Roles of the Translation Initiation Factor eIF2α Phosphorylation in Cell Structure and Function

Sung Hoon Back*

School of Biological Sciences, University of Ulsan, Ulsan 44610, Korea

ABSTRACT. It is often assumed that α-subunit phosphorylation of the eukaryotic translation initiation factor 2 (eIF2) complex is just a mechanism to control protein synthesis. However, eIF2 α phosphorylation induced by multiple kinases can recognize various intracellular and extracellular stress conditions, and it is involved in various other cellular processes beyond protein synthesis. This review introduces the roles of eIF2 α phosphorylation in translational regulation, the generation of reactive oxygen species, changes in mitochondria structure and shape, and mitochondrial retrograde signaling pathways in response to diverse stress conditions.

Key words: eIF2α phosphorylation, Translation, Unfolded Protein Response, Reactive Oxygen Species, Mitochondria

Roles of eIF2α phosphorylation in translational regulation during diverse stress conditions

Suppression of global translation and eIF2α phosphorylation

In translation initiation, delivery of methionyl-initiator tRNA (Met-tRNA\textsuperscript{Met}) to the 40S ribosomal subunit is mediated by the GTP-bound eukaryotic translation initiation factor 2 (eIF2) complex, which consists of α, β, and γ subunits (Proud, 2005). The binding of GTP to eIF2 is the rate-limiting step for Met-tRNA\textsuperscript{Met} delivery and ultimately for protein synthesis (Sonenberg and Hinnebusch, 2009; Wek et al., 2006). Binding of GTP to eIF2 is mediated by guanine exchange activity (GEF) of the eIF2B complex (Wortham and Proud, 2015). In cells exposed to diverse stress conditions such as heme deprivation, viral infection, endoplasmic reticulum (ER) stress, and amino acid starvation, four mammalian protein kinases—HRI/EIF2AK1 (eIF2α kinase 1), PKR/EIF2AK2 (eIF2 α kinase 2), PERK/EIF2AK3 (eIF2α kinase 3), and GCN2/EIF2AK4 (eIF2α kinase 4)—phosphorylate serine 51 of the α subunit of the eIF2 complex (Proud, 2005). The binding of GTP to eIF2 is the rate-limiting step for Met-tRNA\textsuperscript{Met} delivery and ultimately for protein synthesis (Sonenberg and Hinnebusch, 2009; Wek et al., 2006). Binding of GTP to eIF2 is mediated by guanine exchange activity (GEF) of the eIF2B complex (Wortham and Proud, 2015). In cells exposed to diverse stress conditions such as heme deprivation, viral infection, endoplasmic reticulum (ER) stress, and amino acid starvation, four mammalian protein kinases—HRI/EIF2AK1 (eIF2α kinase 1), PKR/EIF2AK2 (eIF2α kinase 2), PERK/EIF2AK3 (eIF2α kinase 3), and GCN2/EIF2AK4 (eIF2α kinase 4)—phosphorylate serine 51 of the α subunit of the eIF2 complex (Proud, 2005). The binding of GTP to eIF2 is the rate-limiting step for Met-tRNA\textsuperscript{Met} delivery and ultimately for protein synthesis (Sonenberg and Hinnebusch, 2009; Wek et al., 2006) (Fig. 1). Recently, Taniuchi et al. tested for additional unknown eIF2α kinases in vertebrates using quadruple knockout cells for all four known eIF2α kinases in 12 different intracellular and extracellular stress conditions, with the exception of heme deprivation, viral infection, and amino acid starvation (Taniuchi et al., 2016). The authors reported finding no additional eIF2α kinases, although the possibility remains that an unknown cell- or stress-specific eIF2α kinase is present in vertebrates. The phosphorylation of eIF2α inhibits the exchange of GDP for GTP by eIF2B on the eIF2 complex and thereby prevents formation of the eIF2-GTP-Met-tRNA\textsuperscript{Met} ternary complex, and therefore delivery of Met-tRNA\textsuperscript{Met} to the 40S ribosomal subunit (Wortham and Proud, 2015). Regardless of the type of stimulus, eIF2α phosphorylation causes an identical reduction in global protein synthesis (Donnelly et al., 2013; Sonenberg and Hinnebusch, 2009; Wek et al., 2006) (Fig. 1). It may therefore permit cells to conserve deficient resources and/or prevent further accumulation of stress-causing materials.

