The impact of the unfolded protein response on human disease

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A central function of the endoplasmic reticulum (ER) is to coordinate protein biosynthetic and secretory activities in the cell. Alterations in ER homeostasis cause accumulation of misfolded/unfolded proteins in the ER. To maintain ER homeostasis, eukaryotic cells have evolved the unfolded protein response (UPR), an essential adaptive intracellular signaling pathway that responds to metabolic, oxidative stress, and inflammatory response pathways. The UPR has been implicated in a variety of diseases including metabolic disease, neurodegenerative disease, inflammatory disease, and cancer. Signaling components of the UPR are emerging as potential targets for intervention and treatment of human disease.

The endoplasmic reticulum (ER) and ER stress

The ER is a vital organelle for production of secretory proteins that are synthesized by ER-bound ribosomes and then modified and folded by a machinery of foldases and molecular chaperones in the ER lumen. Correctly folded secretory proteins exit the ER en route to other intracellular organelles and the extracellular surface. The rates of protein synthesis, folding, and trafficking are precisely coordinated by an efficient system termed "quality control" to ensure that only properly folded proteins exit the ER. Misfolded proteins are either retained within the ER or subject to degradation by the proteasome-dependent ER-associated protein degradation (ERAD) pathway or by autophagy. Many diseases result from protein misfolding caused by gene mutations that disrupt protein-folding pathways.

The ER is the major site for the synthesis of sterols and phospholipids that constitute the bulk of the lipid components of all biological membranes. The ER, therefore, plays an essential role in controlling the lipid composition in membranes, which, in turn, determines the biophysical properties and functions of cell membranes (Fagone and Jackowski, 2009). ER membrane expansion generally reflects the increased secretory capacity of the cell. Lipid homeostasis in membranes maintained by the ER is important for normal functions of secretory cells (Leonardi et al., 2009).

The ER is also the main site for storage of intracellular Ca^{2+}. The concentration of Ca^{2+} in the ER lumen can reach ~5 mM (Stutzmann and Mattson, 2011). The majority of ER-luminal Ca^{2+} is bound to ER molecular chaperones and is required for their optimal function. In addition, ER Ca^{2+} release is sensed by mitochondria as either survival or apoptotic signals in the cell. Deregulation of the ER Ca^{2+} content is reported in a number of diseases including Alzheimer's disease, Huntington's disease, and polycystic kidney disease (Sammels et al., 2010).

The ER is a highly dynamic organelle and responds to environmental stress and developmental cues through a series of signaling cascades known as the unfolded protein response (UPR; Schröder and Kaufman, 2005). The primary signal that activates the UPR is the accumulation of misfolded proteins in the ER lumen (Dorner et al., 1989). As a consequence, the UPR regulates the size, the shape (Schuck et al., 2009), and the components of the ER to accommodate fluctuating demands on protein folding, as well as other ER functions in coordination with different physiological and pathological conditions. Recent studies on the integration of ER stress signaling pathways with metabolic stress, oxidative stress, and inflammatory response signaling pathways highlight new insights into the diverse cellular processes that are regulated by the UPR (Hotamisligil, 2010). The accessibility to genetically engineered model organisms has further advanced our understanding of the physiological and pathological impacts of the UPR in human physiology and disease. Here, we summarize the adaptive and apoptotic pathways mediated by the UPR and discuss how the UPR responds in different physiological and pathological states.

The adaptive role of the mammalian UPR

In mammals, three ER membrane-associated proteins act as ER stress sensors (Fig. 1): (1) the inositol-requiring transmembrane kinase/endoribonuclease 1 (IRE1); (2) the double-stranded

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Abbreviations used in this paper: ATF, activating transcription factor; C/EBP, CCAAT enhancer-binding protein; CHOP, C/EBP homologous protein; eIF2α; eukaryotic translational initiation factor 2α; IRE, inositol-requiring transmembrane kinase/endoribonuclease; PERK, protein kinase-like eukaryotic initiation factor 2α kinase; UPR, unfolded protein response; XBP, X-box binding protein.

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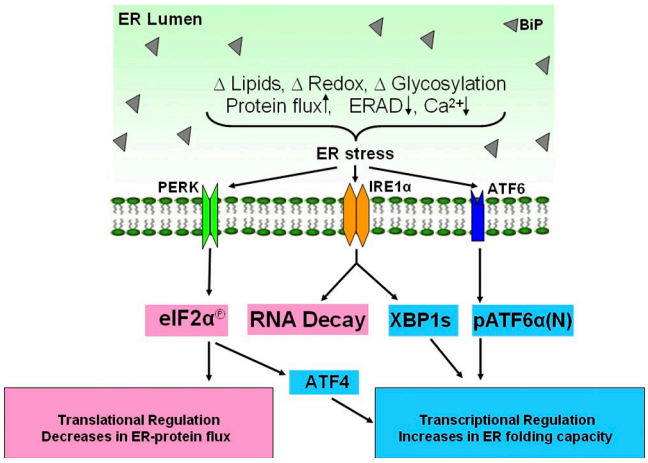

