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REVIEW

Regulatory crosstalk within the mammalian unfolded protein response

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Abstract Increased demands on the protein folding capacity of the endoplasmic reticulum (ER) trigger the unfolded protein response (UPR). Comprised of a tripartite signaling system, the UPR regulates translation and gene transcription to manifest pro-adaptive and, if necessary, pro-apoptotic outcomes. The three UPR pathways, initiated by activating transcription factor 6, inositol requiring enzyme 1, and protein kinase RNA-activated-like ER kinase (PERK), direct distinct downstream signaling events. However, it is becoming increasingly clear that interplay between the cascades is vital in shaping the UPR. In particular, recent discoveries have revealed that PERK-dependent signals mediate both inter- and intra-pathway regulation within the UPR, underscoring the critical role of the PERK pathway in the cellular response to ER stress.

Keywords Endoplasmic reticulum · Unfolded protein response · ATF6α · ATF4 · XBP1 · PERK

Introduction

The endoplasmic reticulum (ER), an elaborate network of flattened sac-like and tubular structures, serves as a factory for protein and lipid production, a specialized protein folding compartment and the major depot for intracellular Ca^{2+} [1, 2]. Hence, cells are exquisitely sensitive to physiologic conditions that either perturb the normal ER environment or that increase demand for synthesis and maturation of secretory pathway proteins. In general, ER stress is considered to occur when the load of client proteins exceeds the folding capacity of the ER. Without appropriate remedy, such stress can be detrimental to the ER, the entire secretory pathway, and the cell as a whole. Physiologic ER stress occurs in a variety of normal processes such as when B lymphocytes differentiate into antibody-secreting plasma cells [3] and pancreatic β cells engage in episodic, high-rate insulin synthesis [4]. Similarly, a number of patho-physiologic situations can stress the ER including the infection of cells by viruses that hijack the secretory apparatus for viral glycoprotein synthesis and replication [5], and the hypoxia and/or glucose deprivation experienced by cancer cells when the available vasculature fails to meet the metabolic needs of growing tumors [6]. Pharmacologic agents such as tunicamycin, an inhibitor of *N*-linked glycosylation, and thapsigargin, an inhibitor of the ER-Ca^{2+} ATPase, grossly disrupt protein folding in the ER, thus potently inducing ER stress. Using such ER poisons as experimental tools, investigators elucidated the molecular details of a multi-faceted cellular response to ER stress, collectively termed the unfolded protein response (UPR) [7] (Fig. 1). Here, the basics of pro-adaptive and pro-apoptotic UPR signaling are reviewed and special emphasis is placed on recent discoveries providing new insight into how the PERK pathway intersects with the entire UPR to influence the fate of cells under ER stress.

Pro-adaptive UPR signaling

When faced with ER stress, cells utilize the UPR to address the problem on multiple fronts (Fig. 1). The UPR can

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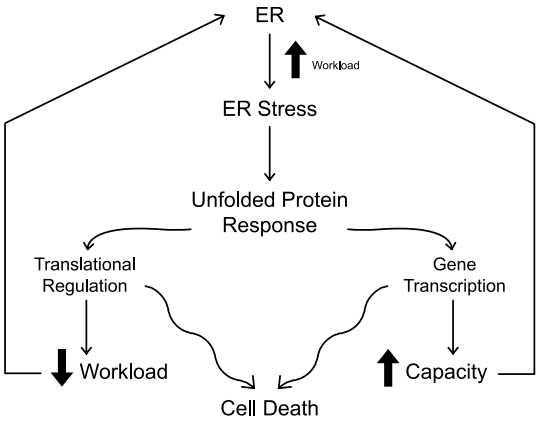

Fig. 1 Conceptual framework for the cellular response to ER stress. An increased workload on the machinery for synthesis, folding, maturation, and transport of secretory pathway proteins renders ER stress, thereby activating the UPR. To adapt to increased demands on the ER, mammalian cells engage translational regulation to reduce the flow of client proteins into the ER and enhance transcription of genes encoding proteins that expand ER capacity. If the pro-adaptive outcomes are insufficient to restore ER homeostasis, the UPR elicits pro-apoptotic mechanisms to promote cell death

slow the flow of nascent polypeptides into the ER lumen, enhance the ER machinery needed for protein folding and assembly, augment the system for disposal of mis-folded client proteins, and coordinate expansion of the ER compartment. The mammalian UPR is composed of three signaling pathways that are separately initiated by the ER transmembrane proteins activating transcription factor 6 (ATF6) [8], inositol requiring enzyme 1 (IRE1, first identified in yeast) [9, 10], and protein kinase RNA-activated (PKR)-like ER kinase (PERK) [11, 12], each of which possesses an ER lumenal domain that senses stress. The transmembrane regions of IRE1 and PERK detect alterations in the lipid composition of the ER membrane [13, 14]. Each of the three primary UPR signaling molecules contains a cytosolic domain that propagates downstream events (Fig. 2) directed toward balancing load with capacity in the ER, thereby restoring ER homeostasis [15].

