

Upstream Open Reading Frames Differentially Regulate Gene-specific Translation in the Integrated Stress Response*

Published, JBC Papers in Press, June 29, 2016, DOI 10.1074/jbc.R116.733899

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Translation regulation largely occurs during initiation, which features ribosome assembly onto mRNAs and selection of the translation start site. Short, upstream ORFs (uORFs) located in the 5′-leader of the mRNA can be selected for translation. Multiple transcripts associated with stress amelioration are preferentially translated through uORF-mediated mechanisms during activation of the integrated stress response (ISR) in which phosphorylation of the α subunit of eIF2 results in a coincident global reduction in translation initiation. This review presents key features of uORFs that serve to optimize translational control that is essential for regulation of cell fate in response to environmental stresses.

Multiple genome-wide analyses, including those utilizing ribosome and polysome profiling and mass spectrometry approaches, have provided evidence demonstrating that translation is a major regulator of gene expression (1–5). In addition to protein coding sequences (CDSs),[2] another class of ORFs suggested to be translated at high frequency consists of short, upstream ORFs (uORFs) that are located within the 5′-leader of mRNAs (3–6). Over 40% of mammalian mRNAs contain uORFs, illustrating that uORFs are prevalent genome-wide and can serve as major regulators of translation (5, 7, 8). Approximation of uORF prevalence has relied upon the use of an AUG to denote the uORF start codon; however, recent ribosome profiling studies indicate that non-canonical initiation codons (*e.g.* CUG, UUG, and GUG) can also serve as competent sites of translation initiation (3, 4, 6). These findings suggest that the magnitude of uORF prevalence and the contribution of uORF translation in the regulation of gene expression have likely been underestimated.

* This work was supported by National Institutes of Health Grant GM049164 (to R. C. W.) and the Ralph W. and Grace M. Showalter Research Trust Fund. The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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[2] The abbreviations used are: CDS, coding sequence; uORF, upstream open reading frame; ISR, integrated stress response; LAP, liver-enriched activating protein; LIP, liver-enriched inhibitory protein; IRES, internal ribosome entry sequence; ER, endoplasmic reticulum.

Typically, uORFs are considered to be inhibitors of downstream translation initiation at CDSs. The inhibitory effect of uORFs is attributed to the fact that in eukaryotes the 43S preinitiation complex binds to the 5′-cap structure of the mRNA, then scans processively 5′ to 3′ and initiates translation at the first encountered initiation codon that is in an optimal context (9). The 43S preinitiation complex is composed of multiple factors including eIF3, eIF1, eIF1A, the eIF2·GTP·Met-tRNA$_i^{Met}$ ternary complex, and the small 40S ribosomal subunit (10). Dissociation of the eIF2 ternary complex and other critical initiation factors during translation of constitutively repressing uORFs is suggested to be the cause of the low levels of subsequent translation reinitiation at downstream coding sequences.

Although uORFs can serve as repressors of CDS translation in a constitutive manner, there are also examples of uORFs that serve as dampeners in a controlled fashion or even promote translation initiation at the CDS in response to environmental stresses (4, 5, 11). Based on these studies, uORFs can have the following core properties that are critical for translational control (Fig. 1). 1) They enhance reinitiation after uORF translation, allowing for degrees of translation initiation at the downstream CDS. For example, translation of short uORFs can allow for retention of critical initiation factors, such as eIF3, which facilitate efficient translation reinitiation (12, 13). 2) They demonstrate direct ribosome elongation stalling during translation of the uORF and, as a consequence, thwart translation at the downstream CDS. Pauses in elongation can be reliant upon RNA secondary structure, codon usage bias, or polypeptide sequences encoded in the uORF, as well as interaction of trans-acting factors (12, 14–17). 3) They promote ribosome dissociation from the mRNA and therefore diminish subsequent CDS translation. Ribosome dissociation from the mRNA post-uORF translation can also be regulated by nucleotide sequences or the polypeptide encoded in the uORF (18–23). 4) They position uORFs out-of-frame with CDSs, resulting in ribosome termination downstream of CDS start codons. Translating ribosomes are suggested to only "back up" in a 3′ to 5′ fashion for a small number of nucleotides, resulting in low translation of the CDS (24–26). 5) They allow for scanning ribosomes to bypass the uORF either in a largely constitutive fashion or upon induction of physiological signals. Ribosome bypass is thought to occur at least in part due to the nucleotide sequences flanking the initiation codon (9, 18, 27, 28). These uORF properties, which can be integrated individually or in combination within mRNAs, help determine the specific mechanism of translational control of a given gene (Fig. 1). This review highlights the mechanisms by which uORFs can modulate translation at CDSs, and the processes by which uORFs with these diverse properties can be integrated individually or in combination into mRNAs to facilitate differential regulation of translation.

