Chapter 5
Role of Protein Translation in Unfolded Protein Response

Surojeet Sengupta, V. Craig Jordan, and Robert Clarke

Abstract The unfolded protein response (UPR) is an adaptive mechanism to maintain protein homeostasis by decreasing the accumulation of unfolded proteins in the endoplasmic reticulum (EnR) of cells. EnR stress activates three distinct sensors, namely, inositol requiring protein 1 alpha (IRE1-α), activating transcription factor 6 (ATF6), and protein kinase RNA-like endoplasmic reticulum kinase (PERK), that collectively mitigate the damaging effects of EnR stress. The downstream signaling from the PERK sensor phosphorylates the eukaryotic translational initiation factor 2 alpha (eIF2α) complex that inhibits global protein translation to restore proteostasis and promote cell survival. However, chronic and unmitigated activation of the PERK pathway leads to apoptosis. Phosphorylation of eIF2α is tightly controlled by the two specific regulatory subunits of protein phosphatase 1 (PP1) complex, (1) growth arrest and DNA damage inducible-34 (GADD34) and (2) constitutive repressor of eIF2α phosphorylation (CReP), that are responsible for dephosphorylation of eIF2α. Phospho-eIF2α also directs preferential translational of stress-related genes such as ATF4 and CHOP. This chapter describes the mechanism by which the PERK pathway regulates the protein translational machinery that plays a critical role in deciding cell fate following endoplasmic reticulum stress.

Keywords ATF4 · ATF6 · CHOP · CReP · Endoplasmic reticulum stress · eIF2α · FoxO1 · GADD34 · IRE1α · Metabolism · Neurodegenerative · PERK · uORF

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Introduction

Protein synthesis is a fundamental mechanism in living organism that translates the information encoded in the mRNA molecule. Protein translation in eukaryotes is an extremely complex, energy consuming, multi-tiered process requiring multiple components and thus it is tightly regulated at many levels [1–3]. The endoplasmic reticulum (EnR) is a key cell organelle where protein is synthesized, folded, and achieves structural maturity [4, 5]. Maintenance of protein homeostasis, also known as 'proteostasis', encompasses a concerted interconnecting network of cellular processes that controls the structural, spatial, and functional integrity of the proteins making up the proteome [6]. The regulatory controls for protein synthesis often operate at the levels of transcription and/or translation. Revolutionary technologies in genomics and proteomics have enabled us to develop a comprehensive understanding of gene regulation at the system-level. Recent studies [7] have revealed that levels of mRNA transcripts are often not sufficient to predict the levels of their translated protein products. Instead, ribosome occupancy serves as a more reliable predictor of protein levels than levels of their mRNA. Therefore, control at the protein translation level plays a critical role in gene regulation. The process of translation can be divided into initiation, elongation, termination, and ribosome recycling [3]. Most of the regulation of protein translation is exerted at the initiation phase, allowing for a rapid and reversible control of gene expression [1, 3].

Initiation of Protein Translation

Protein synthesis initiation requires assembly of ribosomal subunits that are competent for translation elongation in which the anti-codon loop of initiator tRNA (Met-tRNA$^{\text{Met}}{}_{\text{i}}$) base pairs with the initiation codon of an mRNA [1, 3]. This process uses nine different eukaryotic initiation factors (eIFs). A ternary complex (TC) is first formed comprising a 40S ribosomal subunit, eIF2-GTP, and Met-tRNA$^{\text{Met}}{}_{\text{i}}$. This transforms the TC into a 43S pre-initiation complex (PIC) by complexing with certain other eIFs and binding to the capped 5′ proximal region of mRNA. This step involves additional eIFs that are needed to unwind the secondary structure of the mRNA [1]. The 43S complex scans the 5′ untranslated region (5′ UTR) of mRNA in the 5′ to 3′ direction until it recognizes the initiation codon. Once the initiation codon is recognized and the 48S complex is formed, eIF5 and eIF5B promote hydrolysis of the eIF2-bound GTP, the displacement of eIFs, and joining of a 60S ribosomal subunit.