Preferential translation of various specific mRNAs and eIF2α phosphorylation

Global translation reduction is not a unique cellular response to stresses induced by eIF2α phosphorylation. Paradoxically, increased eIF2α phosphorylation promotes translation of diverse selected mRNAs that are inefficiently translated in the absence of stress. Preferentially translated gene transcripts include several basic leucine zipper transcription factors [ATF4 (CREB2) (Kilberg et al., 2009), CHOP (DDIT3/GADD153) (Palam et al., 2011), ATF5 (Zhou et al., 2008), and C/EBPα and β (Calkhoven et al., 2000; Li et al., 2008)], amino acid metabolism-related genes [cations amino acid transporter (Cat-1)] (Fernandez et al., 2002), probable UDP-sugar transporter protein...
SLC35A4 (SLC35A4) (Andreev et al., 2015), bifunctional glutamate/proline-tRNA ligase (EPRS) (Young et al., 2016), a protein phosphatase 1 (PP1) regulatory subunit responsible for eIF2α dephosphorylation [GADD34 (PPP1R15A)] (Lee et al., 2009), and other protective genes [IBTKα (Baird et al., 2014) and CPEB4 (Maillo et al., 2017)] (Fig. 2). Most proteins produced from preferentially translated mRNAs play roles in adapting to cellular stress and restoring homeostasis (Young and Wek, 2016). However, if cellular stress cannot be overcome, transcription factors such as ATF4 and CHOP can induce cell death through increased protein synthesis, resulting in oxidative stress and ATP depletion (Han et al., 2013) or regulated expression of downstream pro/anti-apoptotic target genes (such as DR5, TRB3, Bim, and Bcl-2) (Tabas and Ron, 2011). Furthermore, sustained translational repression by eIF2α phosphorylation is also reportedly deleterious to cells exposed to chronic stress in cognitive and neurodegenerative disorders because repression inhibits normal protein production (Moreno et al., 2012; Zhu et al., 2019).

All the transcripts described above have short upstream open reading frames (uORFs) located in the 5′-untranslated region (5′-UTR) of the mRNA. They are preferentially translated through uORF-mediated mechanisms during diverse stressful conditions in which eIF2α phosphorylation is induced by four eIF2α kinases (Pakos-Zebrucka et al., 2016; Young and Wek, 2016). The uORFs of the transcripts described above can affect downstream coding sequence (CDS) translation in multiple ways. Translation can be affected through the promotion of ribosome re-initiation at the downstream CDS after the uORF translation, the stalling of ribosome elongation while translating the uORF, ribosome dissociation after translation of the uORF, ribosome translation past the CDS start codon resulting in no translation initiation at the CDS, or the bypassing of the uORF by the ribosome (Hinnebusch et al., 2016; Young and Wek, 2016). If a transcript has uORFs that promote ribosome re-initiation or bypass in response to environmental stresses, enhancement of translation initiation will occur at the downstream CDS but low or no translation of the CDS will occur under normal conditions. Thus, eIF2α phosphorylation–dependent translations of the mRNAs described above can be achieved through the proper mixing and matching of uORFs in their 5′-UTRs. However, specific uORF configurations and working mechanisms in each gene were obtained through evolutionary adaptation to relevant stresses. Individual genes may have unique features of uORFs that permit preferential translation in response to eIF2α phosphorylation, implying that there are multiple mechanisms asserted by diverse uORFs. Detailed
working mechanisms of uORFs are well described in two reference papers (Hinnebusch et al., 2016; Young and Wek, 2016).

