CELL BIOLOGY

Beyond the cell factory: Homeostatic regulation of and by the UPR^{ER}

Melissa G. Metcalf*, Ryo Higuchi-Sanabria*, Gilberto Garcia, C. Kimberly Tsui, Andrew Dillin†

The endoplasmic reticulum (ER) is commonly referred to as the factory of the cell, as it is responsible for a large amount of protein and lipid synthesis. As a membrane-bound organelle, the ER has a distinct environment that is ideal for its functions in synthesizing these primary cellular components. Many different quality control machineries exist to maintain ER stability under the stresses associated with synthesizing, folding, and modifying complex proteins and lipids. The best understood of these mechanisms is the unfolded protein response of the ER (UPR^{ER}), in which transmembrane proteins serve as sensors, which trigger a coordinated transcriptional response of genes dedicated for mitigating the stress. As the name suggests, the UPR^{ER} is most well described as a functional response to protein misfolding stress. Here, we focus on recent findings and emerging themes in additional roles of the UPR^{ER} outside of protein homeostasis, including lipid homeostasis, autophagy, apoptosis, and immunity.

INTRODUCTION

Multicellular organisms face a constant barrage of stresses that warrant an effective response, coordinated across diverse tissues. Each cell or tissue must thus be capable of perceiving stresses and signaling distal cells to respond accordingly to mitigate perturbations in cellular function and homeostasis. Furthermore, the distinct membrane-bound environments of the cell require these stress responses to be compartment specific. To maintain homeostasis of these microenvironments, cells have evolved several subcellular stress responses, including the cytoplasmic heat shock response (HSR), the endoplasmic reticulum (ER) unfolded protein response (UPR^{ER}), and the mitochondrial unfolded protein response (UPR^{mt}) (*1*–*3*). Of these responses, the ER's central function in biosynthesis, folding, and modification of membrane-bound and secreted proteins and its major role in lipid synthesis place particular interest on the UPR^{ER}. This interest is highlighted by the fact that defects in ER function are significantly associated with obesity, diabetes, cancer, and age-onset neurodegenerative disease (*4*, *5*).

There are three primary branches of the UPR^{ER}, which enable the ER to maintain normal levels of protein folding, protein secretion, and lipid homeostasis. Each arm of the UPR^{ER} consists of a transmembrane protein containing a luminal-facing domain and transmembrane helix, which act as sensors for induction of a nuclear signal upon detection of ER stress (Fig. 1). The best characterized of the three UPR^{ER} branches involves an endonuclease, inositol-requiring protein 1 (IRE1 in mammals, IRE-1 in *Caenorhabditis elegans*, and Ire1p in *Saccharomyces cerevisiae*. Note: All gene and protein names will use nomenclature pertinent to the organism, and human nomenclature is used as a general terminology when no organism is specified), and a transcription factor, X-box binding protein 1 (XBP1 in mammals, XBP-1 in *C. elegans*, and Hac1p in *S. cerevisiae*). In this branch, unfolded protein stress or lipid disequilibrium is sensed from the ER-localized IRE1, which then undergoes homodimerization and autophosphorylation. This activates IRE1's cytosolic endonuclease domain to splice a specific intron from the mRNA of *XBP1u* to create *XBP1s*. The spliced mRNA is translated into XBP1s, which translocates into the nucleus to mediate expression of protein degradation, protein folding, and lipid metabolism gene targets (*2*, *6*). IRE1 also plays an important role in regulating mRNA levels through regulated IRE1-dependent decay (RIDD). A majority of the identified RIDD mRNA targets encode proteins with signal peptides and transmembrane domains, including several secreted components of the insulin secretory pathway in β cells and mucin 2 in secretory goblet cells, whose reduced translation is expected to reduce the protein-folding load on the ER under conditions of ER stress or damage (*7*–*9*).

The other branches of the UPR^{ER} have different mechanisms of action, namely, the (i) global reduction of protein translation via eIF2α downstream of protein kinase RNA-like ER kinase (PERK in mammals and PEK-1 in *C. elegans*) and (ii) the proteolytic cleavage of an ER-resident protein, which translocates to the Golgi under stress to become a proteostasis-promoting transcription factor, activating transcription factor 6 (ATF6 in mammals and ATF-6 in *C. elegans*) (*2*, *6*). Similar to IRE1, PERK undergoes homodimerization and phosphorylation in response to unfolded proteins and lipid disequilibrium in the lumen. This leads to phosphorylation of eIF2α, which induces a global down-regulation of translation. However, critical mRNA species escape this translational down-regulation, including the activation of transcription factor 4 (ATF4 in mammals and ATF-4 in *C. elegans*), which is up-regulated during ER stress to promote the integrated stress response through remodeling of metabolic and translational programs (*10*). In addition, ATF4 can promote apoptosis during sustained ER stress by up-regulating CCAAT enhancer binding protein (C/EBP) homologous protein (CHOP).

The third arm of the UPR is initiated by ATF6, a type II ER transmembrane protein that translocates to the Golgi upon activation. During stress, the luminal domain of ATF6 loses its association with BiP/GRP78 (HSP-4 in *C. elegans*), which causes translocation of ATF6 into the Golgi. Once in the Golgi, Golgi-resident site 1 protease (S1P) and site 2 protease (S2P) cleave ATF6, allowing the N-terminal cytosolic fragment to translocate into the nucleus and act as a transcription factor to up-regulate target genes, including protein disulfide isomerase (PDI), XBP1, and CHOP (*11*–*13*).

Department of Molecular and Cellular Biology, Howard Hughes Medical Institute, The Glenn Center for Aging Research, University of California, Berkeley, Berkeley, CA 94720-3370, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: dillin@berkeley.edu

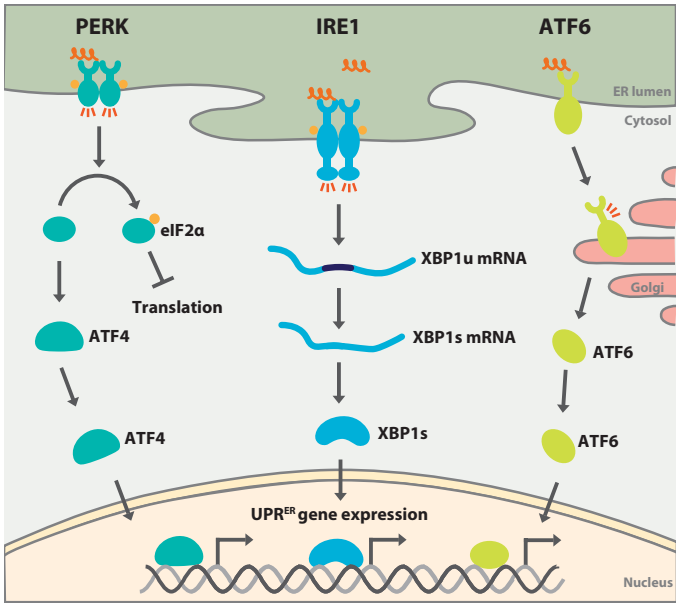

Fig. 1. The three primary branches of UPR^{ER} modulated by IRE1, PERK, and ATF6. There are three branches of UPR^{ER}, each consisting of a transmembrane protein with a luminal-facing sensor for damage, which then signals to the nucleus through a unique transcription factor. When IRE1 senses misfolded protein or lipid stress in the ER, it homodimerizes, is autophosphorylated, and promotes splicing of *XBP1u* mRNA to *XBP1s* which is translated into functional XBP1s, acting as a transcription factor to turn on genes important for restoring ER homeostasis. Similarly, PERK and ATF6 are activated under ER stress. When PERK is activated, it also oligomerizes, causing phosphorylation of eIF2α to inhibit global translation. There is also downstream activation of ATF4, which promotes the expression of ER-restoring genes that escape down-regulation via eIF2α. Unlike the other two ER stress sensors, ATF6 is proteolytically cleaved under ER stress, which causes translocation to the Golgi for further processing, allowing ATF6 to function as a transcription factor.