Unfolded Protein Response

The unfolded protein response (UPR) is a collection of adaptive feedback mechanisms induced following the accumulation of excess unfolded proteins in the lumen of endoplasmic reticulum (EnR) (known as EnR stress) in cells with a high load of protein synthesis [8]. UPR is comprised of three distinct sensors that are activated following EnR stress, namely, **i**nositol **re**quiring protein 1 alpha (IRE1-α), **a**ctivating **t**ranscription **f**actor 6 (ATF6), and **p**rotein kinase RNA-like **e**ndoplasmic **r**eticulum **k**inase (PERK). These sensors activate diverse pathways collectively known as the UPR [8, 9] (Fig. 5.1). Signaling downstream from the UPR sensors coordinate complex cross-talk to restore proteostasis; this signaling is largely cyto-protective [9]. Many downstream signals are transcription factors (such as ATF6, ATF4, CHOP, CReP, XBP1s) that translocate to the nucleus and engage in the transcriptional regulation of a variety of genes that control cell fate [10]. Many of these genes

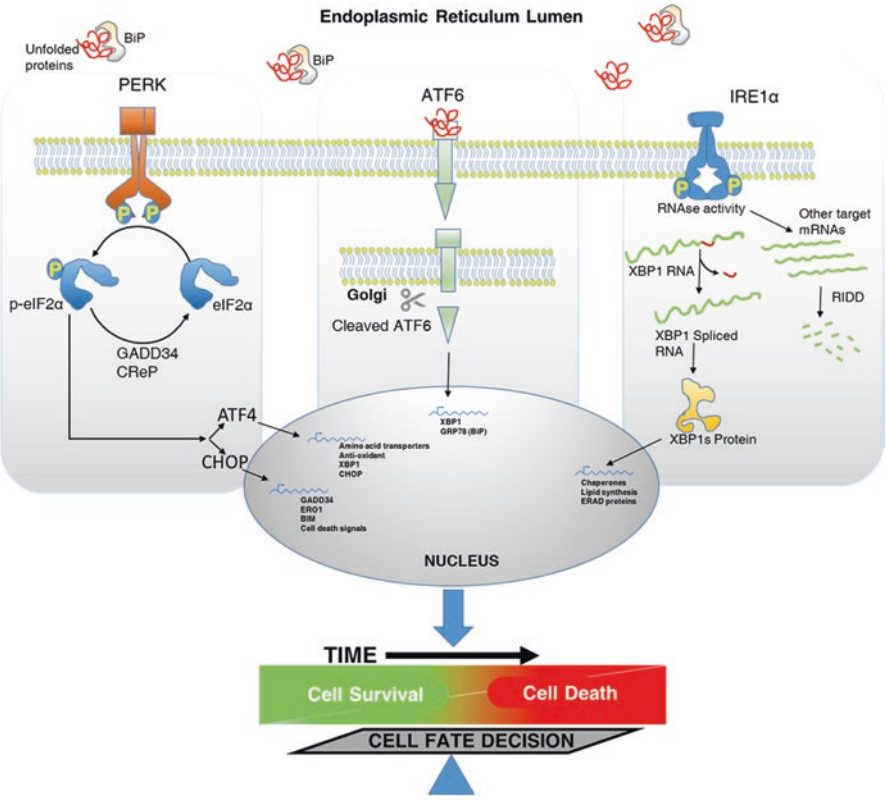

Fig. 5.1 Cartoon depicting the three arms of unfolded protein response (UPR) and its signals converging at the nucleus of the cell. The length of the time of UPR determines the cell fate

help in adaptation to stress, but some may also promote cell death. Therefore, a cell fate decision in favor of adaptive survival or cell death is a consequence of the integrated temporal response initiated by the three distinct sensors of EnR stress over time. For example, chronic and unmitigated UPR leads to apoptosis [11–14], mediated through the mitochondrial/intrinsic pathway [15].

