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Endoplasmic Reticulum Stress and Type 2 Diabetes

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Abstract

Given the functional importance of the endoplasmic reticulum (ER), an organelle that performs folding, modification, and trafficking of secretory and membrane proteins to the Golgi compartment, the maintenance of ER homeostasis in insulin-secreting β -cells is very important. When ER homeostasis is disrupted, the ER generates adaptive signaling pathways, called the unfolded protein response (UPR), to maintain homeostasis of this organelle. However, if homeostasis fails to be restored, the ER initiates death signaling pathways. New observations suggest that both chronic hyperglycemia and hyperlipidemia, known as important causative factors of type 2 diabetes (T2D), disrupt ER homeostasis to induce unresolvable UPR activation and β -cell death. This review examines how the UPR pathways, induced by high glucose and free fatty acids (FFAs), interact to disrupt ER function and cause β -cell dysfunction and death.

Keywords

unfolded protein response; ER stress; free fatty acid; glucose; pancreatic β -cell

INTRODUCTION

Modern lifestyles, characterized by the overconsumption of energy-rich foods and reduced physical activity, have dramatically increased the frequency of type 2 diabetes (T2D). T2D is a major cause of morbidity and mortality, decreasing both the quality of life and life expectancy. In 2010, diabetes affected about 285 million people worldwide and is expected to increase by 1.5 fold (439 million people) by 2030 (1). Between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries. T2D is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose owing to insulin resistance in adipose tissue, muscle, and liver and/or to impaired insulin secretion from pancreatic β -cells (2). Obesity is mechanistically linked to insulin resistance and T2D. Although both obesity and insulin resistance are associated with T2D, the disease only develops in insulin-resistant subjects with the onset of β -cell dysfunction (3). To adapt to the increased metabolic load caused by obesity and insulin resistance, normal pancreatic islets usually respond by increasing β -cell mass through an increase in β -cell proliferation and neogenesis (4) as well as by enhancing β -cell function (5). However, β -cell adaptation eventually fails as a

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consequence of genetic and environmental factors that cause a progressive decline in β -cell function and survival. As a consequence, individuals progress from normal glucose tolerance to impaired glucose tolerance and then to established T2D with reduced β -cell mass (3). Although the molecular mechanisms underlying β -cell failure/death remain to be clarified, recent studies suggest that β -cell loss in T2D results from endoplasmic reticulum (ER) stress responses induced by gluco/lipotoxicity (6) and amyloid accumulation (7). In this review, we examine the mechanisms behind UPR-mediated pancreatic cell death with specific attention to the connection between ER stress and T2D.

PANCREATIC β -CELL AND THE ENDOPLASMIC RETICULUM

The islets of Langerhans constitute ~2% of the total pancreatic mass, and each islet is composed of ~2,000 cells of which insulin-secreting β -cells constitute ~60% of the total (8). Insulin, a blood glucose-lowering hormone, is only secreted by the pancreatic β -cells of the islets of Langerhans. After proinsulin is synthesized in the ER, it is processed into its biologically active form and stored in secretory granules. When blood glucose levels increase, insulin is released from storage granules to maintain a normal blood glucose level (9). The β -cell contains a large pool of cytoplasmic proinsulin mRNA (~20% of the total mRNA), one of the most abundant mRNA species (10). One profound example of physiological fluctuation in the protein-folding load in the ER is the unique translational response of pancreatic β -cells to variations in blood glucose (11). Blood glucose level changes lead to a maximum 25-fold increase in proinsulin synthesis, and $\sim 1 \times 10^6$ proinsulin molecules are synthesized per minute upon glucose stimulation (12). Therefore, proinsulin synthesis imposes a heavy biosynthetic burden upon the β -cell.