The ATF6 pathway

The ubiquitously expressed ATF6 protein localizes to the ER with a type II transmembrane topology [8]. In the 'resting' state, the ER lumenal domain of ATF6 is bound to immunoglobulin binding protein (BiP), an abundant, soluble ER-resident chaperone. Under conditions of ER stress, ATF6 dissociates from BiP, unmasking a Golgi-localization signal in the ATF6 lumenal domain which facilitates its transport to the Golgi [16, 17] via coat protein II-coated vesicles [18]. Reduction of intra- and inter-disulfide bonds in ATF6 oligomers [19], the interaction of its lumenal domain with thrombospondin proteins in the ER [20] and its under-glycosylation and subsequent release from the ER chaperone calreticulin [21] all appear to govern the transit of ATF6 to the Golgi. Upon arrival in the Golgi, ATF6 undergoes regulated intramembrane proteolysis by the site-1 (S1P) and site-2 (S2P) proteases [22], a process that liberates its cytosolic domain from the membrane. This ATF6 fragment, termed ATF6(N), migrates to the nucleus and functions as a basic leucine zipper (bZIP) transcription factor. In the presence of nuclear factor Y, also known as CCAAT-box binding factor, ATF6(N) binds directly to the *cis*-acting ER stress response element (ERSE; CCAAT-N_9-CCACG) [23] found within the promoter regions of many ER stress-responsive genes [24, 25]. Many ATF6-regulated genes encode ER-resident proteins that participate in ER quality control processes. For example, ATF6 targets include molecular chaperones (such as BiP), folding enzymes (such as the protein disulfide isomerase-like protein ERp72), and components of the ER-associated degradation (ERAD) system (such as degradation in ER protein 3, Derlin-3) for disposal of mis-folded proteins [26, 27].

Cells express two isoforms of ATF6, α and β, both of which are functional and responsive to ER stress [8, 28, 29]. Single deletion of either *Atf6α* or *Atf6β* in mice does not disrupt normal development [30, 31]. However, combined deletion of these genes results in embryonic lethality [31], suggesting that ATF6α and ATF6β can compensate for each other, at least during embryonic development. Interestingly, studies of $Atf6\alpha^{-/-}$ and $Atf6\beta^{-/-}$ mouse embryo fibroblasts (MEFs) in vitro indicate that only ATF6α is essential for induction of UPR target genes and cell survival during ER stress [26, 30, 31]. In addition, ATF6α can drive lipid biosynthesis and ER biogenesis [32, 33], and ATF6α has been linked to the physiology of multiple cell types including hepatocytes [30, 34–36], dopaminergic neurons [37], skeletal muscle cells [38], pancreatic β cells [39], and dormant tumor cells [40]. It is important to point out that several other ATF6-like proteins have been identified, including OASIS, LUMAN, BBF2H7, CREBH, and CREB3L4 (Tisp40). These ER transmembrane, bZIP transcription factors appear to mediate specialized UPR functions in specific organs and cell types as they exhibit differences in activating signals, tissue distribution, and response element binding [41]. In this article, further discussion of the ATF6 pathway will center on ATF6α.

The IRE1 pathway

The IRE1 protein contains a serine/threonine kinase module and an endoribonuclease (RNase) domain in its cytoplasmic region [9, 10]. Like ATF6, the ER lumenal domain of inactive IRE1 is bound to BiP and then dissociates when ER stress occurs. In yeast, IRE1 is activated by the