Figure 1. **ER stress and the unfolded protein response.** A number of conditions such as disturbed lipid homeostasis, disturbed calcium signaling, oxidative stress, inhibition of glycosylation, increased protein synthesis, and decreased ER-associated degradation can cause ER stress and activate the unfolded protein response (UPR). The UPR is mediated by three ER membrane-associated proteins, PERK, IRE1α, and ATF6α, to induce translational and transcriptional changes upon ER stress. PERK phosphorylates eIF2α to attenuate general protein translation and decrease protein efflux into the ER. Phosphorylated eIF2α also selectively stimulates ATF4 translation to induce transcriptional regulation of UPR genes. IRE1α cleaves XBP1 mRNA to a spliced form of XBP1 that translates XBP1s to up-regulate UPR genes encoding factors involved in ER protein folding and degradation. ATF6α traffics to Golgi for cleavage by S1P and S2P to release pATF6α(N) that works synergistically or separately with XBP1s to regulate UPR gene expression.

RNA (PKR)–activated protein kinase-like eukaryotic initiation factor 2α kinase (PERK); and (3) the activating transcription factor-6 (ATF6). Each UPR sensor binds to the ER luminal chaperone BiP. When misfolded proteins accumulate in the ER, they bind to and sequester BiP, thereby activating the sensors (Bertolotti et al., 2000; Ma et al., 2002; Shen et al., 2002). However, additional mechanisms that initiate and modulate the activity of individual UPR branches have been reported, in particular for IRE1 (Gardner and Walter, 2011; Promlek et al., 2011), which may explain their diverse responses to different signals and/or in different cell types.

IRE1 is the most conserved branch of the UPR, present from yeast to humans. Mammalian IRE1 has two homologues, IRE1α and IRE1β. IRE1α is expressed in all cells and tissues, whereas IRE1β is specifically expressed in the intestinal epithelium. UPR signaling is mainly mediated through IRE1α, and the function of IRE1β in the UPR is still not clear. Activated IRE1α cleaves a 26-base fragment from the mRNA encoding the X-box binding protein-1 (XBP1; Yoshida et al., 2001). Spliced *Xbp1* mRNA is translated into a potent transcription factor, XBP1s, which targets a wide variety of genes encoding proteins involved in ER membrane biogenesis, ER protein folding, ERAD, and protein secretion from the cell (Lee et al., 2003; Acosta-Alvear et al., 2007). Mouse genetic studies showed that germline deletion of *Xbp1* or *Ire1α* in mice is embryonic lethal (Reimold et al., 2000; Zhang et al., 2005). Recently, a role for IRE1α was suggested in the placenta for oxygen/nutrient exchange between the maternal and fetal circulation (Iwawaki et al., 2009). However, the contribution of XBP1 in the IRE1α pathway to placental development has not been addressed. A recent study identified an inhibitor of IRE1α endoribonuclease activity that did not alter the cellular response to ER stress, but did reduce ER expansion in an exocrine cell model of differentiation. This result suggests that IRE1α may play a more significant role in ER expansion associated with differentiation of secretory cell types than with the adaptation to ER stress (Kaufman et al., 2002; Cross et al., 2012).

PERK is the second arm of the mammalian UPR and is structurally related to IRE1α, with an ER luminal dimerization domain and a cytosolic kinase domain. The immediate effect of PERK activation is the phosphorylation of the α subunit of eukaryotic translational initiation factor 2α (eIF2α) at Ser51 that attenuates global protein synthesis to decrease protein influx into the ER lumen (Shi et al., 1998; Harding et al., 2000b; Scheuner et al., 2001). On the other hand, phosphorylation of eIF2α can change the efficiency of AUG initiation codon utilization (Kaufman, 2004), leading to, for example, preferential translation of activating transcription factor-4 (ATF4) protein over other upstream reading frames in the mRNA (Harding et al., 2000a). ATF4 is a transcription factor that induces expression of genes involved in ER function, as well as ER stress–induced apoptosis, ER stress–mediated production of reactive oxygen species, and an inhibitory feedback loop through dephosphorylation of eIF2α to prevent hyperactivation of the UPR (Harding et al., 2003). PERK was also reported to phosphorylate nuclear erythroid 2 p45-related factor 2 (NRF2) to induce antioxidant response genes including heme oxygenase 1 and glutathione *S*-transferase (Cullinan et al., 2003). Therefore, the PERK–eIF2α arm of the UPR acts to preserve redox balance during ER stress through activation of ATF4 and NRF2.