The ER is the first station of the secretory pathway and the site of synthesis for proteins resident in the ER or destined for the Golgi compartment, endosomes, lysosomes, the plasma membrane, or the extracellular milieu (13). It also serves as a site of biosynthesis for steroids, cholesterol, other lipids, and Ca^{2+} storage, and also as a signaling platform between the nucleus and cytosol (14). Efficient ER function relies on numerous resident quality control factors, such as molecular chaperones and folding enzymes, a high level (several hundred micromolars) of Ca^{2+} , and an oxidative environment. Nascent polypeptides that are translocated into the ER lumen undergo posttranslational modifications and rounds of folding interactions required for optimal function. Properly folded proteins exit the ER and progress through the secretory pathway, whereas irreparably unfolded or misfolded proteins are retro-translocated from the ER and degraded by cytoplasmic proteasomes (15). The flux of nascent polypeptides into the ER is variable because it changes rapidly in response to the physiological state and environmental conditions of the cell. To handle this dynamic situation, cells must adjust their ER protein-folding capacity according to environmental and physiological contexts, thereby ensuring that the quality of membranous and secreted proteins is maintained with high fidelity. Given the importance of ER function for normal cellular function, it is not surprising that altered ER homeostasis affects a diverse number of cellular processes, including transcription, translation, cell cycle control, intracellular signaling, and programmed cell death. When ER homeostasis is altered, signaling pathways are activated, eliciting an adaptive response that is collectively called the unfolded protein response (UPR). The UPR includes expansion of ER size, enhanced folding capacity, reduced protein synthesis through transcriptional and translational controls, and increased clearance of unfolded or misfolded proteins (16). When these mechanisms fail to restore ER homeostasis, cell death signaling pathways are activated (17). There are three transmembrane ER-proximal sensors of unfolded proteins that initiate UPR signaling in mammals: inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK); all three are present in all cell types (Figure 1). These three main UPR sensors are integral membrane proteins that sense the unfolded protein loaded in

the ER lumen and transmit this information across the ER membrane to the cytosol. Considerable progress has been made in our understanding of the signaling pathways and the pathophysiological significance of the UPR. The goal of this review is to summarize our current insight by considering the role of the UPR in T2D as a disease in which there is a structural and pathological change in β -cells.

SIGNALING FROM TRANSMEMBRANE SENSORS OF THE UNFOLDED PROTEIN RESPONSE

Inositol-Requiring Protein 1–Mediated Signaling Pathways

There are two mammalian homologs of yeast Ire1p: IRE1 α , expressed in most cells and tissues with high levels of mRNA expression in the pancreas (18), and IRE1 β , mainly expressed in the epithelium of the gastrointestinal tract (19). IRE1 α , the most fundamental ER stress sensor, is highly conserved in all eukaryotic cells and is well studied (16). The luminal domain of the IRE1 α protein responds to the accumulation of unfolded proteins in the ER and undergoes kinase activation, which triggers a specific endoribonuclease activity in its cytoplasmic domain, where initiation of splicing of the mRNA encoding X-box binding protein 1 (XBP1) occurs (20). β -Cell-specific deletion of *Xbp1* causes β -cell failure in a mouse model, suggesting that IRE1 α -XBP1 signaling is essential for β -cell function (21). Figure 1 provides a schematic view of the IRE1 α -mediated signaling pathway, including the other UPR pathways.

PKR-Like Endoplasmic Reticulum Kinase-Mediated Signaling Pathways

PERK, a type I transmembrane protein located in the ER, has serine/threonine kinase activity in its cytoplasmic domain (Figure 1) (22, 23). The catalytic domain of PERK shares substantial homology with other eIF2 α family kinases (GCN2, HRI, and PKR) (24). Approximately half of *Perk*-null mice, a mouse model of the human genetic disease Wolcott-Rallison syndrome (25), die pre- or postnatally. The surviving half of *Perk*-null mice gradually develop a multitude of metabolic and growth abnormalities, including hyperglycemia, growth retardation, skeletal defects, and atrophy of the exocrine and endocrine pancreas (26, 27). Different from *Perk*-deficient mice, all eIF2 α A/A homozygous neonates die within 24 h after birth and suffer from hypoglycemia, possibly owing to defective gluconeogenesis [low phosphoenolpyruvate carboxykinase (PEPCK) activity], reduced glycogen storage in the liver, and a deficiency of pancreatic β -cells (28). For β -cell function, PERK activity is required to prevent an abnormal increase in insulin translation in islet cells responding to a high-glucose load (26), but eIF2 α phosphorylation is required to control insulin translation in response to both low- and high-glucose conditions (29). Furthermore, it seems that PERK through eIF2 α phosphorylation is a positive regulator of ER chaperone and ER-associated degradation (ERAD) function and thereby contributes to ER and Golgi anterograde trafficking, retrotranslocation from the ER to the cytoplasm, and proteasomal degradation (29–31). Therefore, *Perk* and/or eIF2 α phosphorylation-deficient β -cells show retention of misfolded proinsulin in the ER lumen and defective trafficking of proinsulin, and thereby a reduced number of insulin granules in β -cells, indicating that the mutant β -cells experience ER stress, accompanied by increased cell death, leading to progressive diabetes.