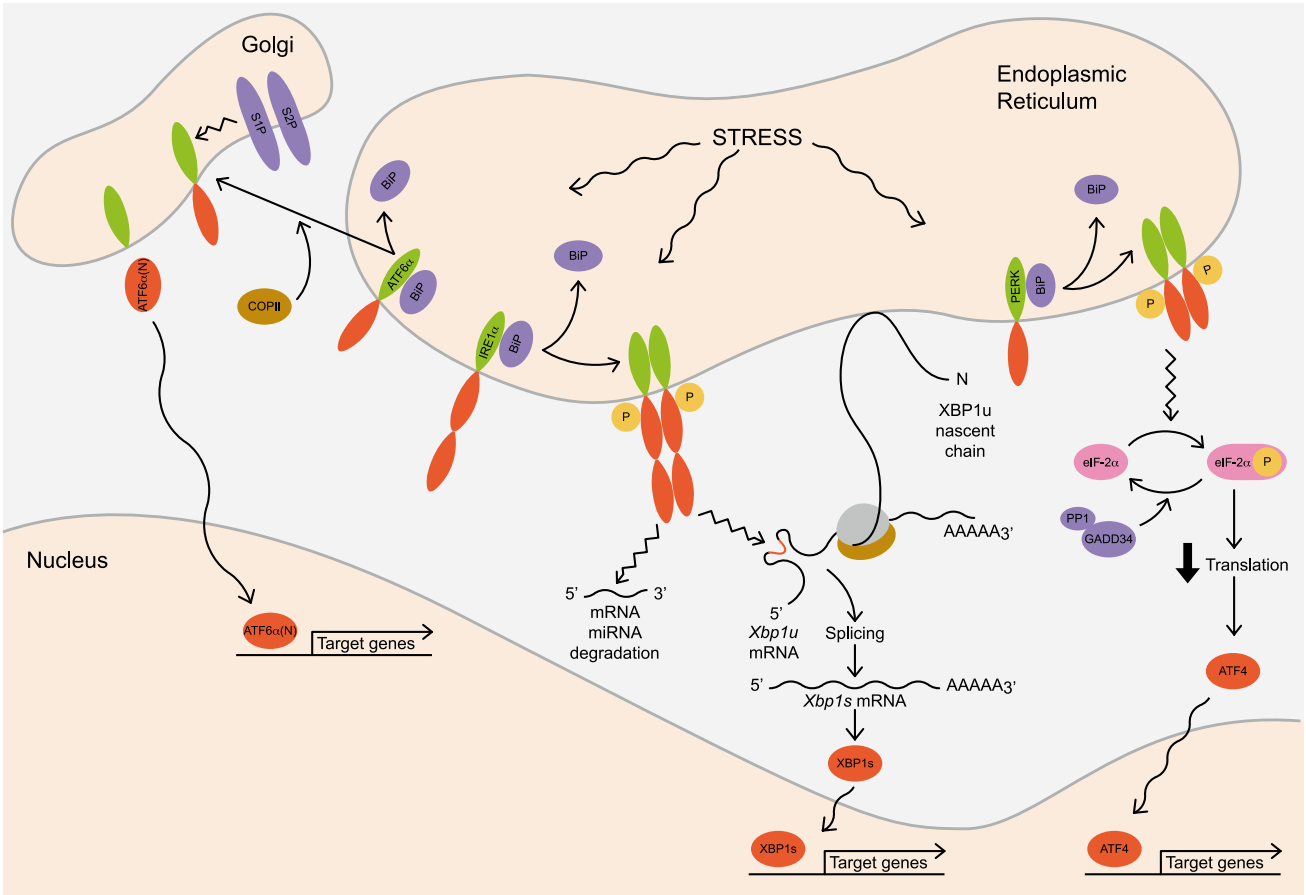

Fig. 2 Signaling pathways of the mammalian UPR. Conditions that increase demand on the ER induce 'stress' and trigger the UPR. Upon release from the chaperone BiP, an exposed Golgi-localization signal and interaction with the COPII complex allows ATF6α to be ferried to the Golgi apparatus where it undergoes regulated intramembrane proteolysis by S1P and S2P. Liberated from the membrane, its cytosolic domain, ATF6α(N), moves into the nucleus where it functions as a bZIP transcription factor to up-regulate expression of genes via the ERSE promoter motif. ATF6α primarily targets genes involved in various ER quality control processes such as protein folding and ERAD. The IRE1α proteins oligomerize when BiP releases, activating their C-terminal endoribonuclease domains that execute site-specific cleavage of *Xbp1* mRNA at two sites. Splicing of the resulting fragments in the cytoplasm yields a transcript with an altered reading frame encoding XBP1s, a bZIP transcription factor that acts on the ERSE and UPRE promoter motifs to up-regulate genes involved throughout the secretory pathway. Translation of *Xbp1* mRNA prior to UPR-directed splicing yields XBP1u, a bZIP factor which lacks a transactivation domain. A hydrophobic region near the C-terminus of nascent XBP1u associates with the ER membrane. This interaction facilitates IRE1α-initiated splicing as it positions ribosome-engaged *Xbp1* mRNA near the ER membrane. Activated IRE1α also cleaves and degrades select mRNA and miRNA. Upon release of BiP, the PERK proteins oligomerize and phosphorylate the translation initiation factor eIF-2α, effectively reducing translation. Translation of ATF4 increases when global protein synthesis decreases. ATF4 acts on the CARE promoter motif to induce a variety of targets including genes involved in cellular redox homeostasis, amino acid metabolism, protein synthesis and apoptosis. The GADD34–PP1 complex de-phosphorylates eIF-2α, allowing translation to resume

binding of unfolded proteins with a groove in its ER lumenal domain [42–44], and the IRE1–BiP interaction fosters appropriate IRE1 triggering [45]. However, in mammalian cells, IRE1 activation does not appear to rely on its association with unfolded proteins [46, 47]. In yeast and mammalian cells, oligomerization and clustering of IRE1 in the ER membrane correlates with onset of IRE1 autophosphorylation and RNase activity [44, 48–50].

One function for the IRE1 RNase is to instigate the demise of various RNA substrates. This mechanism, referred to as regulated IRE1-dependent decay (RIDD), was first shown to target mRNA molecules encoding signal-sequence bearing proteins destined for the secretory pathway [51–53]. Thus, RIDD provides one way for the UPR to stem the flow of nascent polypeptides into the stressed ER [54–56]. Equally important, the activated IRE1 RNase excises a 26-nucleotide intron from X-box binding protein 1 (*Xbp1*) mRNA, initiating a novel spliceosome-independent, cytosolic splicing mechanism that alters the reading frame of the transcript [57–61]. Without modification by IRE1-dependent splicing, the unspliced (u) *Xbp1* transcript encodes XBP1u, a bZIP factor unable to

transactivate genes. In sharp contrast, the spliced (s) transcript yields XBP1s, a bZIP factor with a carboxyl (C)-terminal transactivation domain [58, 59, 61]. As the nascent XBP1u polypeptide is synthesized, a 26-amino acid (AA) peptide at its C-terminus mediates a translational pause, consequently stabilizing the mRNA-ribosome-nascent chain complex. This brief suspension in XBP1u synthesis provides sufficient time for a slightly upstream hydrophobic region that has already emerged from the ribosome to associate with the ER membrane, thereby facilitating IRE1-dependent generation of *Xbp1s* transcripts as ribosome-engaged *Xbp1u* mRNA is brought into the vicinity of ER-localized IRE1 [62, 63].

XBP1s, like ATF6α(N), can regulate gene targets via the ERSE [61]. Additionally, XBP1s activates ER stress-responsive genes via interaction with a promoter motif termed the UPR element (UPRE; TGACGTGG/A) [64, 65], both as XBP1s homodimers and as XBP1s/ATF6α(N) heterodimers [31]. Exerting considerable influence on cellular secretory capacity, XBP1s regulates ER biogenesis [66–68] and enhances expression of genes involved throughout the exocytic pathway. These include gene products that facilitate the entry of nascent polypeptides into the ER (such as SRP54, a component of the signal recognition particle), protein folding and assembly in the ER (such as ERdj4, a co-chaperone and DnaJ/Hsp40 homolog), and vesicular transport (such as SEC23b, an ER-Golgi transport protein) [26, 67, 69, 70].