ATF6α is the third arm of the mammalian UPR that is an ER-associated type 2 transmembrane basic leucine zipper (bZIP) transcription factor. ATF6β is a distant homologue of ATF6α but both are ubiquitously expressed in all tissues. Upon release from BiP, ATF6α traffics to the Golgi apparatus for cleavage by serine protease site-1 (S1P) and metalloprotease site-2 (S2P) to release the transcription-activating form of ATF6, pATF6α(N) (Schindler and Schekman, 2009). The pATF6α(N) can act independently or synergistically with XBP1s for induction of UPR target genes. The role of pATF6α(N) in development is apparently minimal because mice lacking ATF6α are viable without significant abnormalities, although *ATF6α*-null mice are exquisitely sensitive to ER stress (Wu et al., 2007; Yamamoto et al., 2010). Although there has not been a phenotype associated with ATF6β deletion, mice lacking both ATF6α and ATF6β are embryonic lethal, suggesting they display functional redundancy in early development (Yamamoto et al., 2007). Thus, the common role(s) for ATF6α and ATF6β in development needs to be clarified.

In addition to the core components of the UPR, mammals have also evolved some tissue-specific UPR sensors, most of which are transmembrane bZIP transcription factors that are activated by regulated intramembrane proteolysis in a similar manner to ATF6. To date, several of these proteins including cAMP responsive element-binding protein H (CREBH or CREB3L3), CREB3 (Luman), CREB3L1 (Oasis), CREB3L2

(BBF2H7), and CREB4 (Tisp40) have been identified in response to conventional ER stress inducers (Bailey and O'Hare, 2007). Although the exact mechanisms of their activation are not fully understood, it appears that they synergize with the mainstream UPR to expand and/or enhance the diversity of UPR signaling and fine-tune the ER stress response in a temporal and/or cell type–specific manner (Zhang et al., 2006).

The apoptotic role of the mammalian UPR

Chronic or severe ER stress activates the UPR leading to apoptotic death. Most data support the notion that PERK–eIF2α–ATF4 signaling is a primary determinant for apoptosis (Rutkowski et al., 2006). Persistent and/or severe ER stress leads to activation of the PERK–eIF2α–ATF4 pathway and culminates in the induction of the CCAAT enhancer-binding protein (C/EBP) homologous protein (CHOP/GADD153), a proapoptotic factor induced by ER stress (Zinszner et al., 1998). CHOP up-regulates apoptosis-related genes including DR5 (Yamaguchi and Wang, 2004), Trb3 (Ohoka et al., 2005), BIM (Puthalakath et al., 2007), and PUMA (Cazanave et al., 2010) to promote cell death during ER stress. Importantly, CHOP also induces GADD34, a regulatory subunit of protein phosphatase I to dephosphorylate eIF2α and reverse attenuation of mRNA translation. The cytotoxic effects of CHOP are at least in part through GADD34 because CHOP and GADD34 knockout animals are protected from ER stress–induced tissue damage (Marciniak et al., 2004; Malhotra et al., 2008; Song et al., 2008). In addition, selective inhibitors of eIF2α dephosphorylation that target GADD34 can rescue cells from protein misfolding stress (Boyce et al., 2005; Tsaytler et al., 2011). How does translation attenuation divert cells from a cell death pathway to survival during ER stress? One hypothesis is that translation attenuation prevents continued synthesis of unfolded proteins that would exacerbate protein-misfolding stress in the ER leading to a death response.

Under severe stress, activation of IRE1α was implicated in cell death mediated by apoptosis signaling kinase 1 (ASK1) through their interaction with tumor necrosis factor receptor-associated factor 2 (TRAF2; Nishitoh et al., 2002). It was also reported that IRE1α indiscriminately degrades ER-localized mRNAs that can lead to cell death (Hollien et al., 2009; Vecchi et al., 2009). However, the pro-apoptotic signaling molecule(s) that targets activation of this indiscriminate RNase activity of IRE1α has not been identified.

The UPR in health and disease

Many extracellular stimuli and fluctuations in intracellular homeostasis disrupt protein folding in the ER. As a consequence, the cell uses its ER protein-folding status as an exquisite sensor to monitor intracellular homeostasis. Pharmacological insults were initially used to elucidate how cells cope with immediate and severe challenges to the protein-folding quality control system. It is now evident that intracellular signaling, such as insulin anabolic responses, as well as metabolic conditions including hyperlipidemia, hyperhomocysteinemia, hyperglycemia, and inflammatory cytokines all disrupt protein folding in the ER. As a consequence, UPR activation is observed in many human diseases and mouse models of human disease. Therefore, it was proposed that ER stress contributes to the pathology of many human diseases (Kaufman, 2002). Cell death, a physiological consequence of chronic ER stress, is a key to the pathogenesis of many diseases including metabolic disease, inflammation, neurodegenerative disorders, and cancer (Fig. 2). Here, we describe how the use of animal models has contributed to our knowledge of how the UPR impacts cellular homeostasis, normal physiology, and disease pathogenesis.