All three UPR sensors (IRE1α, ATF6, PERK) are transmembrane proteins spanning through the EnR membrane [8]. The luminal domains of these proteins can sense an imbalance in protein folding efficiency inside the EnR and consequently trigger downstream signaling. Specifically, under basal, unstressed conditions the luminal domains of these sensor proteins bind with a chaperone, glucose-regulated protein 78 (GRP78) (also known as binding immunoglobulin protein (BiP) or heat shock protein A5 (HSPA5)) that prevents sensor activation [8, 10].

Activation of the PERK pathway hyper-phosphorylates eukaryotic translational initiation factor 2 alpha (p-eIF2α), which then inhibits global protein translation to restore proteostasis [10]. However, sustained activation of eIF2α can also lead to cell death [8, 16]. The level of eIF2α phosphorylation is tightly controlled by activity of the protein phosphatase 1 (PP1) complex by two specific regulatory subunits, (1) growth arrest and DNA damage inducible-34 (GADD34) and (2) constitutive repressor of eIF2α phosphorylation (CReP), that are responsible for dephosphorylation of eIF2α [16–18] (Fig. 5.2). The other two additional UPR sensors (ATF6 and IRE1α) also contribute to cell fate decisions by complex essential and redundant cross-talk with the PERK arm and modulate the downstream components that may have protective or apoptotic effects [19].

EIF2 Alpha Phosphorylation and Translational Regulation

Formation of the TC during the initiation of protein translation depends upon the availability of GTP-bound eIF2 and its recycling to maintain protein synthesis. Regeneration of GTP-eIF2 is ensured by another initiation factor, eIF2B, which functions as a guanine nucleotide exchange factor. The alpha subunit of eIF2 (eIF2α) can be phosphorylated on serine 51 residue by activated PERK and functions as a major regulatory checkpoint. Phospho-eIF2α can bind strongly to eIF2B and restrict its availability. While phosphorylated-eIF2α can form the TC, its higher levels block regeneration of the active GTP-bound eIF2α by sequestering eIF2B and abrogating its activity. Consequently, the low levels of GTP-eIF2α lead to reduced translation initiation and a suppression of global protein synthesis. Notably, three additional mammalian protein kinases, besides PERK (EIF2AK3) can phosphorylate eIF2α (Fig. 5.2). These three kinases are heme-regulated inhibitor kinase (EIF2AK1), which is significant only in erythroid cells [20]; PKR (EIF2AK2), which can be activated by viral response [21, 22]; and GCN2 (EIF2AK4) that is activated by amino acid starvation [23].