Translation Regulation during the Integrated Stress Response

Phosphorylation of eIF2 on its α subunit at serine 51 (eIF2α-P) inhibits the activity of the guanine nucleotide

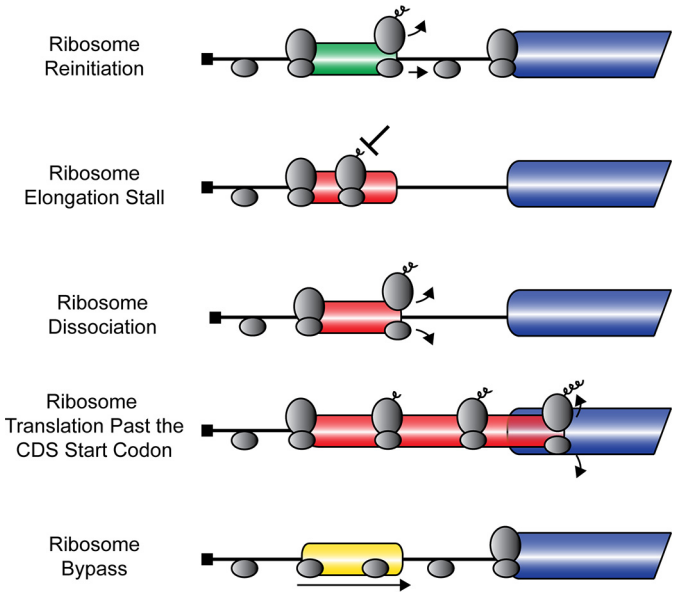

FIGURE 1. **uORFs regulate downstream CDS translation.** uORFs can have multiple core properties, including promoting ribosome reinitiation after uORF translation, ribosome elongation stalling while translating the uORF, ribosome dissociation from the mRNA, ribosome translation of uORFs past the CDS start codon, or ribosome bypass of the uORF. CDSs are indicated by the *blue bar*; positive-acting uORFs are indicated by a *green bar*; negative-acting uORFs are indicated by a *red bar*; and uORFs that have no effect on downstream translation are indicated by a *yellow bar*. Scanning and elongating ribosomes are illustrated by the *gray ovals*.

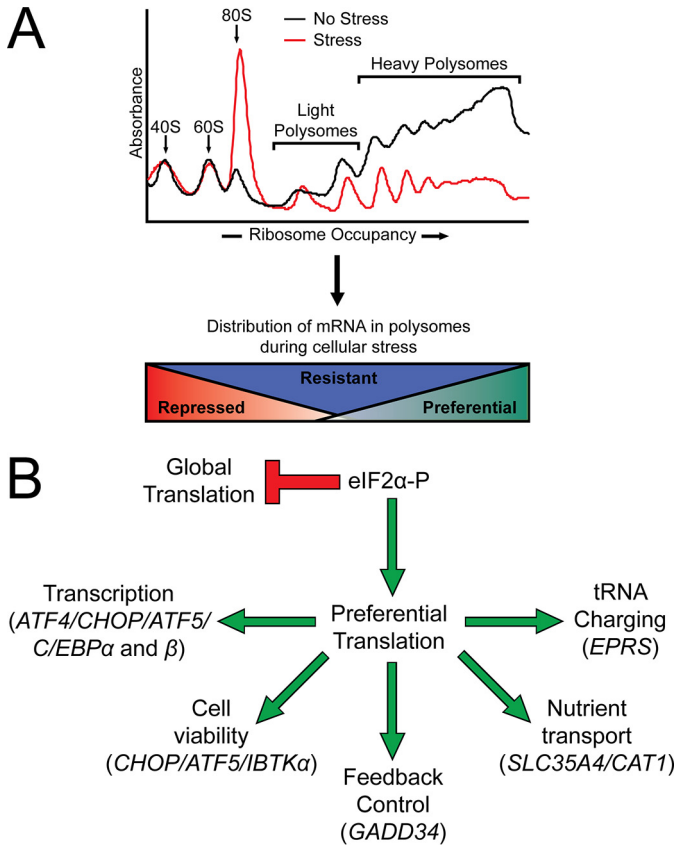

FIGURE 2. **The integrated stress response features a global reduction in translation initiation concomitant with the preferential translation of stress remediation transcripts.** *A*, depiction of polysome profiles as measured by sucrose gradient analyses of lysates prepared from mouse embryonic fibroblast cells that were left untreated (*black line*) or subjected to the ER stress inducer thapsigargin (*red line*). Basal polysome profiles feature distinctive peaks for the 40S and 60S ribosomal subunits and the 80S monosome, with large peaks observed for heavy polysomes, indicative of high levels of global translation. Polysome profiles from cells subjected to ER stress feature decreased heavy polysomes and an elevated 80S monosome peak that is indicative of inhibition of global translation initiation during eIF2α-P. Those mRNAs that are preferentially translated during cellular stress are largely associated with heavy polysomes, whereas those mRNAs that are repressed during cellular stress are largely associated with 80S monosomes and light polysomes. The mRNAs that are translated constitutively are associated with polysomes independent of stress. *B*, depiction of the preferentially translated mRNAs and their function in stress remediation. Multiple preferentially translated mRNAs encode transcription factors that promote stress alleviation (*ATF4*, *C/EBP*α, and *C/EBP*β). If the cellular stress is too great to overcome, a subset of transcription factors promotes a pro-apoptotic signaling cascade (*CHOP* and *ATF5*). Feedback dephosphorylation occurs through the activity of the preferentially translated *GADD34*. Priming of the cell for resumption of global translation occurs through the activity of the preferentially translated nutrient transporters *SLC35A4* and *CAT1*, as well as the glutamyl-prolyl tRNA synthetase *EPRS*. Cell fate regulator *IBTK*α is also preferentially translated through an uORF-mediated mechanism.