Xbp1 is required for proper development of the liver and is therefore essential for embryogenesis [71]. Studies employing tissue-specific *Xbp1* deletion have revealed a vital role for this factor in a number of specialized secretory cell types such as pancreatic acinar cells [66], salivary gland cells [66], antibody-secreting plasma cells [72, 73], and plasmacytoid dendritic cells [74], all of which possess a highly developed ER network. Of the two IRE1 isoforms, IRE1α is ubiquitously expressed [9], while expression of IRE1β has been identified specifically in gut and bronchial epithelium where it optimizes mucin production [75, 76]. Similar to XBP1, deletion of IRE1α causes embryonic lethality due to liver dysfunction [77]. Interestingly, targeted expression of IRE1α in the placenta rescues this defect [78], allowing for the birth of IRE1α-deficient mice that exhibit several mild, but measurable, phenotypes including hypoinsulinemia, hyperglycemia, and reduced antibody levels [79]. For the purposes of this review, further discussion of the IRE1 pathway will focus on IRE1α.

The PERK pathway

The ubiquitously expressed PERK protein utilizes its cytoplasmic serine/threonine kinase domain to signal downstream events [11, 12]. Analogous to the ATF6 and IRE1 proteins, PERK activation correlates with the release of its ER lumenal domain from BiP [16]. One substrate for PERK is nuclear erythroid 2-related factor, a transcription factor involved in cellular redox homeostasis [80, 81]. However, the most heavily studied and well-characterized PERK target is a translation initiation factor, eukaryotic initiation factor-2 (eIF-2) [82]. Normally, eIF-2 binds guanosine triphosphate (GTP) and brings the initiator Met-$tRNA_i^{Met}$ to assembling translational machinery. Phosphorylation of serine 51 on the α subunit of eIF-2 disables the ability of eIF-2B to promote conversion of inactive eIF-2-guanosine diphosphate to active eIF-2-GTP. Consequently, the supply of translation initiation complexes rapidly shrinks [83]. Thus, PERK-mediated phosphorylation of eIF-2α effectively curtails cellular protein synthesis, including the production of nascent polypeptides that would otherwise enter an ER environment unfavorable for folding and maturation [82].

Translational attenuation, however, must be transient if the cell is to efficiently translate new transcripts encoding ER folding assistants and ERAD components that can resolve the problem of mis-folded proteins in the ER. To this end, ATF4 is selectively synthesized through a novel mechanism of translational shunting when the level of phosphorylated eIF-2α (eIF-2α~P) is high and translation initiation complexes are scarce. The ATF4 mRNA contains two small, upstream open reading frames that help direct or shunt the ribosomes to initiate translation at the correct start site for production of ATF4 protein [84, 85]. ATF4 activates gene transcription by binding to CCAAT-enhancer binding protein-activating transcription factor (C/EBP-ATF) response elements (CARE; TGATGXAAX), which consist of half-sites for C/EBP and ATF family members [86, 87]. These CARE motifs are often referred to AA response elements in situations of protein or AA deprivation [88]. A key target for ATF4 is the gene encoding growth arrest and DNA damage-inducible 34 (GADD34) [89], a binding partner for type 1 protein phosphatase (PP1) [90]. GADD34 promotes de-phosphorylation of eIF-2α~P by PP1, allowing for translational recovery [89, 91]. ATF4 also up-regulates expression of many other genes involved in diverse processes including AA metabolism, redox control, protein folding, and autophagy [92–94].

It is important to note that additional stress conditions, besides ER stress, activate distinct eIF-2α kinases, resulting in translational inhibition and induction of ATF4. These eIF-2α kinases include general control nonrepressed 2, activated by nutrient deprivation; heme-regulated inhibitor, stimulated by heme deficiency and oxidative stress; and PKR, turned on by double-stranded RNA in virally-infected cells [82, 83]. With distinct cellular stress responses converging on the eIF-2α~P/ATF4 pathway, this signaling node is considered as the integrated stress response [93, 94].

The PERK pathway provides a means for governance of translation according to the status of the ER, and this is crucial for survival of cells subjected to pharmacologically-induced ER stress in vitro [95]. Moreover, studies of gene-targeted mice revealed that PERK is essential for proper development and function of certain cell types, including specialized secretory cells in the pancreas and skeletal system [96–98]. In humans, loss of PERK causes Wolcott–Rallison syndrome, a disorder involving dysfunction of the exocrine pancreas and liver, neonatal diabetes, skeletal anomalies, and growth retardation [99, 100].

Pro-apoptotic UPR signaling

Despite the concerted efforts of the UPR to adapt to increased demands on the ER, certain ER stress states are incompatible with cell survival. Indeed, under conditions that severely and/or chronically disrupt the ER environment, the UPR can facilitate the execution of stressed cells. Central to ER stress-induced cell death is the mitochondrial apoptosis pathway, a process controlled by the balance of various anti- and pro-apoptotic members of the B cell lymphoma-2 (BCL-2) protein family. These proteins are grouped according to the presence of BCL-2 homology (BH) domains, with pro-apoptotic members harboring BH1, BH2, and BH3 domains or only the BH3 domain [101]. Conformational changes activate the pro-apoptotic BCL-2 family members, BCL-2 associated X protein (BAX) and BCL-2-antagonist or killer (BAK), leading to permeabilization of the outer mitochondrial membrane. Subsequent release of various mitochondrial proteins, such as cytochrome *c*, triggers caspase activation and apoptosis [102]. A number of UPR-mediated events have been linked to the mitochondrial apoptosis mechanism, and these have been the subject of several recent comprehensive reviews [103–106]. Here, a few examples of such connections are highlighted to further set the stage for how crosstalk within the UPR can affect cell fate during ER stress.