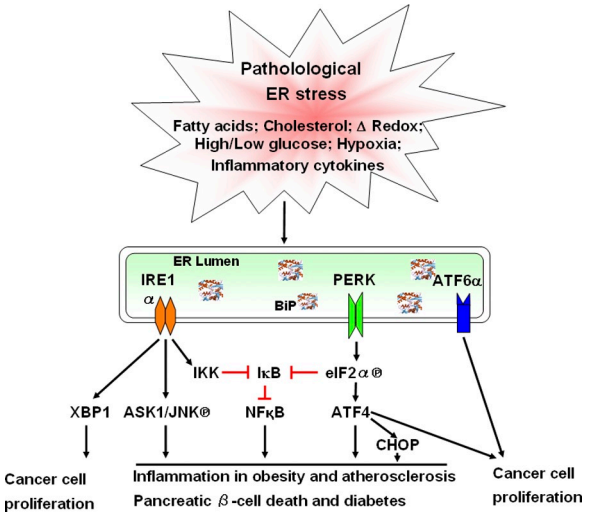

Figure 2. **UPR signaling in diseases.** Pathophysiological conditions such as hypoxia, elevated levels of fatty acids or cholesterol, oxidative stress, high or low glucose levels, and inflammatory cytokines induce ER stress and activate the UPR chronically. UPR signaling is interconnected with oxidative stress and inflammatory response pathways and involved in a variety of diseases including metabolic disease, inflammatory disease, and cancer. The three arms of the UPR, IRE1α-XBP1s, PERK-eIF2α phosphorylation-ATF4, and ATF6 are important for tumor cell survival and growth under hypoxic conditions. The UPR, IRE1α, and PERK can activate c-JUN N-terminal kinase (JNK) and NFκB to promote inflammation and apoptosis that contribute to inflammation in obesity and pancreatic β-cell death in diabetes. In addition, CHOP production in the PERK pathway exacerbates oxidative stress in diabetic states and atherosclerosis to aggravate the diseases.

The UPR in diabetes

Cells that are stimulated to secrete large amounts of protein over a short period of time are highly dependent on a functional UPR. Upon glucose stimulation, the pancreatic β cell increases proinsulin synthesis up to 10-fold (Itoh et al., 1978). The PERK–eIF2α arm of the UPR is indispensable for β cells to adapt to large fluctuations in proinsulin synthesis (Harding et al., 2000b; Scheuner et al., 2001). This is most evident from characterization of Wolcott-Rallison syndrome in which individuals require insulin at the age of three years. This autosomal recessive disease is due to loss-of-function mutations in *PERK* that cause β cell failure. Similarly, whole body inactivation of the PERK signaling pathway in mice causes a defect in β cell expansion during neonatal development and hyperglycemia with reduced serum insulin levels (Harding et al., 2000b; Scheuner et al., 2001). Conditional deletion of *Perk* in β cells further supports a homeostatic role for PERK signaling in β cell survival (Cavener et al., 2010). Consistent with these observations, mice with a Ser51Ala mutation at the PERK phosphorylation site in eIF2α in a homozygous state, or in a heterozygous

state combined with stress of a high fat diet, display β cell loss due to proinsulin misfolding, ER stress, oxidative stress, and apoptosis (Scheuner et al., 2005). In addition, increased protein synthesis in β cells by removing eIF2α phosphorylation caused a reduction in insulin production, which was due to ER dysfunction, oxidative stress, and loss of β cells. Strikingly, feeding an antioxidant diet prevented the β cell failure upon increased proinsulin synthesis (Back et al., 2009). These findings demonstrate that translational control of proinsulin through phosphorylation of eIF2α is required to coordinate proinsulin synthesis with proinsulin folding to maintain β cell homeostasis. Importantly, an increase in proinsulin synthesis alone is sufficient to initiate a series of events including proinsulin misfolding, insulin granule depletion, loss of glucose-stimulated insulin secretion, and oxidative stress, similar to those observed in type II diabetes (Huang et al., 2007; Laybutt et al., 2007).

Recent genetic evidence indicates that proapoptotic components of the ER stress response exacerbate β cell failure in type II diabetes. Deletion of *Chop* improved glucose control and increased β cell mass in heterozygous diabetic *Akita* mice that express a misfolding-prone Cys96Tyr proinsulin (Oyadomari et al., 2002). Furthermore, *Chop* deletion improved β cell function in several mouse models of type II diabetes: (a) high fat diet-fed heterozygous *Ser51Ala eIF2α* mice; (b) mice fed a high fat diet and then given streptozotocin, a compound that kills β cells and induces diabetes; and (c) leptin receptor–null (*db/db*) mice. *Chop* deletion not only protected β cells from apoptosis, but also improved β cell function by reducing oxidative damage and improving protein folding in the ER (Song et al., 2008).