In pancreatic β -cells, the extracellular glucose level modulates the activity of the UPR sensors. PERK phosphorylation is differentially regulated by glucose in the β -cell. In β -cell, eIF2 α phosphorylation is gradually decreased with the increase of glucose levels. Its phosphorylation inversely correlates with the rate of proinsulin synthesis (32). However, both low blood glucose and chronic high blood glucose activate eIF2 α phosphorylation. Chronically high-glucose concentrations stimulate proinsulin transcription and translation.

As a consequence, it is believed that proinsulin synthesis overcomes the ER folding machinery, leading to PERK activation to reduce protein influx into the ER (33). However, there remains some controversy whether chronically high-glucose exposure (more than 18 h) actually causes severe ER stress, activating PERK (33, 34). Yet glucose stimulation of β -cells growing in acute high glucose causes eIF2 α dephosphorylation, likely through a protein phosphatase 1 (PP1)-like phosphatase (32) that dephosphorylates eIF2 α . Although this kinase/phosphatase model can easily explain the changes in eIF2 α phosphorylation in response to glucose, it is not known how PP1 is regulated under these conditions. The kinase responsible for low-glucose eIF2 α phosphorylation has not been identified (31). It is most likely that PERK is the kinase that phosphorylates eIF2 α in low glucose. This is supported by studies from several groups, including Gomez and colleagues (35). Moreover, Gomez et al. (35) propose that PERK may sense levels of cellular ATP/energy in pancreatic β -cells. It has been shown that PERK, but not IRE1 α , is activated by a decrease in glucose concentration or intracellular energy level induced by mitochondrial inhibitors (35). Therefore, it is possible that PERK in pancreatic β -cells is also activated by a mechanism independent of IRE1 α activation or by the unfolded protein accumulation. It was also reported that a decrease in glucose concentration leads to a concentration-dependent reduction in ER Ca²⁺ that parallels the activation of PERK and the phosphorylation of eIF2 α . It was proposed that an ER Ca²⁺ decrease is caused by a decrease in SERCA activity, mediated by a reduction in its cell energy status (154). However, this study did not suggest a precise mechanism that described why IRE1 α is not activated by an ER Ca²⁺ decrease, which is induced by low glucose, although it is possible that PERK and IRE1 α may have different thresholds for activation in response to a decrease in ER Ca²⁺. Clearly, further studies are required to elucidate the precise molecular mechanisms involved in energy/glucose-dependent regulation of eIF2 α phosphorylation and its biological meaning.

It has been suggested that the cytosolic function of PERK is also controlled by P58^{IPK}, first identified as a PKR inhibitor (36, 37). A more recent study (38), however, suggested that P58^{IPK} localizes mainly to the ER lumen and functions as a molecular cochaperone for BiP in the ER lumen. Therefore, if P58^{IPK} is a major regulator of PERK function, it is likely through some chaperone function (38). Thus, the precise inactivation mechanism of PERK remains to be clarified.

Activating Transcription Factor 6–Mediated Signaling Pathways

ATF6 encodes a basic leucine zipper protein (bZIP)-containing transcription factor localized to the ER membrane (39). Upon accumulation of unfolded protein in the ER, ATF6 traffics to the Golgi complex, where it is cleaved by site 1 and site 2 proteases, S1P and S2P, in a process called regulated intramembrane proteolysis. In mammals, there are two *Atf6* genes, *Atf6a* and *Atf6 β /creb-1p/g13* (40). Figure 1 provides a schematic view of the ATF6-mediated signaling pathway. The deletion of *Atf6a* resulted in increased sensitivity to chronic stress in mice challenged with chemical ER stressors, and it was suggested that ATF6 α is required to optimize protein folding, secretion, and degradation during ER stress and thus facilitates recovery from acute stress and confers tolerance to chronic stress (41, 42). Unlike ATF6 α , the role(s) for ATF6 β is still unknown. Moreover, ATF6 β (like ATF6 α) is dispensable in embryonic and postnatal development, and a deficiency of ATF6 β does not alter UPR gene induction (42). However, the double knockout of *Atf6a* and *Atf6 β* causes early embryonic lethality, suggesting that ATF6 α and ATF6 β possess at least overlapping functions that are essential for mouse development. ATF6 α and ATF6 β are ubiquitously expressed (40). However, in recent years, several tissue-specific bZIP transcription factors located in the ER membrane and regulated by regulated intramembrane proteolysis have been identified: CREBH in hepatocytes, the pyloric stomach, and small intestine (43); OASIS in astrocytes (44); BBF2H7/CREB3L2 in most tissues (45); Tisp40

(transcript induced in spermatogenesis) in testis (46); Luman/LZIP/CREB3 in most tissues (47, 48); and CREB4 possibly in most tissues (49). The discovery of these numerous regulated intramembrane proteolysis-activated transcription factors raises a question: Why have cells evolved multiple ATF6-like molecules in the ER? These ATF6-like molecules might be evolutionally chosen to respond to specific conditions of ER stress that can occur in different tissues to activate tissue-specific expression of genes to resolve ER stress. Further studies are required to answer these issues.