While XBP1s is certainly a pro-adaptive/pro-survival factor, IRE1α signaling can also elicit pro-apoptotic outcomes. For example, the interaction of activated IRE1α with tumor necrosis factor receptor-associated factor 2 leads to activation of apoptosis signal-regulating kinase 1 and its target JUN N-terminal kinase (JNK) [107–109]. JNK-mediated phosphorylation reduces the activity of anti-apoptotic BCL-2 family members, but enhances the function of pro-apoptotic members [110]. Thus, the IRE1α-JNK connection, although incompletely understood, may provide a mechanism whereby the UPR modulates the relative amounts of key anti- and pro-apoptotic factors to promote cell death. More recently, evidence has emerged that IRE1α-directed RIDD couples ER stress to apoptosis by selective cleavage of microribonucleic acids (miRNAs). A subset of miRNAs (miR-17, -34a, -96, -125b) that limit translation of caspase-2 (CASP2) are degraded by IRE1α, allowing for increased synthesis of this pro-apoptotic caspase [111]. In turn, CASP2 cleaves and activates the BH3-only protein BID (BH3-interacting domain death agonist), a critical factor in driving BAX/BAK-dependent apoptosis [112, 113]. IRE1α-mediated decay of miR-17 has also been implicated in stabilizing mRNA encoding thioredoxin-interacting protein (TXNIP), a pro-oxidant protein that promotes activation of the NOD-like receptor protein 3 inflammasome and the apoptotic death of pancreatic β cells under ER stress [114, 115].

A growing body of evidence indicates that IRE1α functions as part of a multi-protein signaling platform, referred to as the 'UPRosome', which includes various BCL-2 family members that modulate its activity [116, 117]. For example, the association of BAX and BAK with the cytosolic domain of IRE1α regulates the initiation and duration of IRE1α activity [118]. This checkpoint in IRE1α signaling is calibrated by the levels of the ER transmembrane protein BI-1 (BAX inhibitor), an ER transmembrane protein that antagonizes BAX [119], and bi-functional apoptosis regulator, an ER-associated E3 ubiquitin ligase that promotes BI-1 degradation [120]. In addition, recent work indicates that the BH3-only proteins BCL-2-interacting mediator of cell death (BIM) and p53 up-regulated modulator of apoptosis also interact with IRE1α and sustain its activity as ER stress proceeds [121]. Diminishment of IRE1α activity, concomitant with ongoing PERK signaling, during prolonged ER stress may be pivotal in shifting the UPR toward a pro-apoptotic outcome [122, 123]. Therefore, the apoptosis-unrelated functions of certain BCL-2 family members in managing IRE1α activity may, in fact, affect cell fate determination when the UPR is engaged.

Downstream of PERK, reduced synthesis of secretory pathway cargo and ATF4-induced expression of pro-adaptive genes favors cell survival during ER stress. However, ATF4 also up-regulates expression of C/EBP homologous protein (CHOP, also known as GADD153), a multi-tasking, pro-apoptotic transcription factor [86, 93, 124, 125]. Acting on an ERSE in the CHOP promoter, ATF6α may contribute to transcriptional induction of this factor in the UPR [23]. CHOP collaborates with ATF4 to activate expression of GADD34 and, under conditions of unmitigated ER stress, GADD34-mediated recovery of translation likely exacerbates problems within the ER, leading to cell death [126]. Along these same lines, very recent data indicate that ATF4 and CHOP work together in up-regulating transcription of a large cohort of genes involved in protein synthesis including aminoacyl-tRNA synthetases and translation initiation

factors [127]. In so doing, ATF4 and CHOP promote protein synthesis, and this can lead to oxidative stress and cell death if conditions in the ER remain unfavorable for proper protein folding [127]. These findings further illustrate how the delicate balance between repression and restoration of protein synthesis downstream of PERK signaling influences cell fate in the UPR.

Notably, CHOP has been implicated in the down-regulation of BCL-2 [128], an anti-apoptotic factor that sequesters BH3-only proteins that are required for BAX/BAK-dependent apoptosis [101]. In parallel, CHOP contributes to increased expression of pro-apoptotic BH3-only proteins such as BIM [129]. ER oxidase 1α (ERO1α), another transcriptional target of CHOP, facilitates disulfide bond formation in newly synthesized polypeptides in the ER lumen [126]. During chronic ER stress, however, ERO1α may promote a hyper-oxidizing ER environment, leading to activation of the ER calcium-release receptor inositol tris–phosphate receptor 1 and apoptosis via calcium-sensing calmodulin-dependent protein kinase II [130]. CHOP has been implicated in regulating expression of other apoptosis-associated proteins including death receptor-5 [131], tribbles-related protein 3 [132], and the ATF5 transcription factor [133], although the contributions of these players to the UPR are not fully defined. Clearly, when ER stress conditions persist, CHOP can affect multiple mechanisms to tilt the balance toward apoptosis.