XBP1 is also required for insulin maturation and secretion. *Xbp1* deletion in β cells markedly impaired proinsulin processing and decreased insulin production (A.H. Lee et al., 2011). Enforced expression of XBP1s, as well as ATF6α, inhibited insulin expression and ultimately killed β cells, indicating the importance of homeostatic control of UPR signaling in β cells (Allagnat et al., 2010). Although a mouse model with β cell deletion in *Ire1α* has not been reported, it is also likely required for insulin production, similar to XBP1. However, it was proposed that activated IRE1α degrades proinsulin mRNA to inhibit insulin production (Lipson et al., 2006; Han et al., 2009). The significance of this IRE1α-mediated proinsulin mRNA degradation needs to be confirmed in a physiological setting.

Wolfram syndrome, a rare genetic disorder, provides another link between ER stress, β cell death, and diabetes. Recent genome-wide association studies showed that polymorphisms in *WFS1* are associated with impaired β cell function and risk for type II diabetes (Franks et al., 2008). *WFS1*, a downstream transcriptional target of XBP1, encodes an ER transmembrane protein that negatively regulates ATF6α to prevent β cell death as a consequence of prolonged ATF6α activation (Fonseca et al., 2010). There are reports of ATF6α variants associated with type II diabetes (Thameem et al., 2006; Chu et al., 2007; Meex et al., 2007), suggesting ATF6α might also play a role in β cell function, consistent with recent findings that suggest ATF6α protects β cells from ER stress (Usui et al., 2012).

The UPR in metabolic syndrome

The identification of genetic and environmental factors involved in metabolic syndrome have revealed that ER stress can intensify a variety of inflammatory and stress signaling pathways to aggravate metabolic derangement, leading to obesity, insulin resistance, fatty liver, and dyslipidemia (Fu et al., 2012). In addition to β cells, hepatocytes and adipocytes also significantly contribute to glucose and lipid homeostasis in the body.

ER stress is linked with hepatic steatosis, which is due to either enhanced lipogenesis or decreased hepatic lipoprotein secretion. Overexpression of the protein chaperone BiP in the liver, as what may occur upon activation of the UPR, inhibited activation of the central lipogenic regulator-sterol regulatory element binding protein (SREBP-1c), alleviated hepatic steatosis, and improved glucose homeostatic control in obese mice (Kammoun et al., 2009). ER stress also inhibits hepatic lipoprotein secretion (Ota et al., 2008). Although disruption of any single arm of the UPR aggravated steatosis under pharmacologically induced ER stress, it is not known whether this resulted from increased hepatic lipogenesis or decreased lipoprotein secretion (Rutkowski et al., 2008; Zhang et al., 2011). XBP1s also regulates fatty acid synthesis by inducing expression of critical lipogenic enzymes, including stearoyl-CoA desaturase-1 (Lee et al., 2008). Interestingly, XBP1s interacts with the Forkhead box O1 (FoxO1) transcription factor and the regulatory subunits of PI3K, p85α, and p85β to decrease hepatic gluconeogenesis (Park et al., 2010; Zhou et al., 2011). However, only hypolipidemia, but neither hypoglycemia nor hyperglycemia, was observed in *Xbp1* liver-deleted mice, suggesting that the regulatory role of XBP1 in hepatic metabolism is primarily to maintain lipid, and not glucose, homeostasis.

CREBH, a liver-specific component of the UPR, was originally identified as a central regulator of the acute phase response, a finding that first linked ER stress with innate systemic inflammatory responses (Zhang et al., 2006). Although it was recognized that metabolic control and inflammation were intimately connected (Reddy and Rao, 2006), a mechanism was lacking. As part of a transducer of inflammatory responses in the liver, CREBH was recently demonstrated to also regulate hepatic lipogenesis, fatty acid oxidation, and lipolysis under conditions of metabolic stress (Zhang et al., 2012). In addition, CREBH regulates hepatic VLDL-triglyceride clearance in the plasma by controlling the activity of lipoprotein lipase (Lpl) through up-regulating genes encoding Lpl coactivator apolipoproteins C2, A4, and A5, respectively, and down-regulating the Lpl inhibitor Apoc3 (J.H. Lee et al., 2011). The identification of CREBH as a stress-inducible metabolic regulator is likely significant because multiple nonsynonymous mutations in CREBH produce defective CREBH proteins that were reported in humans with extreme hypertriglyceridemia (J.H. Lee et al., 2011). These findings indicate that CREBH is a molecular link between lipid homeostasis and inflammation. Although CREBH interacts with ATF6α (Zhang et al., 2006), data indicate that they exert opposite effects on gluconeogenesis. ATF6α inhibits hepatic glucose output by competing with CREB for interaction with CRTC2 (Wang et al., 2009), while CREBH promotes gluconeogenic activity in a CRTC2-independent manner via an unknown

mechanism (Lee et al., 2010). In obese (*ob/ob, db/db*) mice, elevated gluconeogenesis was at least in part attributed to decreased levels of ATF6α resulting from chronic ER stress in obese livers (Wang et al., 2009).