Dysregulation of the UPR^{ER} is a common feature of many diseases, including neurodegeneration, metabolic disease, and cancer. During the aging process, UPR^{ER} activation also becomes dysregulated across multiple organisms. For example, in *C. elegans*, the capacity to activate XBP-1–mediated UPR^{ER} in response to protein misfolding stress declines sharply during the aging process (*14*). Similarly, in aged mice, expression of genes involved in ER quality control show marked decline in the brain (*15*, *16*). The decreased function of the UPR^{ER} during aging can lead to the accumulation of damaged and aggregated proteins, which contribute to proteotoxicity and eventual cell death (*17*). Conversely, up-regulation of ER chaperones can protect cells during stress (*18*, *19*), and hyperactivation of the UPR^{ER} can have direct impacts on life span and healthspan: Overexpression of *xbp-1s* in *C. elegans* extends life span and stress resistance (*14*), and increased PERK-eIF2α signaling protects neurons from stress associated with misfolded proteins (*20*, *21*). Many of these studies focus primarily on chaperones and other mechanisms involved in restoring protein homeostasis. However, it is clear that there are other critical downstream targets of the transcription factors involved in up-regulating UPR^{ER}. This review touches on these core machineries outside of protein homeostasis and highlights the open-ended questions involved in how stress affects other functions of the ER, such as lipid and redox homeostasis.

Beyond the UPR^{ER}, there are several other mechanisms involved in maintaining ER homeostasis. Given the major role of the ER in protein synthesis, there are limited proteases that function within the ER. Therefore, proteins that are beyond repair, such as terminally misfolded proteins, are first extracted from the ER by adenosine triphosphate–driven motors and targeted for proteasomal degradation through ER-associated degradation (ERAD). In yeast, where most of the ERAD components have been originally described, transmembrane protein complex including the ubiquitin ligases Hrd1p and Doa10p recognize misfolded proteins and tag them for degradation (*22*, *23*). Upon poly-ubiquitylation via the ERAD machinery, the AAA+ adenosine triphosphatase (ATPase) Cdc48p (p97 or valosin-containing protein in humans) drives extraction of the proteins from the ER into the cytosol, where it is subsequently degraded by the proteasome (*24*). ERAD also plays an important role in maintaining protein quantity control by tagging excess or unnecessary proteins for degradation through similar mechanisms (*25*, *26*). When accumulation of damaged proteins in the ER has exceeded the repair capacity of ERAD, portions of the organelle can be specifically targeted for large-scale degradation through autophagy (ER-phagy). ER-phagy is capable of clearing ERAD-resistant proteins or other ER components, such as lipids, which cannot be cleared by conventional quality control machineries but are generally subject to autophagy through Vps34p/beclin-1–dependent machinery (*27*). It would be of great interest to understand whether ERAD and ER-phagy are critical for maintaining ER function outside of its proteome. It is possible to imagine that eliminating damaged ER via autophagy will also remove toxic lipid species, but can ERAD impose a similar benefit to lipids and other nonprotein components of the ER?

Here, we focus primarily on the UPR^{ER} with specific emphasis on noncanonical roles of UPR^{ER} outside of protein quality control. For a more thorough review on ER quality control machineries outside of UPR^{ER}, refer to (*1*, *28*, *29*).

NOT JUST A PROTEIN FACTORY: LIPID HOMEOSTASIS AND THE ER

Lipids are synthesized and metabolized within multiple organelles; however, specific functions are compartmentalized within organelles to maintain lipid homeostasis. For example, initial fatty acid synthesis primarily occurs in the mitochondria and cytoplasm. Subsequent fatty acid elongation then occurs within the mitochondria, cytoplasm, and ER (*30*, *31*). More complex lipids such as ether lipids are produced by the peroxisome, while sterols, phospholipids, and neutral lipids are synthesized by the ER. Thus, many critical enzymes for lipid metabolism reside in the ER, making the ER a critical hub for lipid homeostasis and a primary source of membrane lipids for all other organelles (*32*, *33*).

Since the ER serves as a critical organelle in regulation of lipid homeostasis, key sensors monitor lipid quality within the ER. These sensors are the same UPR^{ER} transmembrane proteins involved in protein homeostasis: IRE1, PERK, and ATF6. Adjacent to their transmembrane helices, IRE1 and PERK contain an amphipathic helix capable of sensing general ER membrane imbalances and can activate the UPR^{ER} independent of their luminal unfolded protein-sensing domains (*34*, *35*). Within the transmembrane domain of ATF6, a sphingolipid-sensing motif is able to trigger ATF6 activation upon accumulation of dihydrosphingosine or dihydroceramide, also independent of proteotoxic stress (*36*). In combination with basal lipid metabolism transcription factors, these proteins play an integral role in maintaining lipid homeostasis. Activation of UPR^{ER} alters the expression of many lipid metabolism genes. For example,

PERK/eIF2α phosphorylation activates sterol regulatory element–binding protein-1c (SREBP-1c) and SREBP-2, master transcription factors that regulate enzymes of lipogenic pathways (*37*). Mice with compromised eIF2α signaling down-regulate lipogenesis and displayed reduced high-fat diet (HFD)–induced fatty livers (*38*). Furthermore, XBP1s directly up-regulates lipogenic genes, including *Dgat2*, *Scf1*, and *Acc2*, while deletion of *Xbp1* results in hypocholesterolemia and hypotriglyceridemia of the liver (*39*). Last, large-scale sequencing studies in *C. elegans* found that a large subset of genes induced by IRE-1, XBP-1, PEK-1, and ATF-6 under conditions of ER stress were involved in lipid and phospholipid metabolism (*40*).

Two recent, complementary studies found that constitutive activation of UPR^{ER} downstream of *xbp-1s* resulted in notable lipid depletion in *C. elegans*. The original study from our laboratory describing *xbp-1s* overexpression in *C. elegans* identified that overexpression of *xbp-1s* in neurons was sufficient to elicit nonautonomous UPR^{ER} activation in peripheral tissue to promote whole-organism life-span extension (*14*). However, overexpression in other tissues either failed to elicit the same response or was detrimental in some other cases, suggesting that neurons were specialized in sending a specific and beneficial stress signal to other cells. Another unexpected study from our laboratory found that glia could signal a similar beneficial signal to the periphery (*41*).

Following this work, neuron-specific overexpression of *xbp-1s* was found to result in whole-animal depletion of lipids via two mechanisms: (i) up-regulation of lysosomal lipases and desaturases, which resulted in decreased triglycerides and increased oleic acid levels (*42*), and (ii) activation of lipophagy via a conserved RME-1/RAB-10/EHBP-1 (receptor mediated endocytosis-1/ras- related GTP binding protein-10/EH domain binding protein-1) complex, which depletes neutral lipids and decreases lipid droplet size and number, a phenomenon described by our work (Fig. 2, left) (*43*). When *xbp-1s* is overexpressed in neurons, both protein homeostasis and lipid metabolism are activated in peripheral tissue (*14*, *43*). Perturbations of either protein homeostasis or lipid metabolism suppress the beneficial effects of neuronal *xbp-1s* overexpression on life span and ER stress resistance, suggesting that both are essential components downstream of *xbp-1s* to promote ER quality control and organismal health. However, the most notable finding in the latter study is that the beneficial effects of lipid depletion on animal physiology can be uncoupled from protein homeostasis. Overexpression of *ehbp-1* is sufficient to drive lipid depletion and life-span extension but does not promote chaperone induction, suggesting that these two mechanisms can be uncoupled. In the former study, changes in lipid profiles caused by *xbp-1s* overexpression in neurons were sufficient to drive improvements in protein homeostasis. Specifically, supplementation with oleic acid decreased toxicity associated with ectopic polyQ40 expression, suggesting that changes in lipid homeostasis are sufficient to improve protein quality control even in the absence of chaperone induction. Since the ER is composed of both integral lipids and proteins, it is likely that promoting overall ER quality drives global organelle homeostasis, although further studies are required