exchange factor eIF2B that results in a decrease in the exchange of GDP for GTP, thus lowering formation of the 43S preinitiation complex that triggers a global reduction in translation initiation (Fig. 2*A*) (29–31). Because eIF2α-P can direct translational control in response to a range of different environmental stresses, this pathway is often referred to as the integrated stress response (ISR) (32). Lowered protein synthesis would allow cells to conserve nutrients and energy and facilitate reprogramming of gene expression to alleviate stress damage. To aid in reprogramming of gene expression during eIF2α-P, a subset of mRNAs is preferentially translated via uORF-mediated mechanisms. uORFs are similarly distributed among mRNAs that are repressed, resistant, or preferentially translated during cellular stress and eIF2α-P, emphasizing that it is the specific properties of the uORFs that are critical for their regulatory capabilities in translation (Fig. 2*A*) (5). Furthermore, the proper mixing and matching of these uORF features are critical for uORF-mediated translational control mechanisms that appropriately regulate gene expression.

Encoded CDS products of mRNAs that are preferentially translated through uORF-mediated mechanisms play diverse roles in remediation of cellular stress (Fig. 2*B*). Included among the ISR preferentially translated gene transcripts are *ATF4* (*CREB2*), *CHOP* (*DDIT3/GADD153*), *ATF5*, and *C/EBP*α and *C/EBP*β that each encode basic leucine zipper transcription factors that modify gene expression programs to address cellular stress (24, 27, 33–37). GADD34 (PPP1R15A) combines with the catalytic subunit of protein phosphatase 1 (PP1c) to regulate dephosphorylation of eIF2α-P and restore protein synthesis (Fig. 2*B*) (18, 38–41). Nutrient transporters SLC35A4 and CAT1, as well as the bifunctional glutamyl-prolyl tRNA synthetase EPRS, serve to increase available nutrients and prime the cell for resumption of protein synthesis once cellular stress is remediated (Fig. 2*B*) (11, 26). Finally, the cell fate regulator IBTKα was shown to be subject to preferential translation through a mechanism involving uORFs (Fig. 2*B*) (5).

Ribosome Reinitiation in the ISR

The capacity for the ribosome to reinitiate translation downstream has been attributed largely to the ability of the scanning ribosome to retain or reacquire critical initiation factors following uORF translation (42). Extended distance between the

uORF stop codon and CDS initiation codon allows more time for the scanning 40S to reacquire a new eIF2·GTP·Met-tRNA$_i^{Met}$ complex, a significant feature of the delayed translation reinitiation model that was originally identified in the yeast *Saccharomyces cerevisiae* for the transcriptional activator GCN4 (19, 21, 43) and subsequently suggested for the related mammalian ATF4 (24, 33, 34).

Delayed Translation Reinitiation

Both yeast *GCN4* and mammalian *ATF4* encode transcription factors that increase expression of genes involved in nutrient import, metabolism, and alleviation of oxidative stress (32, 33, 44–48). The 5′-leader of *ATF4* contains two uORFs: the 5′-proximal uORF1 that is three codons in length and a 59-codon-long uORF2 that overlaps out-of-frame with the *ATF4* coding region (Fig. 3*A*) (24, 34). The *GCN4* 5′-leader contains four uORFs, each encoding polypeptides two to three residues in length (19, 21). In the delayed translation reinitiation model, the 5′-proximal uORF1 in the *ATF4* and *GCN4* 5′-leaders acts as a positive element that promotes downstream translation reinitiation (19, 20, 24). During nonstressed conditions, the 40S ribosome resumes scanning after translation of uORF1 and reacquires a new eIF2·GTP·Met-tRNA$_i^{Met}$ complex in sufficient time to reinitiate translation at the next uORF initiation codon. In the case of *ATF4*, translation initiation at the overlapping out-of-frame uORF2 results in translation termination and ribosome dissociation 3′ of the *ATF4* CDS, thereby reducing synthesis of ATF4 protein (Fig. 3*A*) (24). Translation of the downstream *GCN4* uORFs 3 or 4 also thwarts expression of the *GCN4* coding region during nonstressed conditions (see Fig. 4*C*) (21, 49). The inhibitory property of uORF4 relies upon a 10-nucleotide sequence 3′ of the uORF4 stop codon that is suggested to interact with the 40S ribosomal subunit to promote ribosome dissociation (49).