Adipocyte differentiation is a crucial step in body weight gain. UPR activation including eIF2α phosphorylation and splicing of *Xbp1* mRNA was detected during adipogenesis. In addition, attenuation of ER stress by treatment with the chemical chaperone 4-phenylbutyrate (4-PBA) inhibits adipogenesis (Basseri et al., 2009). Thus, the ER stress–induced UPR appears to be a stimulus for adipogenesis that requires the IRE1α–XBP1 pathway to enhance the expression of the key adipogenic factor C/EBPα (Sha et al., 2009). On the other hand, CHOP inhibits adipogenesis by interfering with C/EBPα action (Batchvarova et al., 1995). Therefore, the two arms of the UPR apparently exert opposite effects on adipogenesis, raising the question as to how the UPR coordinates adipocyte differentiation in vivo. Further studies are required to address this issue.

Accumulating evidence indicates that ER stress contributes to the development of insulin resistance in obesity. Treatment of obese and diabetic mice with the chemical chaperones PBA or taurine-conjugated ursodeoxycholic acid (TUDCA) alleviated ER stress–induced activation of c-JUN N-terminal kinase, corrected hyperglycemia, and improved systemic insulin sensitivity (Ozcan et al., 2006). PBA treatment also improved glucose tolerance in insulin-resistant humans (Xiao et al., 2011) and TUDCA improved insulin sensitivity in liver and muscle, but not adipose tissue, in obese men and women (Kars et al., 2010). Heterozygous *Xbp1*-deleted mice develop advanced diet-induced insulin resistance due to unresolved ER stress coupled with a compromised UPR (Ozcan et al., 2006). In contrast, BiP heterozygosity attenuated diet-induced obesity and insulin resistance associated with an activated UPR (R. Ye et al., 2010). However, deletion of BiP in the liver is extremely toxic, creating tremendous ER stress and hyperactivation of the UPR (Ji et al., 2011). Therefore, the UPR may be a binary switch between beneficial and detrimental effects to maintain metabolic homeostasis.

The UPR in infectious and inflammatory disease

The role of the UPR in viral infection was well studied in the last decade. Viruses that express high levels of glycoproteins activate IRE1α and PERK. PERK-mediated eIF2α phosphorylation is a frontline defense to viral replication in the host through repressing viral protein synthesis (Cheng et al., 2005). The role of XBP1s in the immune response was first recognized as its description as an essential transcription factor for the differentiation of mature B cells to plasma cells, where XBP1s expands the ER to support a large amount of immunoglobulin synthesis (Reimold et al., 2001). Interestingly, activation of IRE1α was required not only for B cell differentiation, but also for B lymphopoiesis in the early stages, suggesting IRE1α serves additional functions other than splicing XBP1s early in B cell lymphopoiesis (Zhang et al., 2005). Recently, XBP1 was shown to play a protective role in inflammatory bowel disease. Deletion of *Xbp1* compromised ER protein folding capacity to impair antimicrobial peptide production and elevated mucosal inflammatory signals in the intestine (Kaser et al., 2008). In addition, hypomorphic variants of XBP1 are associated with ulcerative colitis and Crohn's disease in humans (Kaser et al., 2008), suggesting the significance of UPR activation in intestinal epithelial cells. Presently, this is an intense area of investigation (Kaser et al., 2011).

The UPR is also involved in innate immune responses. Toll-like receptor (TLR) 4 and TLR2 specifically trigger phosphorylation of IRE1α leading to splicing of *Xbp1* mRNA (Iwakoshi et al., 2007). This TLR-dependent *Xbp1* mRNA splicing is required for maximal production of proinflammatory cytokines, such as interleukin 6 in macrophages (Martinon et al., 2010). In contrast, TLR signaling inhibits ATF6α and PERK activity as well as signaling through ATF4 and CHOP in macrophages (Woo et al., 2009). Another pathogenic effect of chronic ER stress on activation of inflammatory pathways in macrophages is the progression of atherosclerosis in the settings of dyslipidemia. Deletion of *Chop* lessened advanced lesion macrophage apoptosis and plaque necrosis in both the *Ldlr*−/− and *ApoE*−/− models of atherosclerosis (Thorp et al., 2009). However, the effect of TLR-dependent *Xbp1* mRNA splicing on the progression of atherosclerosis requires further investigation.

The UPR in cancer

The UPR is required for tumor cell growth in a hypoxic environment. Inactivation of the PERK pathway by either generating mutations in the kinase domain of PERK or introducing a phosphorylation-resistant form of eIF2α impairs cell survival under extreme hypoxia (Fels and Koumenis, 2006). PERK also promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage through ATF4 (Bobrovnikova-Marjon et al., 2010). Thus, PERK–phospho-eIF2α–ATF4 signaling is critical for tumor cell proliferation and tumor growth (J. Ye et al., 2010). Although a fusion protein of CHOP, the downstream target of ATF4, with an RNA-binding domain was found in all cases of an adipose cell–based tumor (myxoid liposarcoma; Crozat et al., 1993), the function of CHOP in tumorigenesis remains unknown.