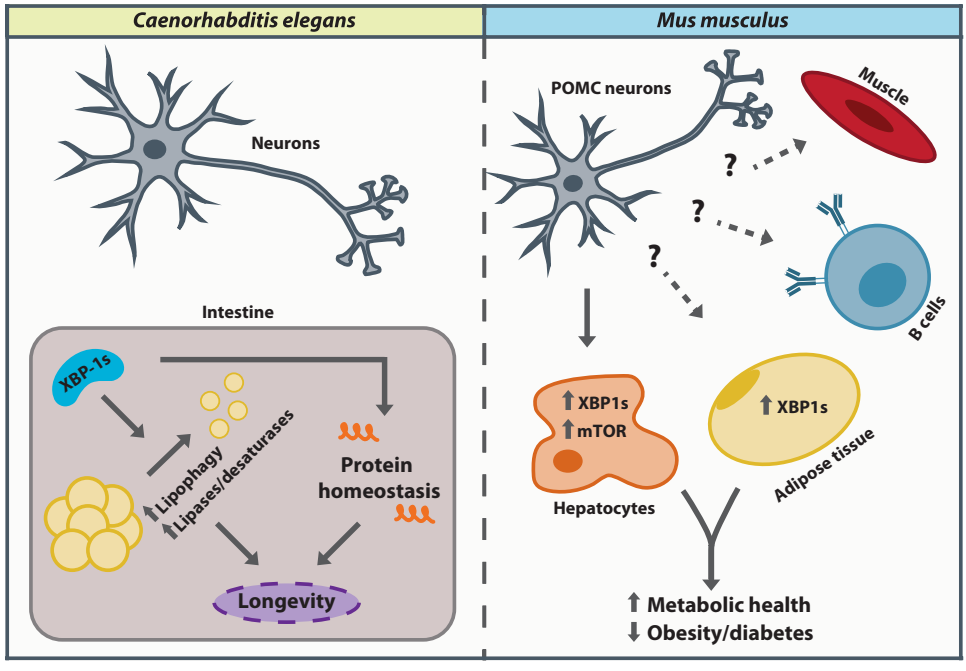

Fig. 2. Activation of the UPR^{ER} in neural cells promotes global changes in ER health in peripheral tissue. In *C. elegans* (left), overexpression of *xbp-1s* in neurons promotes two distinct changes to ER homeostasis in peripheral tissue (intestine): increased protein homeostasis by up-regulation of chaperones and increased lipid metabolism through mobilization of lipids via lipases, desaturases, and increased lipophagy. Both the increase in protein folding and decreased lipids are essential for the life-span extension found in this paradigm. Ectopic expression of *xbp-1s* in glia has also been shown to promote peripheral protein homeostasis and extend life span, although a role in glial signaling in lipid homeostasis has yet to be described. A similar phenomenon was also found in mice (right), where overexpression of *Xbp1s* in *Pomc* neurons (or simply activating *Pomc* neurons via olfactory exposure to food) is sufficient to drive UPR^{ER} in peripheral tissue. Specifically, XBP1s in POMC neurons promotes XBP1s and mTOR signaling in hepatocytes and adipose tissue, resulting in increased metabolic health, including resistance to diabetes and obesity. As UPR^{ER} has been shown to be critical in proper muscle and B cell function, it would be of great interest to investigate whether neuronal XBP1s can signal to elicit a beneficial effect in these and other cell types.

to understand the cross communication of lipid and protein quality control machineries within the ER. Whether this is indirect (i.e., the decreased burden of maintaining lipid homeostasis allows the ER to divert all its energy to protein quality control machineries) or direct (i.e., ER lipid health can directly alter protein folding via a still unknown molecular pathway) is still under investigation. In addition, the specific signal originating from neurons to drive these seemingly separable changes in the periphery also remains to be discovered.

A similar communication from neurons to peripheral tissue is observed in vertebrates. When *Xbp1s* is overexpressed in *Pomc* neurons of the hypothalamus of mice, the UPR^{ER} is up-regulated and has beneficial impacts on metabolic physiology (e.g., improved glucose levels, improved insulin sensitivity, and protection against HFD-induced obesity) (Fig. 2, right) (*44*). In this model, *Xbp1s* increases *Pomc* neuronal activity, which in turn increases energy expenditure by promoting brown adipose tissue thermogenesis and browning of white adipose tissue, which results in an overall decrease in fat mass and body weight, consistent with the findings in *C. elegans*. Conversely, mice with *Xbp1* deleted only in neurons or glia are more susceptible to diet-induced obesity and exhibit elevated levels of insulin and leptin in response to HFD (*45*). In mice, food perception (i.e., smelling of food) was sufficient to drive a *Pomc* neuron response to activate hepatic mammalian target of rapamycin (mTOR) and XBP1 signaling to promote metabolic homeostasis (*46*). Mice with olfactory exposure to food were able to phenocopy *Xbp1s* overexpression in *Pomc* neurons, driving peripheral *Xbp1* activation and its downstream beneficial effects on animal physiology. Both protein homeostasis and lipid homeostasis are activated via peripheral *Xbp1* activation (e.g., hepatic tissue activation upon receiving cues from *Pomc* neurons), and it is unclear whether these two mechanistic pathways can be uncoupled in mammalian models as was found in *C. elegans*.

Determining whether promoting chaperones and overall protein handling in the ER can alter lipid homeostasis and vice versa would be of great interest to understanding the independent roles that lipids and proteins have on mammalian organismal health. Is enhancing lipophagy through EHBP1 sufficient to drive ER stress resistance and organismal healthspan and life span in mammals similar to *C. elegans*? Do there exist divergent nodes of protein and lipid homeostasis downstream of XBP1s, or are these downstream mechanisms overlapping in higher eukaryotes? Under disease conditions, is loss of a single node of XBP1s signaling sufficient to drive pathogenesis? These questions are critical to develop novel therapeutic intervention for diseases that cause dysregulation of UPR^{ER}.

While the activation of the UPR^{ER} has many implications in organismal health and life span, persistent activation of the UPR^{ER} is associated with several metabolic diseases. Chronic UPR^{ER} activation is often observed in the liver or adipose tissue of models of obesity, nonalcoholic fatty liver disease, and diabetes (*47*). Moreover, ER stress within the brain's metabolic control center, the hypothalamus, has been shown to contribute to metabolic changes that promote weight gain and insulin resistance in mice, hallmark symptoms of obesity (*6*, *48*). A major feature of obesity is increased free fatty acids in circulation, which have been linked to UPR^{ER} activation in several models (*49*, *50*). Excessive accumulation of lipids can cause metabolic abnormalities and initiate cell death in response to lipotoxicity, often linked to chronic ER stress and defects in UPR^{ER} signaling. Specifically, saturated fatty acids, such as palmitate, activate the UPR^{ER} and cause detrimental effects in pancreatic β, liver, adipose, and muscle cells.

In primary rat β cells, exposure to palmitate results in increased phosphorylation of eIF2α through PERK activation, increased *Xbp1s* splicing, and increased ATF4 activity (*51*–*53*). Elevated levels of palmitate can result in excessive palmitoylation of proteins, which induce ER stress and activate caspase activity, causing cell death. In addition, excess palmitate can also cause lipotoxicity and ER dysfunction by altering the composition and membrane fluidity of the ER by changing phospholipid composition (*54*), promoting ceramide accumulation (*55*), and altering sphingolipid metabolism (*56*). Regardless of the mechanism, the chronic activation of the ER stress response promotes β cell death through the induction of apoptosis, which often includes the hyperactivation of cytokines, including interleukin-1β (IL-1β), interferon-γ, tumor necrosis factor–α (TNFα), and nuclear factor κB (NF-κB) [reviewed in (*57*)].

Similarly, ER stress through exposure to saturated fatty acids is a major contributing factor in liver lipotoxicity. In several liver cell lines, including HepG2 hepatoma and L02 immortalized liver cells, exposure to saturated fatty acids resulted in activation of PERK and up-regulation of its downstream targets such as ATF4 and CHOP (*58*). Suppression of PERK activation or reducing ER stress load via overexpression of BiP was sufficient to reduce palmitate-induced death (*58*, *59*). Liver cell exposure to palmitic acid results in aberrant phospholipid metabolism and increased membrane saturation (*60*). Alterations in the ER lipid composition and fluidity inhibit ER Ca^{++} signaling (*61*), which can result in aberrant mitochondrial metabolism and increased reactive oxygen species (ROS) production, causing further cellular toxicity (*62*). Restoring ER lipid composition through conversion of saturated lipid species into unsaturated fatty acyl–coenzyme As (CoAs) by overexpressing catalytic enzymes, such as Lpcat3, or restoring Ca^{++} homeostasis by overexpression of sarco-ER calcium ATPase reduces lipotoxicity in liver cells and can improve hepatic function in obese individuals (*61*, *63*). Last, lipid overload impairs autophagic flux in murine models and human patients with nonalcoholic fatty liver disease, suggesting a functional role for autophagy in preventing ER stress–mediated apoptosis (*64*).

Although less understood, muscle cells are also sensitive to lipid-induced ER stress. Mice fed an HFD showed up-regulation of *Xbp1* splicing, BiP, and ATF4/CHOP in skeletal muscle (*65*), while myotubes exposed to high levels of palmitate induced ATF4 and XBP1 activity (*66*). Prolonged lipotoxicity in muscle cells results in increased inflammation and ER stress, which can promote insulin resistance. Overexpression of stearoyl-CoA desaturase 1 (SCD1), a key regulator in lipid metabolism, can restore lipid homeostasis and reduce inflammatory cytokine expression, ultimately preventing insulin resistance in myotubes (*66*). However, a separate study in human and mouse cells showed that restoring ER homeostasis in palmitate-treated muscle cells did not restore insulin signaling, suggesting that palmitate-induced ER stress may not be the cause of reduced insulin signaling (*67*). Another study in human patients on a high-fat, hypercaloric diet showed similar contradicting results. While patients on HFD exhibited glucose intolerance, skeletal muscle biopsies failed to show an increase in ER stress markers, including XBP1, BiP, or PERK (*68*). Thus, further research is necessary to elucidate the connection between lipotoxicity and ER homeostasis in skeletal muscle cells.