**Roles of eIF2α phosphorylation beyond translation regulation**

Although the biochemical mechanism of translation inhibition mediated by eIF2α phosphorylation is well understood, its downstream physiological consequences are less clear at the cellular level. Upon ER stress, viral infection, and other cellular stresses, four eIF2α kinases phosphorylate eIF2α, which leads to transient attenuation of global protein synthesis and transcriptional induction through preferential translation of selected transcription factor genes (such as ATF4, ATF5, CHOP, and C/EBPα and β) (Donnelly et al., 2013; Wek et al., 2006). As the common point of convergence for all the stress stimuli, eIF2α phosphorylation activates an evolutionarily conserved signaling pathway, known as integrated stress response (ISR) to restore cellular homeostasis (Pakos-Zebrucka et al., 2016; Ron, 2002; Wek et al., 2006). Many studies have reported that eIF2α phosphorylation regulates various cellular processes. This review summarizes the roles of eIF2α phosphorylation in oxidative stress and mitochondria function.

**Reactive oxygen species and eIF2α phosphorylation**

Although eIF2α phosphorylation can promptly and robustly change in response to stress, a basal level of eIF2α phosphorylation has also been observed in cells cultured in vitro (Rajesh et al., 2013; Scheuner et al., 2001) and in vivo (Hussain and Ramaiah, 2007; Lewerenz and Maher, 2009). Multiple studies indicate that basal eIF2α phosphorylation prevents oxidative stress by modulating antioxidant levels under normal conditions which may have physiological stress (Choi et al., 2017; Harding et al., 2003; Lewerenz and Maher, 2009; Rajesh et al., 2013). Genetic inactivation of eIF2α phosphorylation in mouse embryonic fibroblasts (MEFs) and immortalized hepatocytes lead to increased intracellular ROS levels (Choi et al., 2017; Rajesh et al., 2013). Furthermore, eIF2α phosphorylation deficiency impairs proliferation and induces premature senescence, which can be prevented by anti-oxidant treatment (Rajesh et al., 2013). Consistent with ROS accumulation in eIF2α phosphorylation-deficient (A/A, homozygous Ser51Ala mutant eIF2α alleles) MEFs, lower average levels of glutathione (GSH), a tripeptide antioxidant that contains L-cysteine, L-glutamic acid, and glycine, can be expected, compared with wild-type (S/S, homozygous Ser51 eIF2α alleles) MEFs. Reduced GSH levels in A/A cells are related to impaired glutathione metabolism because of decreased...
eIF2α phosphorylation–dependent ATF4 translation and subsequent downregulation of the light chain, xCT (encoded by the Slc7a11 gene), of the Xc–cystine/glutamate exchanger (Harding et al., 2003; Lewerenz and Maher, 2009). Several xCT promoter studies have reported that xCT transcription is induced by ATF4 binding to amino acid response elements in its promoter during ER stress or amino acid deprivation (Lewerenz and Maher, 2009; Sato et al., 2004). Moreover, ATF4-deficient cells are impaired in the ability to express genes involved in GSH biosynthesis and anti-oxidative stress. For GSH biosynthesis, these include the heavy chain (Slc3a2) of the Xc–cystine/glutamate exchanger, the glycine transporter (Glyt1), and cystathionine γ-lyase (Cth) (Fusakio et al., 2016; Harding et al., 2003). In response to anti-oxidative stress, the absence of ATF4 reduces expression of mitochondrial superoxide dismutase (Sod2) (Fusakio et al., 2016). Loss of ATF4 therefore results in enhanced oxidative damage (Fusakio et al., 2016; Harding et al., 2003). In line with these results, both eIF2α phosphorylation and ATF4-deficient cells were found to be highly sensitive to oxidative stresses (Choi et al., 2017; Harding et al., 2003). These studies suggest that small but sufficient expression of antioxidant genes by basal eIF2α phosphorylation and its downstream target ATF4 expression are necessary to prevent ROS damage, even in normal conditions (Fig. 3).