During cellular stress, eIF2α-P results in lowered levels of eIF2·GTP required for delivery of Met-tRNA$_i^{Met}$ for reinitiating ribosomes. As a consequence, after translation of uORF1, the scanning 40S ribosomal subunit takes a longer amount of time to reacquire a new eIF2 ternary complex that is required for recognition of the next translation initiation codon in the 5′-leaders of the *ATF4* and *GCN4* mRNAs. The delay in the acquisition of eIF2 ternary complex allows the 40S ribosomal subunit to scan through the inhibitory uORFs in the two mRNAs and instead promote translation initiation at the *ATF4* or *GCN4* CDS, promoting production of ATF4 and GCN4 proteins that serve to transcriptionally enhance genes important for remediation of the stress damage (Fig. 3*A*). Lack of appropriate *GCN4* and *ATF4* expression renders cells susceptible to nutrient deficiencies and oxidative damage (32, 46, 50).

Translation Reinitiation and Differential CDS Translation

Levels of eIF2α-P and the ensuing reduction of eIF2·GTP levels also play a role in start codon selection that regulates the abundance of protein isoforms encoded in the same mRNA. For example, *C/EBPβ* mRNA has four AUG initiation codons that encode three different protein isoforms and one uORF (37). The protein isoforms encoded in *C/EBPβ* are transcription factors that regulate adipogenesis, immunity, and functions of bone and liver tissues (51, 52). All three protein isoforms contain the basic leucine zipper domain, but the two larger isoforms, liver-enriched activating proteins (LAP* and LAP), also contain an N-terminal domain that activates transcription of target genes, which is missing in the smaller isoform, liver-enriched inhibitory protein (LIP) (37, 53). As a consequence, the LAP* and LAP isoforms can dimerize and serve as activators of transcription, whereas production of LIP serves as a transcriptional repressor (52).

The largest protein isoform, LAP*, is translated beginning at the most 5′-proximal AUG (37). The next AUG that follows the LAP* initiation site encodes an out-of-frame uORF. Importantly, the uORF termination codon is located 5′ of the initiation sites for both LAP and LIP and plays a central role in the regulation of translational expression of the LAP and LIP iso-

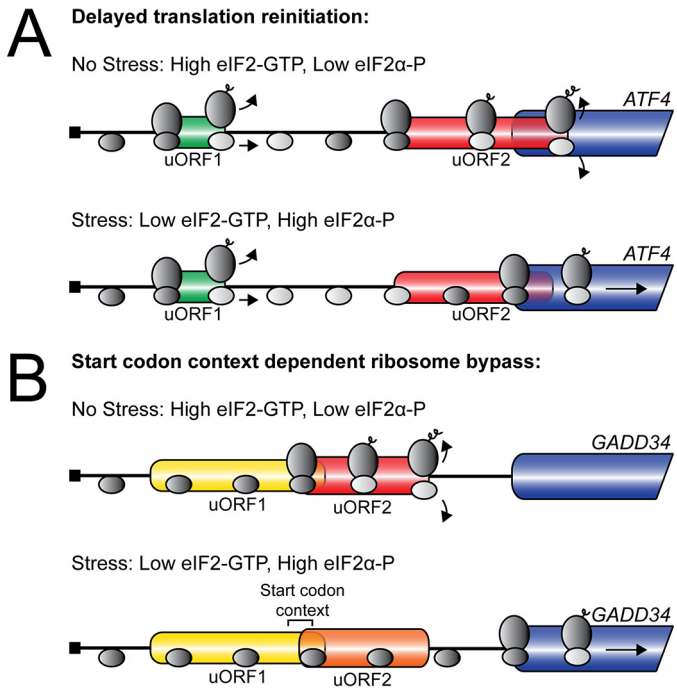

FIGURE 3. **uORFs regulate mRNA translation through diverse mechanisms.** *A*, depiction of the *ATF4* mechanism of preferential translation. During nonstressed conditions, there are low levels of eIF2α-P and high levels of eIF2-GTP. Ribosomes scanning the *ATF4* mRNA initiate at the 5′-proximal uORF1, and following termination, quickly reacquire a new eIF2 ternary complex. Competent 40S scanning ribosomes (*dark gray oval*) then reinitiate translation at uORF2, which overlaps out-of-frame with the *ATF4* CDS. Translation of uORF2 results in ribosome termination and dissociation 3′ of the *ATF4* initiation codon, resulting in low *ATF4* expression. During cellular stress, elevated eIF2α-P results in low levels of eIF2-GTP. Ribosomes scanning the *ATF4* mRNA initiate at uORF1 and post-uORF translation resume scanning. Due to the low levels of eIF2 ternary complex, the 40S ribosome (*light gray oval*) scans pass the initiation codon of the inhibitory uORF2 before reacquiring a new ternary complex (*dark gray oval*). Delayed acquisition of the eIF2 ternary complex results in translation initiation at the *ATF4* CDS and an increase in *ATF4* expression during cellular stress. *B*, depiction of the *GADD34* mechanism of preferential translation. During nonstressed conditions, scanning ribosomes bypass the *GADD34* uORF1 due to its poor start codon context and initiate translation at uORF2. Translation of a Pro-Pro-Gly peptide sequence juxtaposed to the uORF2 stop codon results in an inefficient ribosome termination event that increases ribosome release from the mRNA and causes low levels of basal *GADD34* expression. During cellular stress, elevated eIF2α-P results in a ribosomal bypass of uORF1 due to its poor start codon context and uORF2 due to its moderate Kozak consensus sequence. Bypass of the inhibitory uORF2 by a portion of scanning ribosomes results in increased translation initiation at the *GADD34* CDS and an increase in *GADD34* expression.