Despite these controversies, a recent study in mice showed an interesting role for skeletal muscle in signaling lipotoxicity to other cells. Here, muscle-specific knockout of the lipid droplet–associated protein, perilipin 5, caused an increase in fatty acid oxidation and

reduced ER stress in muscle cells. This resulted in whole-body glucose intolerance and insulin resistance due to reduced secretion of fibroblast growth factor 21 from both skeletal and liver cells, highlighting a critical cross-talk between muscle and liver in ER lipid homeostasis (*69*).

Overall, it is clear that the UPR^{ER} plays a critical role in regulation of lipid homeostasis and metabolic state of the organism. Still to be investigated is whether the impact of UPR^{ER} activity serves to be beneficial or detrimental to organismal health. While many studies have highlighted a beneficial effect of UPR^{ER} activation in neurons (*14*, *41*, *42*, *44*), whole-organism *xbp-1s* overexpression has no beneficial effect on life span in *C. elegans* (*14*). Thus, it is possible that increased UPR^{ER} signaling can be detrimental in some tissue. Next, we describe the potential detrimental impacts of a sustained UPR^{ER}.

TOO MUCH OF A GOOD THING: CHRONIC UPR^{ER} AND APOPTOSIS

Despite many studies providing evidence for UPR^{ER} providing a beneficial role in clearing damage, sustained and unresolved ER stress can result in activation of apoptosis. Hence, chronic and irreversible UPR^{ER} induction can contribute to pathophysiological processes involved in a number of diseases, including neurodegeneration. In unresolved ER stress, the PERK-ATF4 axis of the UPR^{ER} induces the transcriptional activation of proapoptotic machinery, including C/EBP-homologous protein CHOP. CHOP then promotes the downregulation of the antiapoptotic factor, B cell lymphoma 2 (BCL2), and activation of proapoptotic genes, thus inducing the core mitochondrial apoptosis machinery through BCL2-associated X protein (BAX) and BCL2-antagonist/killer 1 (BAK) (*70*).

Under certain conditions, chronic ER stress can also regulate cell death decisions by influencing several mitogen-activated protein kinase (MAPK)–signaling components, including extracellular signal–regulated kinase (ERK), p38 MAPK, and JUN N-terminal kinase (JNK) (Fig. 3) (*71*, *72*). For example, ER stress–induced JNK activation is thought to initiate a proapoptotic pathway. Under ER stress, IRE oligomerizes, activating its kinase domain and increases interaction with TNFα receptor–associated factor 2 (TRAF2), which activates JNK via induction of apoptosis signal–regulating kinase 1 (ASK1). IRE1-TRAF2 promotes ASK1 oligomerization and autophosphorylation, which is required for its kinase activity to promote JNK signaling (*73*). Activation of JNK signaling can promote cell death by promoting de novo synthesis of death receptors and their ligands and by targeting components of the BCL2 family to initiate apoptosis (*74*). Inhibiting the downstream activation of JNK has been shown to promote resistance to ER stress–induced cell death: In human pancreatic β cells, inhibition of JNK significantly decreased eIF2α activity and promoted cell viability under ER stress (*75*); *Ask1*$^{-/-}$ mice showed reduction in JNK activation and decreased apoptosis under ER stress (*76*), and phosphorylation of ASK1 on Ser^{83} decreased its activity, promoting prosurvival by reducing apoptosis (*77*). In addition to the IRE1-TRAF2-ASK1 pathway, JNK can also be activated by the PERK axis of UPR^{ER} through CHOP. CHOP expression promotes the release of Ca^{++} from the ER, which also activates ASK1 through Ca^{++}/calmodulin-dependent protein kinase II (CaMKII) (*78*). JNK activation through CaMKII-ASK1 promotes apoptosis through increased cell surface localization of the death receptor Fas, and in vivo knockout of CaMKII can suppress apoptosis induced via ER stress (*79*).

In contrast to JNK signaling, activation of ERK1/2 signaling serves as a prosurvival cue under ER stress. As a primary signaling molecule downstream of almost all growth factors, ERK1/2 promotes cell survival under numerous stress stimuli by promoting transcriptional activation of several prosurvival proteins, including BCL2 (*80*). Moreover, ERK1/2 activation under ER stress is dependent on IRE1. In gastric cancer cells, *IRE1* knockdown decreased ERK1/2 signaling under ER stress, which results in decreased BiP levels and subsequent induction of cell death (*81*). In mouse embryonic fibroblasts, IRE1 also regulates ERK1/2 signaling by regulating the pool of the Src homology 2/Src homology 3 domain–containing adaptor Nck. Under basal conditions, ER-associated Nck suppresses ERK1 signaling, but upon exposure to ER stress, Nck dissociates from the ER membrane, eliciting IRE1-dependent ERK1 activation to promote cell survival (*82*). However, how IRE1 promotes the activation of ERK1 is still unclear.

ERK1/2 hyperactivation is also found in numerous cancers and is a target for therapeutic intervention (*83*). Several human melanoma cell lines have been shown to be protected from therapeutic interventions that promote ER stress–induced apoptosis due to increased ERK1/2 signaling in these cancers. In some cases, inhibition of ERK1/2 signaling increased sensitivity of cancer cells to ER stress–induced cell death, introducing combined ERK1/2 inhibition and ER stress as a potential therapeutic intervention for these cancers, including melanoma (*84*).

MAPK signaling does not only function downstream of UPR activation but can also promote UPR^{ER} signaling. For example, p38 MAPK can phosphorylate two serine residues found in CHOP, increasing the activity of its transactivation domain (*85*). While the phosphorylation of these serine residues by p38 was not critical for the DNA binding activity of CHOP, they had notable implications in its association with binding partners required to promote cell death machinery (*86*). In cardiomyocytes, ATF6 has also been shown to be a direct substrate for phosphorylation by p38 (*87*). Sustained p38 activity increased ATF6 phosphorylation and promotes its downstream signaling, including the induction of BiP (*88*, *89*).

A recent study from our laboratory elucidated a role for MAPK signaling in maintaining ER stress resistance independent of the UPR^{ER} (*90*). Through whole-genome CRISPR-Cas9 screening in karyotypically normal fibroblasts, the cell surface hyaluronidase transmembrane protein 2 (TMEM2) was identified as a novel regulator of ER homeostasis. Specifically, overexpression of TMEM2 increased resistance to ER stress through ERK and p38 MAPK signaling. While the exact signaling cascade is unknown, it is proposed that the low–molecular weight product of hyaluronic acid produced by TMEM2 converges on the CD44 receptor to activate ERK and p38-dependent cell survival under ER stress. Intriguingly, overexpression of human TMEM2 in *C. elegans* was sufficient to extend life span by more than 20% by preventing the age-associated decline in innate immunity (immunosenescence), similarly dependent on ERK/p38 (PMK-1/MPK-1 in *C. elegans*). Most of the cells in the adult nematode are postmitotic, and MAPK signaling does not play a role in regulating apoptosis in the adult. Rather, the central role of MAPK signaling is in regulating innate immunity (*91*). Perhaps, most notable in the study was that the beneficial effects of TMEM2 were completely independent of all three branches of UPR^{ER}. Therefore, despite numerous studies highlighting notable overlap between UPR^{ER} and MAPK signaling modalities, it is clear that there exist mutually exclusive mechanisms of modulating cell survival under conditions of ER stress.