As in the case of the described in vitro studies, basal eIF2α phosphorylation is required to protect specialized secretory cells, including pancreatic β cells, from oxidative stress in vivo (Back et al., 2009). The acute removal of β cell–specific eIF2α phosphorylation raised uncontrolled translation, ROS accumulation, dysfunction, and death in animal model β cells. The phosphorylation of eIF2α coordinately attenuates translation of glucose-regulated proinsulin mRNA, prevents oxidative stress, and optimizes ER protein-folding in support of insulin production in animal model β cells (Back et al., 2009). However, it is not clear whether reduced expression of ATF4 and its downstream anti-oxidant genes are responsible for observed phenotypes in eIF2α phosphorylation-deficient β cells because the study of whole-body ATF4 knockout mice has shown that ATF4 is not required to preserve β cell function (Back et al., 2009), and further studies using β cell–specific ATF4 knockout mouse models are required to clarify the contribution of ATF4 against oxidative stress in β cells.

However, the eIF2α phosphorylation-ISR pathway
induced by PERK works as an acute or chronic ER stress–induced cell-death pathway (Tabas and Ron, 2011; Wang and Kaufman, 2014). During ER stress, PERK-mediated eIF2α phosphorylation increases the expression of ATF4 and its key downstream target, CHOP (Ron and Walter, 2007). In addition, induced ATF4 and CHOP transcription factors increase transcription of the growth-arrest and DNA damage-inducible protein 34 (GADD34/PPP1R15A) to direct eIF2α dephosphorylation and restore global mRNA translation (Back and Kaufman, 2012; Ron and Walter, 2007). Recently, Han et al. suggested that persistent and strong expression of ATF4 and CHOP increase protein synthesis in GADD34-dependent and -independent manners and cause oxidative stress and cell death (Han et al., 2013) (Fig. 4). For GADD34-independent protein synthesis, ATF4 and CHOP act together to upregulate target genes encoding functions in protein synthesis (such as multiple aminoacyl-tRNA synthetases, ribosomal subunits, and eukaryotic translation initiation factor subunits) to restore general mRNA translation under ER stress (Han et al., 2013). Increased protein synthesis will then push a cell to generate damaging ROS through related cellular organelles, such as the ER and mitochondria (Han et al., 2013). In the ER, ROS are produced as by-products while electrons shuttle through protein disulfide isomerase and ER oxidoreductin 1α (ERO1α) to O2 in oxidative protein-folding pathways for disulfide bond formation (Back and Kaufman, 2012). ERO1α may also hyperoxidize the ER lumen and activate the inositol trisphosphate receptor, causing a release of Ca2+ from the ER (Li et al., 2009). Next, the increase in mitochondrial Ca2+ uptake induces a mitochondrial Ca2+ overload, which leads to enhanced ROS generation by stimulating the TCA cycle and oxidative phosphorylation (Brookes et al., 2004). In addition, CHOP is a well-known proapoptotic transcription factor that can induce several proapoptotic genes (ERO1α, GADD34, DR5, TRB3, and Bim) or repress an antiapoptotic gene (Bcl2) (Fig. 4). Detailed molecular mechanisms of CHOP-induced apoptosis have been fully described (Iurlaro and Munoz-Pinedo, 2016; Tabas and Ron, 2011).