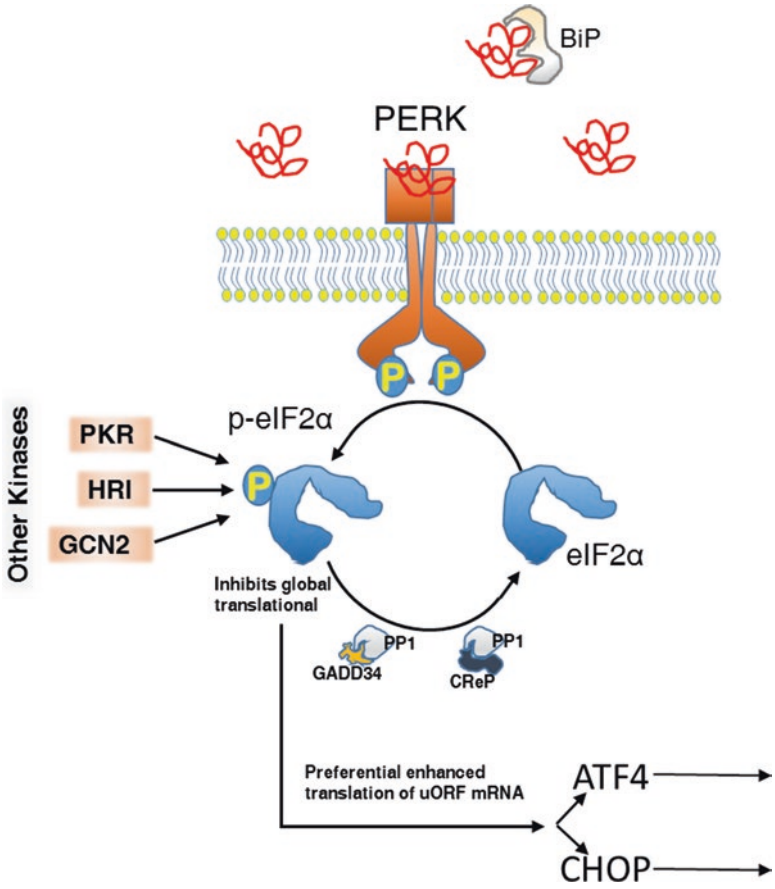

Fig. 5.2 PERK-eIF2α-ATF4/CHOP axis

Regulation of eIF2α Phosphorylation

Phosphorylation of eIF2α is a reversible process and its precise balance in critical for cell survival. To counteract the phosphorylation of eIF2α, two distinct proteins (GADD34 and CReP) function as regulatory subunits forming two distinct holocomplex with protein phosphatase 1 (PP1) to dephosphorylate eIF2α [13]. GADD34 (also known as PPP1R15A) is an inducible factor downstream of PERK activation and functions as a feedback loop [8, 13, 18, 24]. Conversely, CReP is the constitutively expressed regulatory protein of PP1 complex that dephosphorylates eIF2α and is responsible for maintaining the balance of phosphorylated and non-phosphorylated eIF2α in unstressed cells [8, 19, 25]. Activated IRE1α also cleaves other EnR mRNAs (besides XBP1) [26–29], ribosomal RNA [30], and microRNAs

[31, 32] that share specific nucleotide sequences as in XBP1 [28] in a process known as regulated IRE1α-dependent decay (RIDD). Notably, CReP mRNA is cleaved by activated IRE1α, establishing unique cross-talk between different UPR sensors that can increase phospho-eIF2α levels and reduce protein load [33].

Preferred Protein Translation of Selective Messenger RNA

The primary goal of UPR is to maintain ER proteostasis. Proteostasis is chiefly attained by global suppression of protein translation to reduce newly synthesized protein-load into EnR [34]. However, a distinct subset of messenger RNAs coding for certain proteins and transcription factors evade the global suppression of translation triggered by eIF2α phosphorylation. Paradoxically, these mRNAs are translated at a higher rate in stressed cells [35]. Most of these factors are required to respond to the consequences of EnR stress and include ATF4 [36, 37], GADD34 (PPP1R15A) [38], ATF5 [39–41], and CHOP (DDIT3) [42, 43]. The mechanism of enhanced protein synthesis of these factors is attributed to the small inhibitory upstream open reading frames (uORF) located within the 5′-leader (untranslated region) of their mRNA [44, 45]. Genome-wide ribosomal profiling has revealed that over 40% of mammalian mRNA contain uORFs that may serve as a major regulator of translation and protein levels [44, 46, 47].

Multiple mechanisms exist by which uORF-containing mRNAs can be translated preferentially in stressed cells with high levels of phospho-eIF2α [35]. The capacity to reinitiate translation from a downstream start codon, known as ribosome reinitiation, depends upon the ability of the scanning ribosome to acquire or retain the essential initiation factors following translation of uORF. The distance between the initiation codon of uORF and the initiation codon of the coding protein plays a critical role, as it allows more time to re-acquire a new eIF2-GTP-Met-tRNAimet [48]. For example, the transcription factor ATF4 mRNA contains uORFs that restrict ribosome access to the coding sequence (CDS) in unstressed cells but allows increased access and translation of the ATF4 protein in stressed cells with higher levels of phospho-eIF2α [36, 37]. This occurs because reduced eIF2α-GTP levels in stressed cells delay reinitiation of ribosomes that enables skipping of the inhibitory uORFs and increases ribosome access to the start codon of the ATF4 coding sequence [37].