Beyond apoptosis, chronic activation of PERK signaling can result in sustained repression of translation through eIF2α, which can also be detrimental. For example, in animal models, hyperactivation of PERK promotes synaptic failure and neuronal death in prion disease

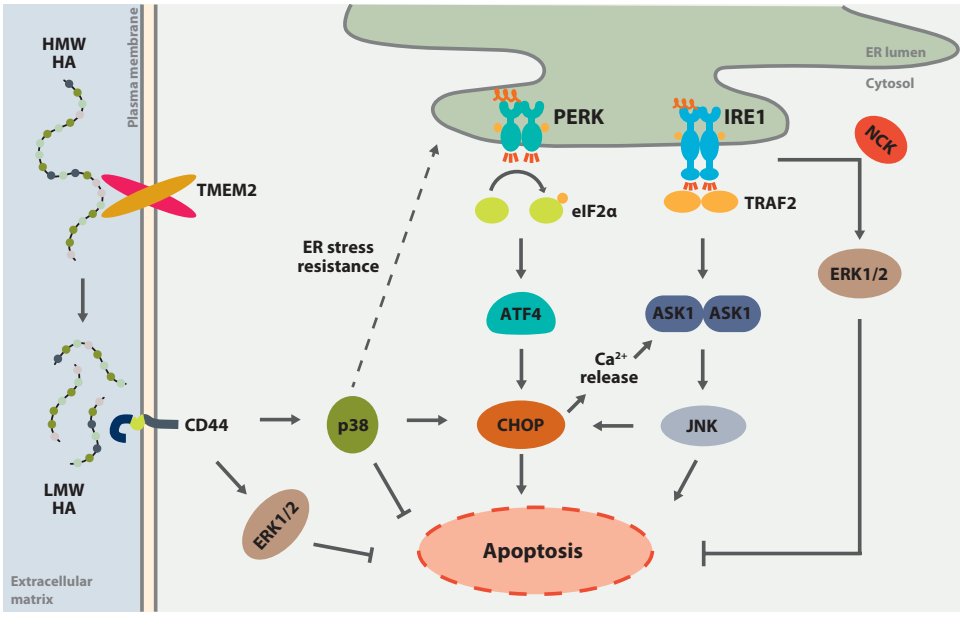

Fig. 3. UPR^{ER} in apoptosis and cell survival signals. Functionally, the UPR^{ER} serves as a quality control mechanism to restore ER form and function under conditions of stress. However, under sustained and unresolved ER stress, UPR^{ER} can actually promote cell death through apoptosis. For example, sustained PERK signaling can promote the activation of CHOP through ATF4, which activates proapoptotic signals. The other branches of UPR^{ER} can also modulate MAPK signaling, which feeds into cell survival or apoptotic cues in various ways. For example, IRE-1 can activate both prosurvival signals through activation of ERK1/2 and proapoptotic signals through JNK depending on the ER stress conditions. Beyond the UPR^{ER}, extracellular cues can promote cell survival under ER stress. Specifically, the cell surface hyaluronidase, TMEM2, cleaves high–molecular weight hyaluronic acid (HMW HA) into low–molecular weight hyaluronic acid (LMW HA), which acts as a ligand to the CD44 receptor and activates downstream p38 and ERK1/2 prosurvival signals.

mouse models, which suggests that decreasing UPR^{ER} activity could be a potential therapeutic intervention by restoring protein synthesis in neurons (*58*). In triple-negative breast cancers, hyperactivation of XBP1 can also promote tumor growth, and inhibition of IRE1/XBP1 was shown to be beneficial (*59*). Thus, it is clear that UPR^{ER} signaling is complex and context specific, highlighting the importance of dissecting the molecular mechanisms downstream of UPR^{ER} activation for therapeutic intervention.

ER AND IMMUNITY

ER stress is commonly found in inflammatory diseases, such as diabetes, atherosclerosis, and inflammatory bowel disease (*92*). Accumulating evidence links the activation of the UPR^{ER} in inflammatory signaling cascades, including the activation of cytokine release (*93*). In addition, several studies indicate that inflammation itself augments ER stress responses (Fig. 4). For example, exposure to proinflammatory cytokines, such as TNFα, IL-1β, and IL-6, induced ER stress, promoted XBP1s expression, and activated UPR in mouse livers and fibrosarcoma cells (*94*, *95*). In addition, lipopolysaccharide (LPS) stimulation resulted in the activation of XBP1s, ATF4, and CHOP in mice (*96*). These studies strongly link the connection between ER stress and immunity.

Perhaps the first identified role of UPR^{ER} in the immune system was in the development of specific immune cells. For example, XBP1 is critical for the development of immunoglobulin-secreting plasma cells, such that mice lacking *Xbp1* fail to mount antibody responses, have decreased levels of all immunoglobulins, and are more susceptible to infections that are normally cleared by antibody-mediated immune responses (*97*). Subsequent studies have shown that functional B cells splice *Xbp1* mRNA and up-regulate UPR target genes, including BiP, upon exposure to LPS (*98*, *99*). It is likely that the massive induction of UPR in B cells is critical to expand the ER and promote protein synthesis to meet the new secretory demands of a mature B cell (*100*). Both XBP1 activity and ATF6 activity reach maximal levels once Ig synthesis and secretion are induced in B lymphocytes (*101*). PERK is not activated upon LPS stimulation, and B cells lacking *Perk* develop normally and are fully capable of Ig synthesis and antibody secretion, providing further evidence that the purpose of UPR^{ER} activation in B cells is primarily to meet the increased secretory demands of these cells (*102*).

Similar to B cells, T cell differentiation is also highly dependent on a functional UPR. During viral or bacterial infection, expansion of antigen-presenting CD8$^+$ T cells requires splicing of *Xbp1* mRNA downstream of IL-2 signals. Unlike B cells, T cells exhibit increased *Atf4* mRNA, suggesting that the PERK/eIF2α pathway is also activated during T cell differentiation (*103*). *Xbp1* splicing is also critical in maintaining dendritic cells (professional antigen-presenting cells), as loss of XBP1 leads to reduced numbers due to increased apoptosis of dendritic cells, whereas overexpression of *Xbp1s* promotes their survival (*104*). In addition to promoting survival in these cell types, ER stress also plays a critical role in antigen presentation, although the exact mechanism is not yet understood (*105*, *106*). Increased levels of triglycerides have been found in dendritic cells in both mice and human patients with tumors (*107*, *108*). Lipid accumulation occurs in dendritic cells due to up-regulation of receptors involved in extracellular lipid uptake, which has detrimental effects in dendritic cell function (*109*). It would be of particular interest to determine whether hyperactivation of XBP1 can promote lipid depletion in dendritic cells similar to the neuronal XBP1 signaling paradigms

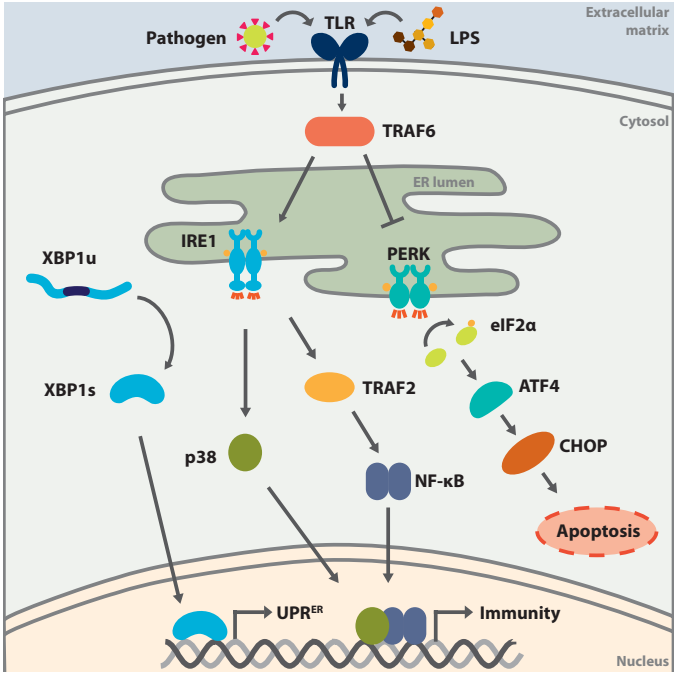

Fig. 4. Role of UPR^{ER} in immune response. The immune response and the UPR^{ER} have both been shown to affect the other. Mounting an immune response requires the synthesis of many proteins, including several secreted factors, which makes a functional ER imperative during pathogenic infection. Thus, under exposure to pathogens, UPR^{ER} is activated to promote protein homeostasis. In addition, to avoid cell death, immune signals may dampen the PERK arm to inhibit apoptosis. UPR^{ER} components can also alter immunity through IRE1-mediated activation of TRAF2, which can promote cytokine signaling through NF-κB or directly alter transcription of immune response genes through p38 MAPK signaling.

described in mice and nematodes. Can *Xbp1* overexpression promote dendritic cell survival and function by preventing accumulation of triglycerides? Pharmacological normalization of lipid levels on dendritic cells restored their functional activity and promoted immune response (*109*).