forms (37). After translation of the uORF, a number of 40S ribosomes are suggested to scan past the initiation codon of the LAP isoform before reacquiring a new eIF2·GTP·Met-tRNA$_i^{Met}$ complex and begin translation at the initiation codon of LIP. LIP expression is also elevated after UV irradiation, suggesting that decreased eIF2 ternary complex levels during cellular stress exacerbate this phenomenon (54). The related gene *C/EBPα* and its encoded protein isoforms are also translationally regulated by a 5′-proximal uORF (28, 37, 55).

Ribosome Bypass in the ISR

Selection of the translation start site relies largely on the nucleotide context surrounding the start codon. The optimal sequence, termed the Kozak consensus sequence, is GCC(A/G)CCAUGG of which the most important residues are the purines in the −3 and +4 positions, and the initiation codon is underlined (9, 56). These two residues have been shown to interact with the eIF2α subunit and the 18S rRNA contained within the small ribosomal subunit and are proposed to promote recognition of the initiation codon (57). Additionally, poor uORF start codon context has been associated with those mRNAs that are preferentially translated, whereas mRNAs that are repressed during eIF2α-P typically contain an uORF in a strong Kozak consensus sequence (5). These findings suggest that start codon context plays a significant role in uORF-mediated translation regulation.

Ribosome Bypass Is Dependent on Start Codon Context

An important example in the ISR of ribosome bypass of an uORF is that of *GADD34* (18, 38). GADD34 functions in feedback control of the ISR (39–41). The *GADD34* mRNA contains two uORFs (Fig. 3*B*). The uORF1 is considered to be a constitutive, modest dampener of downstream *GADD34* expression (18, 38). uORF1 is in a poor Kozak consensus sequence and is largely bypassed both basally and during stress conditions. Translation of uORF2, however, results in a significant decrease in translation initiation at the CDS, indicating that uORF2 is largely inhibitory to downstream translation (Fig. 3*B*) (18, 38). The inhibitory nature of *GADD34* uORF2 relies in part upon a Pro-Pro-Gly sequence juxtaposed to the stop codon (18). Translation of the Pro-Pro-Gly sequence in uORF2 is suggested to result in an inefficient termination that increases ribosome release from the mRNA, promoting low levels of *GADD34* expression during basal conditions (Fig. 3*B*). This was an interesting finding, because polyproline and Pro-Pro-Gly sequences have been shown to require the activity of an additional factor, eIF5A, for efficient translation (58).

Preferential translation of *GADD34* in response to eIF2α-P and cellular stress occurs by scanning ribosome bypass of uORF2 by a process involving, at least in part, the poor start codon context of this inhibitory uORF (Fig. 3*B*) (18). Appropriate inhibition of *GADD34* translation by uORF2 and its regulated bypass is critical for the magnitude and duration of translational control during the progression of a stress response. For example, deletion of the inhibitory *GADD34* uORF2 results in an inappropriate increase in *GADD34* expression even in the absence of stress that serves to sharply diminish levels of eIF2α-P even upon induction of the ISR (18). As a consequence, there are continued high levels of protein synthesis and increased cell sensitivity to cellular stress.

Ribosomal bypass of an uORF is also central for preferential translation of *CHOP* in the ISR (27, 35). Prolonged expression of *CHOP* during times of chronic stress and continued eIF2α-P can induce apoptosis (59–62). Analogous to *GADD34*, bypass of the inhibitory *CHOP* uORF during eIF2α-P is also suggested to rely at least in part upon poor start codon context of the uORF (14, 27). The inhibitory nature of the *CHOP* uORF during basal conditions is centered upon an elongation stall at an encoded polypeptide segment (14). Emphasizing the importance of the uORF for appropriate *CHOP* expression in the ISR, mutations rendering the uORF nonfunctional lead to overexpression of CHOP and its downstream pro-apoptotic target genes, enhancing cell death during stress (14).