Another mechanism by which preferential translation is regulated in stressed cells with high levels of phospho-eIf2α is reported for the GADD34 protein [49], which functions as a feedback control for eIF2α dephosphorylation [24, 50, 51]. The GADD34 mRNA contains two uORFs. uORF1 is constitutive but is bypassed due to a poor kozak sequence. uORF2 is the main inhibitory sequence as deletion of this uORF increases the expression of GADD34 [49]. Under stress conditions, the

ribosome bypasses uORF2 due to "poor start codon context," allowing higher translation from the CDS start codon [49]. This mechanism is also evident during the preferential translation of CHOP in chronically stressed cells [43, 52]. Expression of CHOP protein in cells with unmitigated stress and continued elevated levels of phospho-eIF2α can induce apoptosis [53–55]. Notably, GADD34, is regulated at the translational level and its rate of transcription is increased by ATF4 [56] and CHOP [57]. Both ATF4 and CHOP are preferentially translated in response to EnR stress and hyper-phosphorylation of eIF2α [37, 52] indicating a coordinated mechanism to remediate stress.

A recent study shows a new mechanism that relies on eIF2α independent and non-AUG starting codon in the uORF to regulate translation in stressed cells [58]. This study discovered that an alternative initiation factor, eIF2A, and non-AUG ORF is required for translation of GRP78 (HSPA5; BiP) during stress response [58]. In addition, the internal ribosome entry sequences (IRES) may also play a role in expression of GRP78 protein [59].

Role of PERK in Breast Cancer

Multiple studies have implicated a central role for the UPR in several cancers including breast cancers [60]. In breast cancers, integration of UPR, EnR stress, and autophagy drives the cell fate in endocrine therapy resistance [10]. For example, depletion of estrogen receptor [61] and GRP78 [62], a key component of the UPR pathway, restored endocrine sensitivity in the endocrine therapy resistant breast cancer cells. Inhibition of autophagy potentiates antiestrogen therapy in the resistant breast cancers [63]. In addition, XBP1, another key component of UPR, not only contributes to estrogen-mediated cell proliferation [64] but also plays a vital role in conferring endocrine resistance upon breast cancer cells [65–68].

In particular, the PERK pathway plays a critical role in oncogenic development, survival, progression, and invasion of cancers [69–73]. In breast cancers, PERK signaling is associated with invasion and metastasis [74], and selectively sensitizes cancer cells that have undergone an epithelial-to-mesenchymal transition to an EnR stress (EMT) [75].

High doses of estrogen were used as a therapy for estrogen receptor positive breast cancers before the discovery antiestrogens [76, 77]. Laboratory studies have confirmed that estrogen can induce apoptotic cell death in select LTED (long-term estrogen-deprived) breast cancer cells, both in vitro and in vivo [78, 79]. UPR, specifically, the PERK-eIF2α axis, is involved in estrogen induced apoptosis [80–82]. Studies have confirmed that prolonged and unmitigated phosphorylation of eIF2α can induce apoptosis in endocrine therapy resistant breast cancer cells using the same estrogen-mediated mechanism (Sengupta et al. in press, 2019).

Conclusions and Future Direction

Protein translation control has emerged as a critical mechanism to maintain the integrity of cells and allows them to adapt to numerous stress. Increasingly, this control is being recognized as a critical feature in development and various diseases including several cancers. Precise understanding of mechanisms governing the preferential translation of numerous proteins after activation of the PERK-eIF2α pathway will be immensely helpful in determining the factors responsible for cell fate decisions. In addition, the cross-talk between the different components of UPR pathway and its influence on the PERK pathway may predict a prosurvival or prodeath outcome. Clearly, further studies are needed to develop a unified model integrating all components of UPR signaling and its role in cell fate determination.

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