UPR^{ER} also affects innate immunity. Exposure to ER stress activates many inflammatory signaling cascades, including NF-κB, which is considered a major mechanism for inducing the innate immune response. Under ER stress, IRE1 interacts with inhibitor of nuclear factor κB (IκB) kinase through TRAF2, which enhances TNFα and NF-κB activation (*110*). NF-κB can also be activated via PERK, which promotes NF-κB by translational inhibition of IκB via eIF2α (*111*). UPR^{ER} activation also occurs in macrophages, one of the primary immune cell types involved in innate immunity through phagocytosis of infectious agents. Upon exposure to pathogens, Toll-like receptors (TLRs) detect microbes to activate immune responses in macrophages. TLR2 and TLR4 specifically activate IRE1/XBP1, which are critical for sustained production of inflammatory cytokines in macrophages. IRE1 is activated upon TLR ligation via interaction with TRAF6, which promotes its phosphorylation to sustain IRE1 function (*112*). Mice lacking XBP1 in macrophages display increased sensitivity to infection due to impaired production of IL-6 and TNF (*113*). In addition to activating the IRE1/XBP1 branch of UPR, TLR activation promotes suppression of the ATF4/CHOP branch of UPR downstream of PERK. Prolonged PERK activation triggers cell death through CHOP as described above, and thus, TLRs play a critical role in suppressing ATF4/CHOP-mediated apoptosis to promote survival of macrophages (*114*).

Since *C. elegans* lack an adaptive immune system, resistance to pathogenic infection is dependent on PMK-1 (MAPK)–mediated innate immunity responses, which potentially induce ER stress in the organism because of the increased secretory demand of the response (*91*). It has been shown that XBP-1 plays an essential role in protecting nematodes during pathogenic infection. For example, animals lacking *xbp-1* exhibit major defects in ER morphology and larval lethality when exposed to *Pseudomonas aeruginosa* infection (*115*). Moreover, the increased sensitivity of *xbp-1* mutants to *P. aeruginosa* exposure was exacerbated with simultaneous loss of *pek-1* both in larval stages and during adulthood, suggesting that PEK-1 and XBP-1 function together to protect against immune activation (*116*). Similarly, exposure to pore-forming toxins, the most common proteinaceous exotoxin produced by bacteria, activates the IRE-1/XBP-1 pathway in a p38/MAPK-dependent manner. Loss of *ire-1*, *xbp-1*, and, to a lesser extent, *atf-6* resulted in severe sensitivity of animals to pore-forming toxins (*117*). UPR^{ER} activation during pathogenic infection is controlled by neuronal G protein–coupled receptors (GPCRs). Specifically, the octopamine GPCR, OCTR-1, expressed in sensory neurons serves as a negative regulator of UPR, such that mutations in *octr-1* increases UPR activation and promotes immunity (*118*, *119*). Therefore, UPR^{ER} serves as a critical means to maintain ER homeostasis during pathogen infection in nematodes.

Similar to other stress responses, the innate immune response declines in function during the aging process in *C. elegans*. Termed immunosenescence, a decline in p38/MAPK signaling occurs during intestinal aging, allowing bacterial proliferation in the gut, which is the leading cause of death (*91*). As described above, promoting p38/MAPK signaling can prevent immunosenescence and extend life span independent of the UPR^{ER}. However, it is also likely that promoting canonical UPR^{ER} can promote resistance to pathogenic invasion and prevent immunosenescence. A forward genetic screen in *C. elegans* identified that dominant mutants of vitellogenin proteins (homologs of human apolipoprotein B-100) caused ER stress and increased sensitivity to pathogenic infection. Specifically, accumulation of mutant vitellogenins in the intestine caused collapse of the proteome and caused massive ER stress, decreasing the secretory capacity of the intestine, which is essential for mounting an efficient innate immune response. An up-regulated UPR counteracts the toxic effects of the ER stress associated with the accumulation of lipoproteins, while inhibition of UPR^{ER} via *xbp-1* or *ire-1* knockdown resulted in a notable increase in sensitivity to pathogens in this model (*120*). Moreover, another study found that overexpression of *xbp-1s* was sufficient to drive increased secretion of vitellogenins from the intestine, which suggests that these animals would perform better against infection (*43*).

UPR^{ER} IN OXIDATIVE STRESS RESPONSE

The matrix of the ER is under highly oxidizing conditions in comparison to the cytosol to allow for oxidation of cysteine residues required to form intramolecular disulfide bonds during protein folding. Moreover, many enzymes that catalyze the formation of these disulfide bonds, including phosphodiesterases (PDIs), become reduced during their activity and need to be reoxidized to promote further reactions. Thus, additional enzymes, such as endoplasmic reticulum oxidoreductin 1 (ERO1), exist to provide oxidizing environments within

the ER [reviewed in (*121*, *122*)]. Ultimately, the primary functions of protein folding in the ER itself can serve as a major source of ROS and oxidative stress, especially under ER stress. Thus, under conditions of ER stress, global down-regulation of protein translation can mitigate ER oxidation and promote resistance to ER stress. In contrast, cells lacking *Perk* fail to down-regulate global translation through eIF2α and accumulate endogenous peroxides within the ER and experience increased oxidative stress (*123*).

In metazoans, the nuclear factor erythroid 2–related factor 2 basic leucine zipper (NRF bZIP)–family transcription factors (NRF1/2/3 in mammals and SKN-1 in *C. elegans*) serve to promote activation of oxidative stress defense genes. Under basal conditions, NRF2 remains in the cytosol via association with Keap1. Upon exposure to ER stress, PERK-dependent phosphorylation of NRF2 promotes NRF2 dissociation from Keap1, allowing subsequent nuclear transport and activation of NRF2 targets, including glutathione (GSH) synthesis genes responsible for buffering ROS from the ER (*124*, *125*). While these studies highlight a clear connection between UPR^{ER} and oxidative stress response, it is unclear whether NRF2 can directly affect quality control of the ER or simply serves as a means to clear ER-induced oxidative stress. A comprehensive analysis of SKN-1 targets in *C. elegans* identified several UPR^{ER} targets activated directly by SKN-1. Specifically, in animals lacking functional SKN-1, ER stress failed to increase the expression of major UPR^{ER} targets, including chaperones, autophagy, calcium homeostasis, lipid homeostasis, and even UPR transcription factors themselves. Due to the failure to mount an appropriate UPR^{ER}, *skn-1* mutants also exhibited increased sensitivity to multiple forms of ER stress, providing direct evidence that SKN-1 can affect ER quality control beyond its indirect roles in redox buffering (*126*). Perhaps most surprising in this study is that the core UPR machinery was also required for SKN-1–mediated oxidative stress response. All three branches of the UPR were shown to affect *skn-1* transcriptional expression, and functional IRE-1 was required for nuclear localization of SKN-1 under arsenite-induced oxidative stress (*126*).

Similar findings in human cells and *Drosophila* suggest that the integrated signaling of UPR^{ER} and oxidative stress are conserved across eukaryotes. In *Drosophila*, increased ER folding capacity by UPR^{ER} promotes long-term tissue homeostasis by enhancing redox response through JNK and the Nrf2 homolog CncC (*127*). In human HepG2 cells, NRF1 and NRF2 were shown to be required to promote the activation of ER stress signaling in response to ER stress. Specifically, *NRF1* knockout cells had a diminished response to tunicamycin by ATF6, IRE1, and PERK, and partial loss of all three UPR^{ER} responses was found in *NRF2* knockout cells (*128*).

Beyond the regulation of *NRF2*, UPR^{ER} components have also been shown to directly affect the transcriptional output of redox homeostasis genes. For example, ATF4 is essential for GSH synthesis to maintain redox balance of the ER (*123*). Moreover, XBP1 can stimulate the hexosamine biosynthesis pathway (HBP), which promotes synthesis of glycosylation products that can increase defense against ROS (*129*). Through these studies, it is clear that oxidative stress response and UPR^{ER} are tightly linked (Fig. 5), which begs the question of why such an extensive overlap between two distinct processes would have evolved. Perhaps the simplest explanation is that the ER serves as a major source of ROS production through its protein-folding capacity and the requirement to maintain a highly oxidative environment within its matrix, and thus, modulating NRF2 activity is critical. Beyond this, it is possible that the NRF2-UPR axis serves as a bidirectional signal between the ER and cytoplasm about its homeostatic state. As a hypothetical example, under ER stress, the UPR activates NRF2 to prepare the cytoplasm for the potential toxic effects downstream of ROS production under protein misfolding condition. Similarly, when cytoplasmic stress is high, it would be advantageous to activate a robust UPR response to promote protein folding of essential homeostatic regulators (e.g., chaperones) while also down-regulating global protein translation through eIF2α.