Collectively, both hypo- and hyper-eIF2α phosphorylation are responsible for cellular ROS accumulation and cause cell death because of hypo- or hyper-expression of downstream target genes. The level of eIF2α phosphorylation should therefore be in a balanced range to promote successful adaptation to cellular stress (Wek and Anthony, 2009).
Mitochondrial structure and shape and eIF2α phosphorylation

Although eIF2α phosphorylation appears to affect only cytosolic translation initiation, the effect is not restricted to translation because eIF2α kinases and eIF2α-dependent genes are involved in various cellular responses from multiple places in a cell. Recent reports suggest that mitochondria can be regulated through eIF2α phosphorylation in response to diverse stresses (Back et al., 2009; Balsa et al., 2019; Lebeau et al., 2018; Zheng et al., 2012). The structural role of PERK in stabilizing ER-mitochondrial contacts (Munoz et al., 2013) implies that PERK-deficient cells suffer from defects in mitochondrial functions. PERK silencing reportedly stimulated mitochondrial respiration, whereas overexpressing PERK suppressed mitochondrial respiration and led to fragmented or rounded mitochondria (Munoz et al., 2013). However, the eIF2α phosphorylation requirement was not checked in Munoz’s results. In addition, opposite effects of PERK signaling have been reported in mitochondrial respiration and morphology (Back et al., 2009; Balsa et al., 2019; Lebeau et al., 2018; Zheng et al., 2012). Although further studies are required to clarify the discrepancies in the described reports, PERK signaling–deficient cells show perturbed responses to ER and nutrient stress, including defects in respiratory supercomplex formation, increased mitochondrial cristae (Balsa et al., 2019) and mitochondrial hyperfusion (Lebeau et al., 2018), all of which are important to mitochondrial integrity and homeostasis. Recently, the functional organization of electron transport chain (ETC) complexes was explained by a plasticity model, in which individual ETC complexes coexist with superassembled structures (including I+III+IV, I+III, and III+IV) called supercomplexes (SCs) (Cogliati et al., 2016). This organization of complexes allows for more efficient transportation of electrons. Balsa et al. suggested that mitochondrial respiratory activity is elevated through increased cristae formation and SC levels to satisfy energetic and metabolic demands during glucose deprivation and ER stress (Balsa et al., 2019). These structural changes are driven by activation of the PERK signaling pathway. The PERK-eIF2α axis transcriptionally controls expression of supercomplex assembly factor 1 (SCAF1/COX7A2L) through one of its downstream targets, which is translationally expressed ATF4 transcription factor (Balsa et al., 2019) (Fig. 5). Because SCAF1 mediates the interaction between CII and CIV, its presence determines the formation of two SCs I+III+IV (the respirasome) and III+IV (Lapuente-Brun et al., 2013). As with SCAF1 null cells, PERK, eIF2α phosphorylation, or ATF4-deficient cells displayed bioenergetics defects such as reduced SCs, mitochondrial respiration, and ATP levels and then compromised their proliferation during ER stress and glucose-deprived conditions (Balsa et al., 2019). SC formation and proliferation were restored in ATF4 null cells, but not in PERK null cells, by overexpressing SCAF1, suggesting that SCAF1 may not be the only factor that promotes assembly of SCs through the PERK-eIF2α axis during ER and nutrient stress (Fig. 5). Further investigation is required to identify other assembly factors. In addition, whether other eIF2α kinases-eIF2α pathways can remodel mitochondrial ultrastructures to activate oxidative phosphorylation in the response of different cellular stresses remain an open question.

Mitochondrial elongation reportedly protects mitochondria from autophagic degradation during nutrient starvation (Gomes et al., 2011; Rambold et al., 2011). In parallel with increased SC assembly, the PERK-eIF2α axis also promotes stress-induced mitochondrial hyperfusion to protect mitochondria during ER stress (Lebeau et al., 2018). Hyperfusion protects mitochondria from pathologic fragmentation and increases metabolism to facilitate recovery from acute ER stress. Unlike SC assembly, PERK-mediated mitochondrial elongation does not require ATF4 transcriptional activity but does need eIF2α phosphorylation–dependent translational attenuation (Fig. 5). Furthermore, ER stress–dependent elongation requires SLP2 and YME1L in a mitochondrial large-protease complex that includes the membrane scaffold SLP2, rhomboid protease PARL, and ATP-dependent mitochondrial inner membrane protease YME1L (Lebeau et al., 2018), reflecting the importance of degrading a short-lived mitochondrial protein in elongation. No known molecular mechanism explains why PERK-eIF2α phosphorylation needs SLP2 and YME1L proteins, although they are important factors in elongation during ER stress (Fig. 5). The involvement of GCN2-eIF2α pathways in mitochondrial elongation induced by amino acid starvation (Gomes et al., 2011; Rambold et al., 2011) should also be studied.