Regulatory crosstalk in the UPR: much work for PERK

Given the complexity of the ER stress response and its myriad downstream effects, it is not surprising that a variety of regulatory interactions occur within and among the three UPR pathways. For example, while ATF6α(N) and XBP1s are responsible for up-regulating distinct sets of ER stress-responsive genes [26, 31], ATF6α(N)/XBP1s heterodimers induce a separate batch of targets, many of which are involved in ERAD [31]. In contrast, XBP1u can partner with and accelerate the cytosolic degradation of both XBP1s and ATF6α(N) [134–136]. Other potential, albeit incompletely understood, regulatory relationships include negative regulation of ATF6α activation by the ER stress-inducible proteins nucleobindin-1 [137] and Wolfram syndrome 1 [138], enhancement of PERK signaling by a cytosolic splice variant of BiP [139], and inhibition of PERK activity by p58 inhibitor of protein kinase [140, 141], an ER co-chaperone up-regulated by ATF6α(N) and XBP1s [31, 142]. A series of recent publications have reported mechanisms by which PERK-dependent signals can modulate both pro-adaptive and pro-apoptotic UPR outcomes, and these are discussed in detail below.

Cranking-up the ATF6α pathway: PERK holds the keys

Studies of ER stress-responsive gene expression in MEFs lacking PERK [30, 93] and in MEFs expressing a non-phosphorylatable form of eIF-2α (eIF-2α^{Ser51Ala}) [97] uncovered the initial clues of a relationship between the PERK and ATF6α pathways. Surprisingly, transcriptional induction of ER chaperones such as BiP, well characterized as ATF6α gene targets, was found to be defective in these cell types deficient in PERK-dependent signaling. ATF4 does not bind to the *cis*-acting ERSE vital for transcriptional activation of such genes [124], suggesting that another event downstream of PERK might be necessary for the activation and/or function of ATF6α.

Adachi and colleagues [26] provided the first direct evidence linking PERK to ATF6α activation. These investigators demonstrated that ER stress-induced cleavage of full-length ATF6α to generate the active ATF6α(N) transcription factor is weak and not sustained in PERK-deficient MEFs. Extending these studies, Teske et al. [143] showed that ATF6α activation is defective in MEFs expressing non-phosphorylatable eIF-2α^{Ser51Ala} and in MEFs lacking ATF4. Furthermore, they evaluated the ATF6α pathway in the livers of wild-type and liver-specific PERK knockout mice after intraperitoneal injection of the ER stress-inducing agent tunicamycin. In this situation of ER stress in vivo, the generation of ATF6α(N) and induction of ATF6α target genes were found to be severely attenuated in PERK-deficient liver tissue [143]. Therefore, an intact PERK/eIF-2α~P/ATF4 pathway is necessary for successful activation of the ATF6α pathway in vitro, and this appears to hold true for in vivo models of ER stress.

While the mechanism(s) that couples PERK-mediated signaling to ATF6α activation is not fully defined, experiments in the Teske et al. [143] study revealed a major role for ATF4 in this process. First, ATF4 promotes increased transcription of *Atf6α* during ER stress, thereby helping to replenish full-length ATF6α as it is being cleaved to yield ATF6α(N). Next, ATF4 is required for proper movement of ATF6α from the ER to the Golgi during ER stress. Finally, in keeping with its role in ATF6α trafficking, ATF4 is essential for maximal expression of certain genes involved in ER-Golgi transport that are transcriptionally activated in a PERK-dependent manner during the UPR. Together, these data indicate that the PERK/eIF-2α~P/ATF4 cascade 'cranks-up' the ATF6α pathway by driving ATF6α synthesis and supplying the machinery to ferry it to the Golgi for proteolytic activation (Fig. 3). Knowledge of this regulatory circuit provides further insight into the phenotypic characteristics of both PERK-deficient cells and mice. Whether reliance on the PERK pathway is a feature for all physiologic modes of ATF6α activation awaits further study.