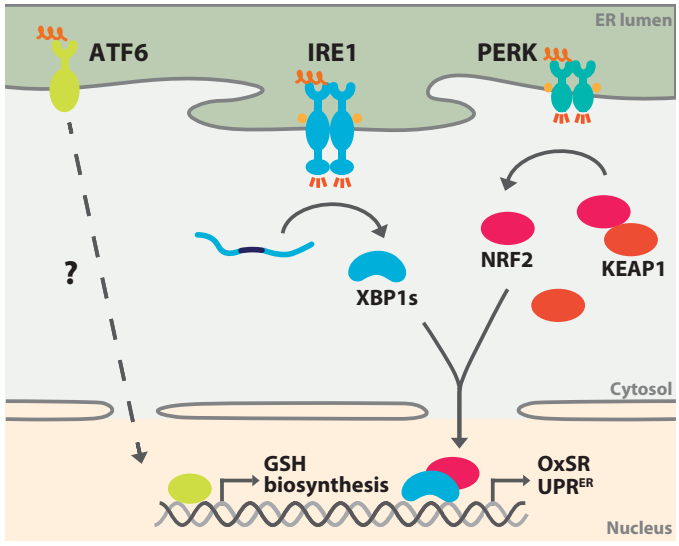

Fig. 5. Cross communication of the UPR^{ER} and OxSR. It is becoming increasingly clear that cellular stress responses are not completely separate, and there exist notable cross communication and interdependent regulation. The UPR^{ER} and oxidative stress response (OxSR) have been shown to functionally affect the other, such that targets of XBP1s affect redox homeostasis and targets of NRF2 affect ER homeostasis. One study in *C. elegans* showed that transcriptional output of SKN-1 was, to a certain extent, dependent on XBP-1s function and vice versa. There are also some studies in mammalian systems that hint to similar signaling pathways, where NRF2 promotes ER quality control genes and XBP1s promotes genes involved in redox homeostasis. Another study found that glutathione synthesis genes (GSH) were potentially downstream of ATF6 signaling.

UPR^{ER} IN THE REGULATION OF AUTOPHAGY

The UPR^{ER} and autophagy are two cellular processes that respond to both intra- and extracellular stressors. Both of these processes work to maintain organellar and cellular homeostasis. While it is clear that autophagy can play a role in regulating ER homeostasis by mediating lysosomal degradation of damaged ER through ER-phagy, the interplay and cross-talk between UPR^{ER} and autophagy remain poorly understood.

Autophagy is a cellular degradative process that removes damaged or unnecessary proteins and organelles to recycle macromolecules such as amino acids and lipids. Autophagy requires the coordination of more than 30 autophagy-related genes, which are involved in the formation of the autophagasome, generation of the autophagic vesicle, and fusion with the lysosome (*130*). Autophagy is activated under times of nutrient deprivation, mitochondrial and ER stress, cell fate and lineage decisions, and pathogen infection (*131*). Under conditions of ER stress, misfolded proteins accumulate in the ER and can lead to the activation of autophagy to reestablish cellular homeostasis. For example, aggregated polyglutamine in the cytosol

can cause ER stress–induced activation of PERK, which induces conversion of microtubule-associated protein light chain 1 (LC1) to LC3, inducing apoptosis in an eIF2α-dependent manner (*132*). Recent studies have shown that under conditions of ER stress, PERK can actually mobilize the major autophagy transcription factors, transcription factor EB (TFEB) and transcription factor E3 (TFE3), to translocate to the nucleus. TFEB/TFE3 activation not only leads to the induction of autophagy and lysosomal genes but also induces ATF4 and CHOP, making them more resistant to ER stress–induced apoptosis (*133*).

In addition, the IRE1/XBP1 pathway has been implicated in the activation of autophagy (Fig. 6). In cancer cells, XBP1s has been shown to induce autophagy through regulation of expression of Beclin2, an antiapoptotic protein, which interacts with Beclin1 to inhibit the nucleation of autophagy (*134*, *135*). Similarly, sustained XBP1s activation in endothelial cells can promote autophagic vesicle formation, conversion of microtubule-associated protein LC1 to LC3, and expression of Beclin1. Conversely, XBP1 deficiency in mouse endothelial cells reduces LC3 expression and decreases autophagosome formation (*136*). IRE1 can also induce autophagy via a TRAF2-mediated pathway similar to the apoptosis machinery by inducing JNK activation and downstream Beclin1 transcription by c-Jun (*137*). In contrast to these studies, depletion of IRE1/XBP1 activity has also been shown to enhance autophagy and promote viability in cells obtained from patients with amyotrophic lateral sclerosis (ALS). XBP1s deficiency leads to increased forkhead Box O1 (FOXO1) expression and increased autophagy in neurons, and neuron-specific XBP1 ablation is sufficient to drive disease resistance in mice (*138*). These contrasting effects of the IRE1/XBP1s branch on autophagy indicate the complex interplay between the two mechanisms and highlight the importance of further research to consider targeting UPR^{ER}-autophagy cross communication as a potential avenue of therapeutic intervention.

Recent work in *C. elegans* has shown that activation of lysosomal activity downstream of constitutive UPR^{ER} activation via *xbp-1s* overexpression in neurons is crucial for *xbp-1s*–mediated longevity (*139*). Both cell autonomous, via intestinal *xbp-1s* overexpression, and cell nonautonomous, via neuronal *xbp-1s* overexpression, activation of UPR^{ER} induce lysosomal gene expression. In addition, *xbp-1s* overexpression leads to increased lysosomal activity and acidity within the intestine, which is necessary for the enhanced life span and proteostasis found in this long-lived paradigm. These processes may be mediated by HLH-30, the *C. elegans* homolog to mammalian TFEB, as *hlh-30* knockdown is sufficient to suppress the life-span extension of neuronal *xbp-1s* animals. Another study has found that HPL-2, a chromatin-modifying protein, plays a critical role in ER homeostasis through autophagy. Specifically, knockdown of *hpl-2* increases resistance to ER stress by promoting autophagy (*140*). Further, transcriptional profiling of worms deficient in phosphatidylcholine (PC) synthesis, which causes ER stress through lipid dysregulation, also induced autophagy in an IRE-1/XB-1–dependent manner (*141*). This is highly similar to a process previously described in yeast, where inhibition of PC biosynthesis activates microlipophagy downstream of UPR^{ER} (*142*). These studies highlight the critical impact of UPR^{ER} on autophagy beyond canonical protein misfolding stress in the ER.

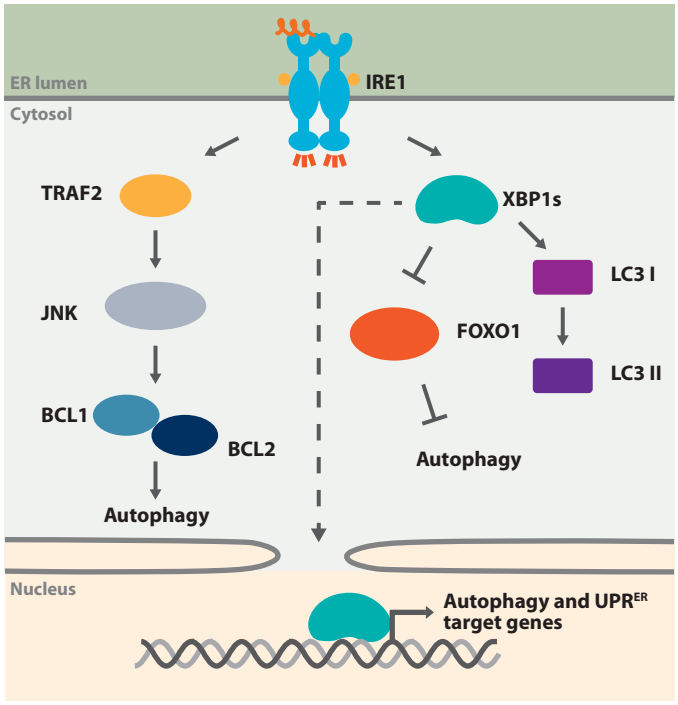

Fig. 6. The role of the IRE1/XBP1 pathway in modulating autophagy. The IRE1/XBP1 pathway has been shown to regulate autophagy both through direct transcriptional regulation of autophagic genes downstream of XBP1s and indirectly through other signaling molecules, including FOXO1 and JNK. IRE1 can promote JNK signaling through TRAF2-mediated pathways similar to the apoptosis machinery and thus activate BCL1/2 to promote autophagy. XBP1s can also activate autophagy either by inhibiting FOXO1 signaling, which releases its inhibitory effect on autophagy, or by promoting conversion of LC3 I to LC3 II.