Mitochondrial retrograde signaling pathways and eIF2α phosphorylation

Mitochondria communicate with the cell through mitochondrial retrograde signaling pathways (MRSP), which signal mitochondrial dysfunction to the cytosol or nucleus and then induce anterograde mechanisms to protect mitochondria and restore cellular homeostasis (Kasai et al., 2019; Melber and Haynes, 2018). In mammals, several branches of the MRSP, including mitochondrial unfolded protein response (UPRmt), mitophagy, and the ISR pathway are known. However, many experimental paradigms indicate that the UPRmt can be cross-regulated with ISR (Kasai et al., 2019; Melber and Haynes, 2018). This review discusses the regulation and function of MRSP with a focus on the ISR pathway.

In the life cycle of a cell, transient inhibition of global protein synthesis prevents unwanted translation during mitosis (Sivan and Elroy-Stein, 2008). It is assumed that eIF2α phosphorylation can repress global translation during
the G2/M phase (Kim et al., 2018). However, which kinase is responsible for eIF2α phosphorylation and how an eIF2α kinase is activated during the mitotic phase are not fully understood. Kim et al. suggest that PKR is a responsible enzyme that can be activated by cellular double-stranded RNAs (dsRNAs) during mitosis (Kim et al., 2018). PKR induces an immune response and phosphorylates eIF2α by sensing viral dsRNAs (Donnelly et al., 2013; Wek et al., 2006). However, PKR signaling is also assumed to be regulated by various cellular dsRNAs, such as mitochondrial RNAs (mtRNAs), inverted Alu repeats containing mRNAs (IRAlus mRNAs), and noncoding RNAs (ncRNAs) (Elbarbary et al., 2013; Golec et al., 2019; Kim et al., 2018; Murad et al., 2006). The ncRNAs and IRAlus mRNAs originate primarily in the nuclear genome but mtRNAs are found in the mitochondria genome. Kim et al. indicated that mtRNAs are a major class of PKR-interacting cellular dsRNAs and their increased expression may account for PKR phosphorylation during the mitotic phase (Kim et al., 2018). They also suggested that PKR can localize in both cytosolic and mitochondrial regions and be activated by the mtRNAs in the mitochondria matrix under normal conditions, including the M phase (Kim et al., 2018). However, Kim did not provide a mechanism to explain how PKR has mitochondria localization and PKR activated by mtRNAs phosphorylates cytosolic eIF2α. Besides PKR-eIF2α signaling activated by mtRNAs residing in mitochondria, regulation can be a result of mtRNAs released to the cytosol during stress conditions (Kim et al., 2018). Treatment of two stress inducers, okadaic acid (OA, PKR phosphatase inhibitor) and staurosporine (STP, non-selective protein kinase inhibitor), leads to the cytosolic release of mtRNA by disruption of the mitochondrial membrane. Cytosolic interaction of PKR-mtRNA can then induce an immune response and eIF2α phosphorylation-mediated signaling, both of which can be used in beneficial ways. However, under stressful conditions during which PKR is overactivated by mitochondrial dysfunction, it is possible that mtRNA-mediated PKR activation can trigger inflammation and cell death (Rath et al., 2012).

The MRSP is not limited to regulation of a cytosolic translation factor such as eIF2α phosphorylation but also extends to expression of several transcription factors to control nuclear gene expression (Kasai et al., 2019; Melber and Haynes, 2018). In mammalian cells, CHOP in association with C/EBPβ, regulates expression of UPRmt genes.