We do not yet understand the mechanisms by which selection of translation start sites can be affected by eIF2α-P. It was suggested that eIF2α-P disrupts the stability of the interaction between the scanning ribosomal subunit and the mRNA at the −3 position of the start codon (63). Another possibility is that eIF2α-P may modify the nature of the interaction between eIF2 and the Met-tRNA$_i^{Met}$ that regulates start site selection (63). Alternatively, the effects of eIF2α-P on start codon selection may be indirect. For example, eIF2α-P may alter the expression and stoichiometry of other initiation factors, such as eIF1, which play a role in translation start site selection (64). The recently characterized small molecule ISRIB (integrated stress response inhibitor), which renders eIF2B largely insensitive to eIF2α-P (65–67), may provide an important tool for discerning genome-wide changes in initiation codon selection afforded by eIF2α-P.

Ribosome Bypass and Non-canonical Start Codons

Recent ribosome profiling studies have illustrated that previously uncharacterized uORFs with non-canonical initiation codons are more commonly translated than previously thought (3, 4, 6). This observation has been followed by the characterization of multiple mechanisms involving bypass of uORFs due to their non-canonical initiation codons that regulate gene-specific translation during cellular stress conditions (4, 26, 68).

One such example is the translation mechanism for glutamyl-prolyl tRNA synthetase, EPRS. Although *EPRS* expression is required for normal protein homeostasis, preferential translation of *EPRS* is suggested to increase the appropriately charged tRNA pool and prime the cell for resumption of protein synthesis once the cellular stress is remediated (26, 69). Of the five non-canonical initiation codons in the 5′-leader of *EPRS* only the 5′-proximal UUG and the subsequent CUG are considered to be the main regulators of *EPRS* preferential translation (26). The uORF containing the CUG initiation codon overlaps out-of-frame with the *EPRS* CDS and was shown to be inhibitory to *EPRS* expression, presumably by ribosome dissociation from the mRNA past the initiation codon for the CDS (26). The uORF encoded by the UUG, on the other hand, terminates 51 nucleotides 5′ of the CDS start codon, and was suggested to allow for a portion of the translating ribosomes to reinitiate at the downstream *EPRS* CDS (26). Importantly, both inhibitory uORFs are bypassed due to their non-canonical ini-

tiation codons to a moderate extent during basal conditions and experience increased bypass efficiency during eIF2α-P and stress (26).

Another gene recently identified as containing a functional uORF with a non-canonical initiation codon is that of *GADD45G*, which regulates cell growth and apoptosis (4). The *GADD45G* mRNA contains an overlapping out-of-frame uORF with a CUG initiation codon. The uORF serves as a barrier to downstream translation during nonstressed conditions and is bypassed due to its noncanonical initiation codon during eIF2α-P (4). Also recently described is the model of translation control for *BiP* (*GRP78*/*HSPA5*) that participates in protein folding in the ER (68). The 5′-leader of the *BiP* mRNA features two uORFs that are both encoded by non-canonical initiation codons, as well as possible internal ribosome entry sequences (IRES), which may function in conjunction with uORFs to facilitate *BiP* translation during cellular stress (68, 70–72).

Other Complexities of Gene Expression Regulated by uORFs

The observation that the 5′-leader of *BiP* mRNA contains both uORFs and an IRES that coordinate expression of *BiP* emphasizes that other mRNA structural features also contribute to translation regulation involving uORFs. The mRNA encoding the cationic amino acid transporter CAT1 contains both an uORF and an IRES that are required for induced expression of the *CAT1* CDS during cellular stress (73, 74). The 5′-leader of *CAT1* forms a stable secondary structure that prevents translation during nonstressed conditions. However, translation of the uORF during eIF2α-P is suggested to unfold the RNA structure, yielding an IRES that serves to increase translation of the *CAT1* CDS (75).

The mRNA encoding the HER2 proto-oncogene contains an uORF that represses translation initiation at the primary *HER2* initiation codon due to insufficient time for the scanning 40S ribosomal subunit to reacquire a new eIF2 ternary complex (76, 77). Instead translation reinitiation occurs at alternative downstream initiation codons that lead to the synthesis of N-terminally truncated HER2 protein isoforms (77). However, full-length *HER2* expression is enhanced in in both breast and ovarian cancers (76). This regulatory scheme is suggested to occur via interactions between RNA secondary structures in the 3′-UTR of the *HER2* mRNA and the terminating ribosome at the uORF stop codon. This interaction is thought to retain the terminating ribosome and associated initiation factors on the *HER2* mRNA until a new eIF2 ternary complex is acquired, thereby facilitating translation at the most immediate 5′-proximal AUG that leads to full-length HER2 protein synthesis (76).

The three major regulators of mRNA abundance, transcription, mRNA processing, and mRNA degradation, also play major roles in uORF-mediated regulation of ISR-induced preferential translation (54, 78–80). For example, *ATF4* expression is potently induced during endoplasmic reticulum stress, but there are only low levels of *ATF4* expression during UV irradiation (54, 81). Both conditions induce robust eIF2α-P, and preferential translation of *ATF4* mRNA occurs with either stress (81). However, increased LIP expression and binding to the *ATF4* promoter during UV irradiation repress *ATF4* transcription (54). Lowered levels of *ATF4* mRNA available for translation during UV stress thus result in negligible ATF4 protein and transcriptional activity. These findings illustrate that *ATF4* mRNAs with identical 5′-leaders and uORF configurations have sharply different induction capabilities in response to different stress conditions despite having comparable levels of eIF2α-P.