UPR^{ER} IN N-LINKED GLYCOSYLATION

Besides the well-characterized ER chaperones and ER quality control genes, XBP1s can also transcriptionally up-regulate genes involved in N-glycan biosynthesis (*143*, *144*) and the HBP, which generates uridine diphosphate (UDP)–*N*-acetylglucosamine (UDP-GlcNAc), an essential substrate for both N- and O-linked glycosylation (*145*, *146*). N-linked glycosylation begins in the ER, in which a preassembled oligosaccharide is transferred to selective asparagine residues on newly synthesized polypeptides. These oligosaccharides are essential for protein folding and maturation through the secretory pathway, and blockage of ER N-glycosylation leads to ER stress [for a detailed review, see (*147*, *148*)]. Intriguingly, activation of XBP1s up-regulates not only genes required for ER N-glycosylation but also glycotransferases and sugar transporters in the ER and Golgi that modulate N-glycan maturation, resulting in remodeling of N-glycan structures on cell surface and secreted proteins (*149*). While the functional role of XBP1s-induced glycoproteome remodeling is unclear, it likely influences how cells interact with the extracellular environment and may be used to communicate ER stress between cells.

Glycosylation also regulates cytosolic and nuclear proteins via O-linked GlcNAc modifications, a dynamic posttranslational modification analogous to phosphorylation. Activation of HBP, either by XBP1s induction or by increased expression of HBP rate-limiting enzymes, enhances cellular O-GlcNAc modifications and has been shown to protect cardiomyocytes from ischemia/reperfusion injury in mice and promote proteostasis in *C. elegans* (*145*, *146*). However, the specific O-GlcNAc–modified proteins that mediate such protective effects are yet to be identified. In contrast, O-GlcNAc modification on eIF2α inhibits downstream activation of UPR^{ER}, preventing

ER stress–induced apoptosis (*150*). Additional studies will be required to understand how glycosylation changes on specific proteins during ER stress may modulate UPR^{ER} and intertissue ER stress signaling.

ER-PEROXISOME CROSS-TALK

Peroxisomes are organelles that aid in lipid metabolism and neutralizing or using hydrogen peroxide to oxidize substrates. These functions often overlap with other cellular compartments, such as the cytosol and mitochondria, because of their overlap in metabolic processes. For example, the cytosol houses several ROS scavengers, while the mitochondria contain critical enzymes in β-oxidation of fatty acids and fatty acid derivatives (*87*). Peroxisomes also communicate with other organelles to mediate these processes through cellular signaling pathways, vesicular trafficking, and membrane-membrane interactions. Through these complex interorganellar communications, peroxisomes regulate cellular aging in multiple ways: maintenance of the lipid bodies within the cell, exchange of metabolites between peroxisomes and other organelles, maintenance of ROS homeostasis and oxidative stress, and recycling of tricarboxylic acid cycle intermediates [refer to (*151*) for a more comprehensive review]. Similar to all other membrane-bound organelles, the peroxisome has a tight link with the ER, as the ER serves as the primary site for lipid and protein biogenesis of the organelle.

While there are numerous studies highlighting the importance of the ER and functional ER in maintaining peroxisomal function and biogenesis [reviewed in (*152*)], much less is known about the function of the peroxisome under ER stress and how UPR^{ER} affects this organelle. One study found that peroxisome deficiency can activate ER stress signaling, primarily through PERK and ATF4 signaling, which can lead to lipid dysregulation and dysfunction in cholesterol homeostasis. Specifically, peroxisome-deficient *PEX2* knockout mice exhibited UPR^{ER} activation, which results in dysregulation of the endogenous sterol pathways through SREBP-2 (*153*). In addition, peroxisome-deficient mice showed increased peroxisome proliferator–activated receptor α (PPARα), which can cause increased expression of both SREBP-2 and the transcriptional regulator p8, leading to increased ER stress. Sustained p8 and UPR^{ER} activity can contribute to the development of hepatocarcinogenesis (*154*). Despite these studies highlighting a link between ER and peroxisomes, it is still unclear how peroxisome dysfunction leads to ER stress. Are the effects simply indirect where lipid dysregulation upon peroxisome dysfunction leads to ER stress? Or is there a causative link between ER and peroxisome function?

CONCLUDING REMARKS AND OPEN QUESTIONS

The current state of the literature has made it evident that the ER serves numerous critical functions outside of protein homeostasis. As such, the quality control machineries dedicated to preserving ER form and function, such as the UPR^{ER}, are essential in homeostatic regulation of these alternative functions, including lipid metabolism, autophagy, apoptosis, redox homeostasis, and glycosylation. Here, we briefly discussed how the UPR^{ER} affects these other functional roles of the ER independently. However, a critical question is how these functional roles overlap and whether the homeostatic regulation of these pathways can be separated. It is clear that when the UPR^{ER} is activated, many downstream targets are simultaneously regulated. For example, under conditions of protein misfolding stress, lipid homeostasis genes downstream of IRE1/XBP1 are activated in addition to chaperones and protein repair machinery. Thus, is it sufficient to promote a single component downstream of UPR^{ER}, or is it essential to simultaneously maintain all functions of the UPR^{ER}? Alternatively, if lipid homeostasis of the ER is enhanced in the absence of protein quality control machinery, would that be detrimental? Is there an essential balancing act that occurs between all the functional roles of the ER? And if so, how does the cell modulate this balance?

Beyond the beneficial roles of the UPR^{ER}, we also discussed how sustained and unresolved UPR^{ER} signaling can be detrimental. However, often the detrimental effects of the UPR^{ER} are described under conditions where there is unresolved ER stress. Hyperactivation of the UPR^{ER} in the absence of stress is generally a beneficial phenomenon and promotes metabolism, organismal health, and life span [reviewed in (*6*)]. Note that there do exist some specific circumstances where even UPR^{ER} hyperactivation in the absence of stress can also be detrimental. For example, overexpression of *xbp-1s* in the muscle of *C. elegans* decreases life span (*14*), and overexpression of *HAC1s* (the *S. cerevisiae* homolog of *XBP1*) can perturb cell cycle progression (*155*). Therefore, how does a cell differentiate between a beneficial and detrimental UPR^{ER} signature? Do there exist other transcriptional regulators that function with canonical UPR^{ER} transcription factors to alter the downstream signaling cascade? We briefly discussed the interplay between SKN-1 and XBP-1 in *C. elegans*. What are the other transcriptional cofactors of the canonical UPR^{ER} transcription factors, and how do they serve as sensors to inform the cell of when UPR^{ER} activation is beneficial or damaging?

An additional concern in studying quality control mechanisms is that, historically, research is generally focused on a single, organelle-specific machinery. However, current research has made it apparent that communication between homeostatic and stress response machineries is not only common but also critical. For example, we described the complex interplay between the oxidative stress response and the UPR^{ER} that is impossible to disconnect. Moreover, as the ER is not the only organelle responsible for producing ROS, it comes as no surprise that mitochondrial quality control machineries are also highly interconnected to the oxidative stress response (*156*). How then do all these quality control machineries communicate with one another? Under conditions of competing needs, such as through general stress where several organelles are damaged, which stress response pathway is preferentially activated? Can all cellular stress responses be mutually activated in a way that is beneficial to the cell? Hyperactivation of a single stress response is generally sufficient to promote organismal healthspan and life span [reviewed in (*1*)]. In these models, is it possible that other quality control machineries are also activated? Or would hyperactivating multiple stress response pathways simultaneously have a compounded effect and create a super long-lived organism? Conversely, is it possible that hyperactivating too many stress response pathways would be detrimental for an organism?

Last, we still know relatively little about cross communication of the stress signals identified here across cell and tissue types. While cell nonautonomous signaling has generally been heavily studied in the realm of the UPR^{ER}, most of these studies focused primarily on the canonical role of the UPR^{ER} in protein homeostasis. Very recent studies have now emerged in how nonautonomous communication of UPR^{ER} from the nervous system to the periphery can promote lipid homeostasis in distal tissues, as described above. Even in these studies, the actual signaling events that happen across tissues are still

poorly understood. Do there exist similar cell-to-cell communication events for regulation of autophagy, immunity, oxidative stress response, etc.? If so, are the signaling molecules and receptors involved similar to or distinct from those already identified? Answering these questions described above is critical in furthering our understanding of the impacts of manipulating the UPR^{ER} for therapeutic intervention. Because of the pleiotropic effects of the UPR^{ER} described here, it is clear that targeting the master regulators of UPR^{ER} activation is unwise. However, downstream targets of UPR^{ER} can be targeted for specific diseases, ideally in specific tissue types of interest.

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