Fig. 5. PERK-eIF2α phosphorylation-dependent mitochondria hyperfusion and respiratory supercomplex formation during ER stress and glucose deprivation conditions.
(such as nuclear genes encoding Hsp60, Hsp10, mtDNAJ, and ClpP) in response to the accumulation of a deleted mutant form of the mitochondrial matrix protein, ornithine transcarbamylase (OTC) (Zhao et al., 2002). ER unfolded protein stress response (UPR$_{ER}$) is not involved in the CHOP-mediated gene expression of the UPR$_{mt}$. Nevertheless, eIF2α-phosphorylation dependent ISR pathway reportedly can interact with multiple points of the UPR$_{mt}$ (Fiorese et al., 2016; Kasai et al., 2019; Melber and Haynes, 2018; Quiros et al., 2017). Other mitochondrial dysfunctions (mtDNA depletion by EtBr, ethidium bromide treatment, mitochondrial translation inhibition by doxycycline treatment, analogous to ATFS-1 in $C. elegans$ (Fiorese et al., 2016). The transcriptional expression of ATF5 is induced by both ATF4 and CHOP and its translation is regulated by eIF2α phosphorylation (Teske et al., 2013; Zhou et al., 2008). The expression of ATF5 is responsible for upregulation of mitochondrial chaperone genes (Hsp60 and mtHsp70) and the protease gene (Lonp1) in responses to oxidative stress or oxidative phosphorylation inhibition (Fiorese et al., 2016), which also induces an ER stress response (Wang et al., 2018). These findings suggest that there are significant degrees of overlap in target gene regulation between the eIF2α-phosphorylation-dependent ISR pathway and the UPR$_{mt}$. Furthermore, a multiomics analysis in HeLa cell mitochondrial stress models identified ATF4, which is a target translationally regulated by eIF2α phosphorylation, as a key regulator of stress response (Quiros et al., 2017). All four different drugs that alter mitochondrial homeostasis in a different way (FCCP/proton ionophore, actioninin/mETC protein stability modifier, doxycycline/mitochondrial translation inhibitor, MitoBloCk-6/ mitochondrial protein import inhibitor) activated the eIF2α phosphorylation–dependent ISR pathway. However, the ISR pathway activation was not dependent on the four known eIF2α kinases. Still, during mitochondrial stress, the ISR-ATF4 pathway activated the expression of many cytoprotective genes, including amino acid metabolism and GSH metabolism to maintain cell proliferation and provide protection against mitochondrial stress (Back and Kaufman, 2012; Quiros et al., 2017) (Fig. 6). The IRS-ATF4 pathway is therefore an important player in MRSP, regulating both cytosolic translation and mitochondria homeostasis. However, upregulation of canonical UPR$_{mt}$ genes mediated by ATF5 (Quiros et al., 2017) has not been reported and it is possible that the ISR pathway in MRSP can be divided into ATF4-dependent and independent pathways, although further study is required.