Expression of the ISR transcriptional regulator *ATF5* features two different mRNA isoforms (79). The more abundant transcript, *ATF5*α, contains two uORFs that serve to promote preferential translation by delayed translation reinitiation (36, 80). *ATF5*β encodes the same *ATF5* CDS, but contains an alternative 5′-leader that does not contain any conserved uORFs and is not translationally regulated in a stress-dependent manner (80). Thus, expression of *ATF5* can also be modulated through the differential production of mRNA isoforms (79). Recent genome-wide evidence has suggested that regulation of translation through mRNA splicing also plays a significant role in the presence of different 5′-leaders in mRNAs that can affect translational expression (78).

uORF translation can also result in activation of the mRNA decay pathways, thus adding another layer to the mechanisms in which uORFs can negatively regulate downstream translation (82). For example, *CHOP* was identified as a target of the nonsense-mediated mRNA decay pathway that recognizes the presence of a premature termination codon (83). Depletion of the nonsense-mediated mRNA decay machinery from cells results in the stabilization of *CHOP* mRNA levels (84). *CHOP* mRNA half-life was also increased more than 2-fold in cells in which the two initiating codons of the *CHOP* uORF were mutated (14). Combined, these studies suggest that the presence of an uORF can also serve to repress expression of the CDS through mechanisms involving mRNA decay.

Evolutionary Conservation of uORF-mediated Translation Mechanisms

Many uORF-mediated translation control schemes that rely on eIF2α-P are conserved in eukaryotes. For example, the uORF-mediated translational control mechanism for *IBTK*α is suggested to be largely conserved among mammals (Fig. 4*A*) (5). Interestingly, the *Homo sapiens IBTK*α mRNA has four uORFs, but only the two key uORFs that confer *IBTK*α translational control are consistently conserved among mammals (Fig. 4*A*) (5). This finding indicates that those uORFs that are retained throughout species likely maintain functional significance. This idea is emphasized in genome-wide analyses of uORF conservation in which the presence of an uORF and the regulatory nature of uORF(s) is suggested to be conserved throughout species (6, 7, 85–87).

An example in which the regulatory function of an uORF is retained, but the underlying mechanisms vary among species is the inhibitory uORF located in the 5′-leader of *GADD34* mRNA (18, 38, 88). The 5′-leaders of the *Drosophila melanogaster* and *Mus musculus GADD34* transcripts each contain two uORFs, with the first uORF considered to be largely dispensable for *GADD34* translation control (Fig. 4*B*) (18, 38, 88). uORF2 in *D. melanogaster* overlaps out-of-frame with the *GADD34* CDS and is considered to be inhibitory by promoting