The IRE1α–XBP1 axis of the UPR is also important for tumor cell survival and growth under hypoxic conditions. In a mouse glioma model, IRE1α inhibition decreased tumor growth and reduced angiogenesis and blood perfusion, which correlated with increased overall survival in glioma-implanted recipient mice (Auf et al., 2010). Deletion of *Xbp1* increased sensitivity to hypoxia-induced cell death and reduced tumor formation (Fujimoto et al., 2007). IRE1α–XBP1 transcriptional induction of proangiogenic factors, such as vascular endothelial growth factor, was suggested to promote tumorigenesis (Ghosh et al., 2010). Inhibiting the IRE1α–XBP1 axis may be a promising approach for anticancer therapy (Koong et al., 2006). Treatment with STF-083010, a selective inhibitor of the IRE1α RNase activity, demonstrated significant antimyeloma activity in human multiple myeloma xenografts (Papandreou et al., 2011). MKC-3946, another small molecule that inhibits IRE1α-mediated XBP1 splicing, was also reported to strongly suppress multiple myeloma cell growth in vivo (Mimura et al., 2012). In addition, ATF6α plays a pivotal

Table 1. **Physiological functions of UPR components in mouse models and their genetic association with human disease**

Gene	Factors that regulate expression	Phenotypes of knockout mouse model	Genetic association with human diseases	References
IRE1α	N.A.	(1) Embryonic lethality at E12.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia	(1) Human somatic cancers	Zhang et al., 2005, 2011; Greenman et al., 2007
XBP1s	XBP1s and ATF6α	(1) Embryonic lethality at E13.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia; (3) Intestinal epithelial cell deletion: enteritis; (4) Pancreatic acinar cell deletion: extensive pancreas regeneration; (5) Pancreatic β cell deletion: hyperglycemia; (6) Neuron deletion: leptin resistance	(1) Inflammatory bowel disease; (2) Schizophrenia in the Japanese population; (3) Bipolar disorder; (4) Ischemic stroke	Kakiuchi et al., 2003b, 2004; Kaser et al., 2008; Yilmaz et al., 2010
ATF6α	N.A.	(1) Susceptible to pharmacologically induced ER stress	(1) Type 2 diabetes and pre-diabetic traits; (2) Increased plasma cholesterol levels	Chu et al., 2007; Wu et al., 2007; Meex et al., 2009
CREBH	PPARα, HNF4α, and ATF6α	(1) Hypoferremia and spleen iron sequestration; (2) Hyperlipidemia; (3) Liver knockdown: fasting hyperglycemia	(1) Extreme hypertriglyceridemia	Zhang et al., 2006; Vecchi et al., 2009; J.H. Lee et al., 2011
PERK	N.A.	(1) Neonatal hyperglycemia	(1) Wolcott-Rallison syndrome; (2) Supranuclear palsy	Delépine et al., 2000; Höglinger et al., 2011
ATF4	CHOP	(1) Delayed bone formation; (2) Severe fetal anemia; (3) Increased insulin sensitivity; (4) Defects in long-term memory	N.A.	Elefteriou et al., 2006; Costa-Mattioli et al., 2007; Yamaguchi et al., 2008
CHOP	ATF4 and ATF6α	(1) Protected from pharmacologically induced ER stress; (2) Protected from type 2 diabetes; (3) Protected from atherosclerosis; (4) Protected from leukodystrophy Pelizaeus-Merzbacher disease	(1) Early-onset type 2 diabetes in Italians	Oyadomari et al., 2002; Marciniak et al., 2004; Silva et al., 2005; Gragnoli, 2008; Song et al., 2008
WFS1	XBP1s	(1) Diabetes due to insufficient insulin secretion; (2) Growth retardation	(1) Wolfram syndrome; (2) Risk of type 2 diabetes in Japanese and European populations	Karasik et al., 1989; Inoue et al., 1998; Ishihara et al., 2004; Mita et al., 2008
ORMDL3	N.A.	N.A.	(1) Ulcerative colitis; (2) Risk of childhood asthma	Hjelmqvist et al., 2002; Breslow et al., 2010; McGovern et al., 2010
Grp78 (BiP)	ATF6α and ATF4	(1) Embryonic lethality at E3.5 due to impaired embryo peri-implantation; (2) Liver deletion: simultaneous liver damage and hepatic steatosis	(1) Bipolar disorder	Kakiuchi et al., 2005; Luo et al., 2006; R. Ye et al., 2010
SIL1	XBP1s	(1) Adult-onset ataxia with cerebellar Purkinje cell loss	(1) Marinesco-Sjogren syndrome; (2) Alzheimer's disease	Tyson and Stirling, 2000; Anttonen et al., 2005; Zhao et al., 2005, 2010
Grp94	XBP1s, ATF6α, and ATF4	(1) Embryonic lethality at E7; (2) B cell deletion: reduced antibody production; (3) Bone marrow deletion: hematopoietic stem cell expansion	(1) Bipolar disorder	Kakiuchi et al., 2007; Mao et al., 2010
P58 IPK	XBP1s and ATF6α	(1) Diabetes	N.A.	Ladiges et al., 2005
Calnexin	XBP1s and ATF6α	(1) Postnatal death; (2) Motor disorder	N.A.	Denzel et al., 2002
Calreticulin	XBP1s and ATF6α	(1) Embryonic lethality at E14.5	(1) A case of schizophrenia	Aghajani et al., 2006
Selenoprotein S	N.A.	(1) Disturbed redox homeostasis in the liver and cataract development in eyes	(1) Inflammatory response; (2) Non-small cell lung cancer	Hart et al., 2011; Kasaikina et al., 2011