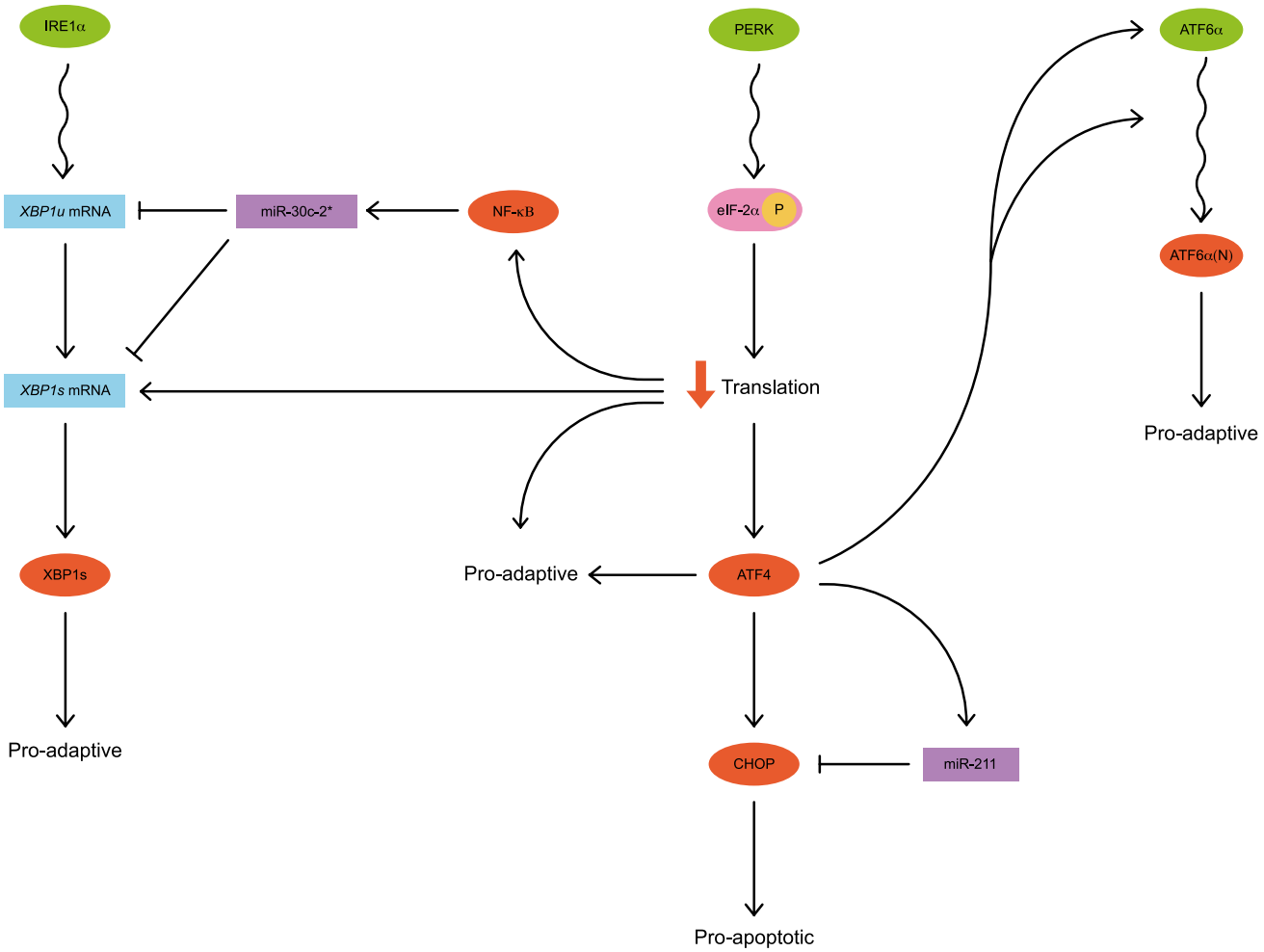

Fig. 3 PERK-dependent signals mediate inter- and intra-pathway regulatory crosstalk within the UPR. Translational repression, downstream of PERK, is pro-adaptive as it reduces entry of new client proteins into the stressed ER. In parallel, translational repression leads to induction of ATF4, a transcriptional activator that up-regulates expression of the pro-apoptotic transcription factor CHOP. ATF4 induces expression of miR-211 which negatively regulates CHOP expression in the early UPR. Thus, the ATF4/miR-211/CHOP relationship is an example of intra-pathway regulation within the PERK cascade of the UPR. Furthermore, ATF4 up-regulates expression of ATF6α and promotes trafficking of ATF6α to the Golgi apparatus where it is activated, yielding the pro-adaptive transcription factor ATF6α(N). Hence, ATF4 mediates inter-pathway regulatory crosstalk between the PERK and ATF6α pathways of the UPR. Finally, PERK-dependent translational inhibition leads to stabilization of *Xbp1s* mRNA, thereby maximizing synthesis of the pro-adaptive transcription factor XBP1s. PERK signaling, via a mechanism involving NF-κB, increases expression miR-30c-2* which can negatively regulate *Xbp1* mRNA. Therefore, PERK-dependent signals converge on *Xbp1* mRNA, mediating inter-pathway regulatory crosstalk between the PERK and IRE1α pathways of the UPR

Optimizing XBP1s levels: PERK makes it happen

Similar to the initial discovery of the PERK–ATF6α connection, studies of PERK-deficient MEFs [58] and MEFs expressing the non-phosphorylatable eIF-2α^{Ser51Ala} [144] provided the first hints that the PERK/eIF-2α pathway governs expression of *Xbp1*. Calfon and colleagues [58] showed that PERK knockout MEFs fail to up-regulate *Xbp1* mRNA during ER stress, but the underlying mechanism was unclear. While ATF6α(N) can induce *Xbp1* transcription [59, 61], ATF6α is not essential for induction of *Xbp1* mRNA in the UPR [26, 31]. Thus, the inability of PERK-deficient MEFs to up-regulate *Xbp1* mRNA cannot be easily attributed to a lack of ATF6α activation in these cells. The Hatzoglou laboratory demonstrated that accumulation of XBP1s protein in the UPR is at least partially dependent on eIF-2α~P [144], suggesting an event(s) downstream of PERK/eIF-2α~P has a positive impact on the generation, stability, and/or translation of *Xbp1s* mRNA.

To investigate this phenomenon further, the Hatzoglou group examined the relationship between *Xbp1* mRNA

levels and translational control during the UPR. Using MEFs and thapsigargin-induced ER stress as an experimental system, these investigators tied PERK/eIF-2α~P-mediated translational control to the regulation of *Xbp1s* mRNA turnover [145]. Specifically, *Xbp1s* mRNA is stabilized during early (initial 3 h of thapsigargin treatment), but not late (≥7 h of thapsigargin treatment), ER stress. Stabilization of *Xbp1s* mRNA requires eIF-2α~P and the corresponding inhibition of protein synthesis. Interestingly, for *Xbp1s* mRNA half-life to increase during the UPR, these transcripts must be derived by IRE1α-mediated cytoplasmic splicing of *Xbp1u* mRNA. The authors proposed that cytoplasmic splicing somehow 'marks' *Xbp1s* transcripts for stabilization in the early UPR when protein synthesis is repressed [145]. Such a mechanism would allow the cell to stockpile *Xbp1s* mRNA for translation when repression is eventually lifted, thus ensuring maximal levels of XBP1s protein are achieved. These data spawn a number of intriguing questions. For example, what is the molecular composition of the proposed 'marks' on *Xbp1s* mRNA bestowed by the cytoplasmic splicing process, and does this putative modification shield these transcripts from a degradative mechanism? Also, is turnover of *Xbp1s* mRNA similarly regulated in physiologic processes involving XBP1s, but in which the PERK/eIF-2α pathway is not engaged, such as the development of antibody-secreting plasma cells [146, 147] and Toll-like receptor-mediated activation of macrophages [148]?