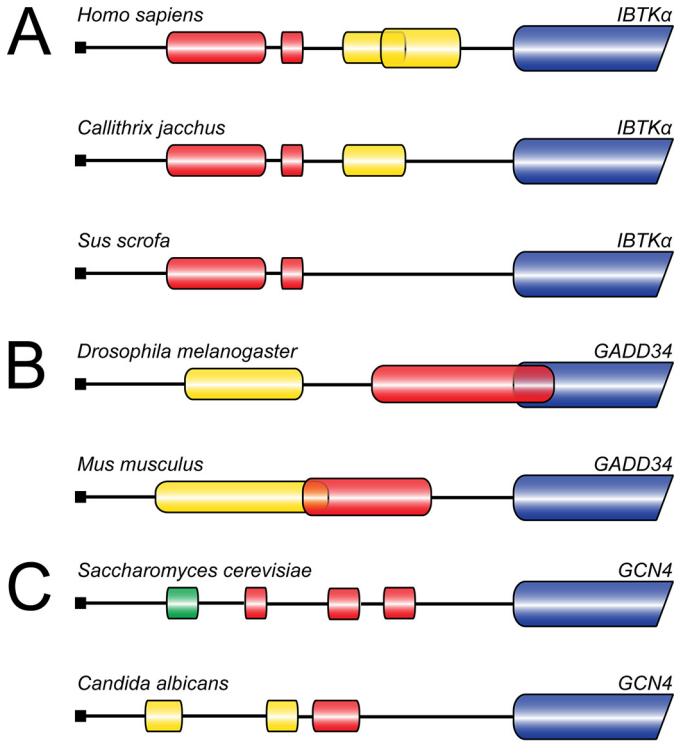

FIGURE 4. **uORF mechanisms of translation control are evolutionarily conserved.** *A*, illustration of the *IBTKα* 5′-leader in multiple species including: *H. sapiens*, *Callithrix jacchus*, and *Sus scrofa*. Translation of *IBTKα* mRNA is regulated by a bypass mechanism. The inhibitory uORFs 1 and 2 (*red bars*) repress *IBTKα* CDS translation during nonstressed conditions. The inhibitory effects of uORF1 and 2 are overcome during eIF2α-P, facilitating the preferential translation of *IBTKα* (*blue bar*). uORF 3 and 4 (*yellow bars*) are considered to be dispensable for *IBTKα* translation control and are not conserved between species. *B*, depiction of the 5′-leaders for *D. melanogaster* and *M. musculus GADD34* mRNAs. The 5′-leader of *GADD34* mRNA in both species contains a dispensable uORF1 (*yellow bar*) that is largely bypassed independent of cellular stress. uORF2 (*red bar*) in both mRNAs is translated during basal conditions and is inhibitory to downstream *GADD34* CDS translation. uORF2 in *D. melanogaster* overlaps out-of-frame with the *GADD34* CDS (*blue bar*) and promotes ribosome dissociation from the mRNA 3′ of the initiation codon of the *GADD34* CDS. The *M. musculus* uORF contains an inhibitory Pro-Pro-Gly sequence juxtaposed to the uORF2 termination codon that promotes inefficient termination that increases ribosome dissociation from the mRNA. During cellular stress, the inhibitory uORF2 in either *D. melanogaster* or *M. musculus* is bypassed, resulting in increased translation initiation at the *GADD34* CDS and increased GADD34 protein synthesis. Bypass of *M. musculus* uORF2 relies upon its moderate start codon context, whereas bypass of *D. melanogaster* uORF2 may rely upon additional factors. C, illustration of the 5′-leader of *GCN4* in fungal species *S. cerevisiae* and *C. albicans*. Translation control of *S. cerevisiae GCN4* relies on a delayed translation reinitiation model in which translation of the positive acting uORF1 (*green bar*) promotes translation reinitiation at downstream uORFs. Translation of the following uORFs 2, 3, and 4 (*red bars*) in the *S. cerevisiae GCN4* 5′-leader are inhibitory to downstream translation by promoting ribosome dissociation from the mRNA in nonstressed conditions. During cellular stress, low levels of eIF2 ternary complex levels allow the scanning 40S ribosome to scan through the inhibitory uORFs in *GCN4* post-uORF1 translation, resulting in translation initiation at the *GCN4* CDS (*blue bar*). *C. albicans* translation control relies on a bypass mechanism in which only uORF3 (*red bar*) is required for regulation of *GCN4* expression. In nonstressed conditions, translation of uORF3 precludes the ribosome from initiating translation at the *C. albicans GCN4* CDS, presumably through ribosome dissociation from the mRNA. During cellular stress, eIF2α-P promotes bypass of the inhibitory uORF3, thereby facilitating an increase in translation of the *GCN4* coding region (*blue bar*).

uORF translation termination 3′ of the start codon for the *GADD34* CDS (88). By comparison, *M. musculus* uORF2, which terminates 23 nucleotides upstream of the *GADD34* CDS, prohibits reinitiating downstream due to the inefficient translation termination (Fig. 4*B*) (18). Both inhibitory uORFs have been suggested to require ribosome bypass during cellular stress for preferential translation of *GADD34* (18, 88).

Assessment of the *GCN4* mRNAs in 12 fungal species revealed that uORFs range from three to six in number and are not positionally conserved (25, 89). Furthermore, the mechanism of *GCN4* translation control for *Candida albicans* is reliant upon bypass of a single inhibitory uORF whereas *GCN4* in *S. cerevisiae* relies on a mechanism involving delayed translation reinitiation that features multiple uORFs (Fig. 4*C*) (19, 25). These findings suggest that even among gene orthologs in different species, different uORF configurations and mechanisms can be implemented to achieve preferential translation in response to eIF2α-P.

These examples of the evolutionary conservation of uORF-mediated translation control emphasize that there are multiple features of uORFs that can be combined in specific ways to generate uORFs of similar functions in regulation of translation. Furthermore, the proper composition and position of uORFs and their features are critical for uORF-mediated translation control mechanisms that direct regulated gene expression for optimal adaptation to environmental stress.

A future challenge is the application of key tenets from uORF-mediated mechanisms described herein to genome-wide assessments of translation. The ubiquitous placement of uORFs throughout the genome indicates that the specific properties of uORFs themselves determine whether uORFs regulate gene expression in a positive or negative fashion. How can the specific features of uORFs be used to predict patterns of translation control? Furthermore, although uORFs regulate mRNA translation in a cis manner, can some uORF peptides function in trans to facilitate cellular homeostasis? Finally, base modifications in RNA, such as N^6-methyladenosine, can occur differentially in the 5′-leaders of mRNAs in response to cellular stress (90, 91). Additionally, N^6-methyladenosine can promote translation of select mRNAs by binding to eIF3 and recruiting ribosomes independent of the 5′-cap. Can RNA base modifications in specific mRNAs alter ribosome scanning and uORF utilization affecting translational control in the ISR?

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