N.A., not applicable.

survival role for dormant tumor cells through activation of mTOR signaling (Schewe and Aguirre-Ghiso, 2008). Increased expression of BiP/GRP78, which is primarily regulated by ATF6α, correlates with chemotherapeutic resistance and is observed in aggressive cancers (Lee, 2007).

Most significantly, the success of proteasome inhibition with bortezomib in multiple myeloma (Dimopoulos et al., 2011) supports the notion that targeting protein homeostasis may be therapeutic in a number of cancers that are associated with excessive expression of secretory proteins, such as epithelial

tumors. Because the UPR is highly activated in cancer cells, chemotherapeutic agents that cause ER stress such as brefeldin A, bortezomib (Velcade), and geldanamycin could be effective by exacerbating UPR activation to activate apoptosis in cancer cells (Nawrocki et al., 2005; Healy et al., 2009). Therefore, the proapoptotic effects of the UPR may be harnessed as a means to treat cancer.

The UPR in neurodegenerative disorders

In contrast to the indispensable role of the UPR in secretory cells, its function in the physiology of the nervous system is not fully understood. Studies in *Xbp1*-null neurons revealed that XBP1s regulates the induction of GABAergic markers including somatostatin, neuropeptide Y, and calbindin through brain-derived neurotrophic factor signaling to control the neurite extension (Hayashi et al., 2007, 2008). A polymorphism in the *XBP1* promoter was linked to a risk factor for bipolar disorder and schizophrenia (Kakiuchi et al., 2003a). Interestingly, translational control of ATF4 mediated by GCN2–eIF2α phosphorylation appears important for hippocampal synaptic plasticity and memory (Costa-Mattioli et al., 2005). Targeting inactivation of ATF4 can enhance synaptic plasticity and memory storage (Chen et al., 2003). Nevertheless, the function of ATF6 and PERK–eIF2α phosphorylation in the nervous system remains to be determined.

Although the role of the UPR in the nervous system remains speculative, activation of the UPR is observed in a number of neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, prion-related disorders, and Alzheimer's disease, and demyelinating neurodegenerative autoimmune diseases such as multiple sclerosis, Pelizaeus-Merzbacher disease, and transverse myelitis (Doyle et al., 2011; Matus et al., 2011). Interestingly, the pathogenic contribution of the UPR is highly specific in different disease models. *Chop* deletion shortened lifespan and increased oligodendrocyte death in mice with Pelizaeus-Merzbacher disease, whereas *Chop* deletion attenuated neurotoxin-induced Parkinson's disease (Gow and Wrabetz, 2009). In addition, deletion of *Xbp1* delayed ALS disease onset and increased life span due to an increase in autophagy (Hetz et al., 2009), whereas deletion of *Xbp1* did not affect neuronal loss or animal survival in a prion-related disorder disease mouse model (Hetz and Soto, 2006). Thus, the importance of the UPR in neurodegeneration appears disease specific, which introduces challenges to study the functional significance of ER stress in the pathogenesis of these disorders.

Future perspectives

Although by no means exhaustive, Table 1 summarizes the physiological functions of the UPR components in mouse models and the genetic association of these components with human disease. The UPR contains considerable sensitivity and flexibility to exquisitely regulate ER activity and adapt cells to different physiological conditions. As the phase of the UPR shifts from a protective stage to proapoptotic, the UPR commits cells to death, which can concurrently intersect with inflammatory signaling pathways to either initiate or exacerbate pathogenic states. Despite tremendous progress in understanding the physiological significance of the UPR as well as the cross talk between the UPR, metabolic, inflammatory, and other signaling pathways, real-time analysis of protein folding in the ER and UPR activation has only been performed in yeast (Merksamer et al., 2008). Thus, the mechanisms involved in stimulating and sustaining UPR signals in the pathogenesis of different diseases is still unknown. Further studies on identifying these mechanisms will greatly facilitate approaches to modulate UPR activity to reach a desired therapeutic benefit.

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