Making matters more complicated is recent work linking a miRNA up-regulated downstream of PERK to negative regulation of *Xbp1* expression in the UPR. Employing computational tools and bioinformatics analyses, Byrd and colleagues [149] identified miR-30c-2* (also known as miR-30c-2-3p) as having potential regulatory activity toward a target site in the *Xbp1* mRNA 3′ untranslated region. These investigators provided evidence that miR-30c-2* has the capacity to limit the level of *Xbp1* mRNA and XBP1s protein, thereby tempering the magnitude of XBP1s-mediated gene transcription in the UPR. Intriguingly, expression of miR-30c-2* increases during ER stress in a PERK-dependent fashion via a mechanism involving NF-κB [149], a transcription factor activated under many conditions including PERK-dependent translational inhibition [150, 151]. Whether miR-30c-2* activity participates in the intricate mechanisms controlling *Xbp1* mRNA half-life remains to be determined, as does its potential significance in physiologic processes involving XBP1s. While many questions are unanswered, the emerging story is that PERK/eIF-2α~P-mediated translational control intersects with the IRE1α–XBP1 pathway by fostering robust expression of XBP1s, as well as fine-tuning the amount of this pro-adaptive transcription factor as cells cope with ER stress (Fig. 3).

Timing CHOP expression: PERK keeps the clock

Among the many puzzles in UPR research are the ways cells appropriately coordinate pro-survival and pro-apoptotic signals during ER stress. For example, certain PERK-dependent events, such as translational control and induction of ATF4 target genes that maintain redox homeostasis, clearly promote adaptation and survival, whereas ATF4-driven CHOP expression favors apoptosis. It follows that balancing these distinct signaling outcomes would be fundamental in determining cell fate depending on the severity and duration of a given ER stress condition.

Shedding new light on this aspect of UPR control, Chitnis and colleagues [152] recently identified an ER stress-responsive miRNA that negatively regulates expression of CHOP [encoded by DNA damage-inducible transcript 3 (*Ddit3*)]. Using MEFs and thapsigargin treatment as the primary model of ER stress, these investigators showed that induction of miR-211 is dependent on PERK/eIF-2α~P-mediated translational inhibition and ATF4. Acting through two sites in the proximal *Ddit3* promoter, miR-211 increases histone methylation and impedes *Ddit3* transcription, thus limiting synthesis of pro-apoptotic CHOP. Importantly, specific suppression of miR-211 hastened CHOP-dependent apoptosis of cells under ER stress, indicating that *Ddit3* is a significant target for miR-211 [152]. Therefore, the PERK/eIF-2α~P/ATF4 pathway mediates both positive and negative regulation of CHOP expression (Fig. 3). This seemingly paradoxical arrangement appears to make sense within the timing of the UPR. After increasing during the initial 5 h of thapsigargin-induced stress, miR-211 levels decrease thereafter, and this corresponds with accumulation of CHOP [152]. Based on these data, the authors proposed that PERK/ATF4-dependent miR-211 induction in the early UPR guards against a premature buildup of CHOP, thereby allowing sufficient time for restoration of ER homeostasis before initiation of a terminal apoptotic outcome. Sustained ER stress, however, leads to diminishment of miR-211, consequently ensuring maximal CHOP production and tilting the balance toward apoptosis. Defining the mechanism by which ATF4 and potentially other factors mediate differential expression of miR-211 in early and late ER stress is of obvious interest as this may represent a decisive 'switch' in cell fate determination in the UPR. Likewise, it will be important to evaluate the possible function of miR-211 during physiologic settings of UPR activity such as the tumor microenvironment [6]. In this regard, the Chitnis and colleagues study [152] included evidence that elevated levels of miR-211 in mouse mammary tumors correlate with increased PERK expression and reduced CHOP mRNA. Certainly, the miR-211/CHOP connection, an exciting example of intra-pathway regulation within the PERK branch of the UPR, is ripe for further analysis.

Concluding remarks

Approximately 15 years ago, the PERK protein was identified and characterized [11, 12], providing a molecular explanation for the drastic attenuation of global protein synthesis known to occur when cells encounter ER stress [153, 154]. Since then, a number of excellent studies have yielded a wealth of information regarding how this ER-localized kinase functions through its substrate eIF-2α and downstream effector ATF4 as cells respond to conditions that challenge the ER environment. With effects extending well beyond its immediate impact on translation, the PERK pathway influences the entire UPR as it facilitates ATF6α activation, optimizes XBP1s synthesis, and coordinates temporal expression of CHOP. Thus, the PERK pathway interconnects with both the ATF6α and IRE1α pathways and modulates itself to affect pro-adaptive and pro-apoptotic outcomes at multiple points in the complex circuitry of the UPR.

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