Although the involvement of eIF2α kinases was not carefully studied in the conditions of the MRSP described above, data indicate that activated eIF2α kinases are responsible for eIF2α phosphorylation in mitochondrial stress conditions. Multiple studies have reported that an endocrine hormone, fibroblast growth factor 21 (FGF21), is expressed in an ATF4-dependent manner in response to mitochondria stresses induced by mETC inhibition, mitophagy defects, and defects in mitochondrial dynamics (Joe et al., 2018; Kim et al., 2013a; Kim et al., 2013b). Metformin, which can inhibit mitochondrial complex 1, increased FGF21 expression via the ISR-ATF4 pathway activated by PERK (Kim et al., 2013a). Metformin-induced ATF4 activation was inhibited by treatment with the mitochondrial antioxidant Mito-TEMPO, suggesting a role for the mitochondrial-derived ROS-PERK axis. In addition, rotenone (a mitochondrial complex I inhibitor) or antimycin A (a mitochondrial complex III inhibitor) also induced FGF21 gene expression via eIF2α phosphorylation–ATF4 activation in C2C12 myotubes (Kim et al., 2013b). In muscle-specific KO mice of ATG7 or mitofusin 1 (Mfn1) and 2 (Mfn2), mitochondrial function was decreased by defects in mitophagy or mitochondrial dynamics, respectively (Kim et al., 2013b). Mitochondrial dysfunctions were also responsible for FGF21 induction by eIF2α phosphorylation–ATF4 activation. These effects are most likely mediated by the activation of the PERK pathway because the knockout of ATG7 (Antonucci et al., 2015; Yang et al., 2010) or Mfn2 (Sebastian et al., 2012) causes ER stress and activates PERK (Fig. 6). During mitochondrial stresses cells use eIF2α phosphorylation–mediated translation regulation and the ISR pathway to preserve cellular energy and restore mitochondrial homeostasis. However, an eIF2α phosphorylation–mediated response can produce unwanted and contradictory results. In Drosophila melanogaster, overexpression (O/E) of the mitochondrial mutant protein pret/ups1p induces dendritic regression of Drosophila peripheral sensory neurons by mitochondrial dysfunction (Tsuyama et al., 2017). A mechanical study of cell regression revealed that pret O/E-mediated mitochondrial dysfunction induced prolonged translation suppression in neurons in a PERK-eIF2α phosphorylation–dependent manner, which led to dendrite loss, whereas it contributed to the maintenance of cellular ATP levels through translation inhibition (Tsuyama et al., 2017). Collectively, these observations indicate that successful adaptation to cellular stress requires eIF2α phosphorylation to be in a balanced range (Wek and Anthony, 2009).

**Conclusions**

Mammalian cells are equipped with four eIF2α kinases to sense and control diverse intracellular and extracellular stresses. The cells activate a common adaptive pathway, the ISR, through eIF2α phosphorylation, to restore cellular homeostasis, and eIF2α phosphorylation is involved in
multiple biological processes. In protein synthesis, eIF2α phosphorylation suppresses translational initiation of general mRNAs and activates it from specific mRNAs in response to stress. However, regulation of protein synthesis by eIF2α phosphorylation does not always work as intended. In effect, too much is as bad as too little. Hyper and prolonged phosphorylation of eIF2α can increase expression of proapoptotic genes through downstream transcription factors, which can make matters worse, and sustained translational suppression can disrupt cellular homeostasis through deficiency of cell type–specific proteins or important cellular function–maintaining proteins. Beyond translation regulation, both hyper- and hypo-phosphorylation of eIF2α cause ROS damage because of both translational dysregulation of general mRNAs and the absence or over-expression of its downstream target genes. In addition, eIF2α phosphorylation can modulate mitochondrial structure and shape to maintain homeostasis and preserve its integrity through the PERK–eIF2α axis during ER stress. Mitochondria appear to use eIF2α phosphorylation to communicate with the cytosol and nucleus and protect and restore mitochondria homeostasis. Mitochondrial RNAs are recognized by PKR to regulate cytosolic translation through eIF2α phosphorylation during the mitotic phase and mitochondrial stress conditions. In mitochondria-nuclear communication, diverse mitochondrial dysfunctions, except for accumulation of a misfolded mitochondrial protein, activate eIF2α phosphorylation–dependent ISR pathways, which can be divided into ATF4-dependent and -independent pathways. However, only further study can shed light on what induces eIF2α phosphorylation during mitochondrial dysfunctions. MRSP induced by eIF2α phosphorylation does not always protect or ameliorate but can deteriorate mitochondria stress. The level of eIF2α phosphorylation should be in a balanced range to promote successful adaptation to cellular stress.

Acknowledgments. This work was supported by the Basic Science Research Program (2017R1D1A1B03028229), the Bio and Medical Technology Development Program (2017M3A9G7072745), and the Priority Research Centers Program (2014R1A6A1030318) of the National Research Foundation of Korea, which is funded by the Korean government.

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(Received for publication, March 31, 2020, accepted, April 24, 2020 and published online, April 29, 2020)