Wolfram syndrome (which is caused by mutations in the *Wfs1* gene, encoding wolframin) is a disorder of progressive neurodegeneration and β -cell failure leading to diabetes. Wolframin is a transmembrane ER protein involved in preventing ER stress signaling. Recently, Fonseca et al. (50) reported that WFS1 negatively regulates ATF6 α through the ubiquitin-proteasome pathway during ER stress. In the absence of ER stress or in late stages of ER stress, WFS1 may limit ER stress by recruiting ATF6 α to the E3 ubiquitin ligase, HRD1, for ubiquitination-mediated degradation of ATF6 α . Therefore, *Wfs1*-null murine pancreata display higher levels of ATF6 α protein and lower levels of Hrd1 protein compared with those in the control littermates. Additionally, another study suggested that the ectopic overexpression of an active form of ATF6 α in β -cells causes apoptosis (51). These results imply that dysregulated ER stress signaling through hyperactivation of ATF6 α is one pathogenic pathway for diseases involving chronic, unresolvable ER stress, such as pancreatic β -cell death in diabetes. Therefore, understanding the role of ATF6 α in diabetic mouse models is an important step toward understanding the physiological roles of ATF6 α in pancreatic β -cells.

UNFOLDED PROTEIN RESPONSE-MEDIATED CELL DEATH

Activation of each arm of the UPR initiates adaptive mechanisms to relieve the stresses accumulating in the ER. The adaptive UPR responses, signaled through activating transcription factor 4 (ATF4), cleaved ATF6 α , and spliced XBP1, include inhibition of general mRNA translation by rapid PERK-mediated phosphorylation of eIF2 α as well as induction of genes to increase the ER protein folding capacity and remove misfolded proteins from the ER. In 2006, it was recognized that preemptive quality control may also reduce the burden of misfolded substrates entering the ER by inhibiting translocation of many, but not all, polypeptides into the ER lumen (52). Thus, the adaptive pathways maintain cellular function and avoid apoptosis during ER stress. However, if ER stress is severe and chronic, the UPR-mediated efforts to correct the protein-folding defect fail, and then several apoptotic pathways are activated (53). Although both mitochondrial-dependent and -independent cell death pathways (17) execute apoptosis in response to ER stress, it is proposed that the ER serves as an important compartment where apoptotic signals are generated and integrated to elicit cell death in response to the unresolvable accumulation of unfolded proteins. Below, we briefly describe two UPR sensor-mediated cell death pathways.

PERK-Mediated Cell Death Pathways

During unresolvable ER stress, sustained activation of the PERK pathway also induces the proapoptotic pathway, similar to IRE1 α (Figure 2). There are no reports that show PERK association with adaptors or modulators involves apoptosis. The persistent phosphorylation of eIF2 α by PERK increases proapoptotic CHOP/GADD153 (*C/EBP* homology protein/growth arrest and DNA damage 153) expression through the transcription factor ATF4. CHOP is a member of the *C/EBP* family of bZIP transcription factors (54). The major inducers of the UPR (ATF4, ATF6, and XBP1) regulate *Chop* through both an ER stress response element and a *C/EBP*-ATF composite site (55–57). *Perk*- and *Atf4*-null cells, as well as *eIF2 α* (*S51A*) knock-in cells, fail to induce CHOP (28, 58, 59), whereas *IRE1 α* -

Xbp1-, and *Atf6a*-null cells or mouse liver tissues show increased and/or persistent *Chop* expression during ER stress (41, 42, 60, 61), suggesting that the PERK-eIF2 α -ATF4 pathway is the main contributor toward ER stress-dependent CHOP expression. The deletion of the murine *Chop* gene attenuates ER stress-induced cell death in cultured fibroblasts and partially protects mice from renal toxicity owing to pharmacological induction of ER stress by tunicamycin (62). Furthermore, *Chop* deletion (a) prevents neuronal apoptosis induced by ischemia (63) and neuronal oxidative injury in a model of Parkinson's disease (64) and (b) protects the murine liver from ER stress and oxidative damage induced from clotting factor VIII expression (65), which is prone to mis-folding and pancreatic β -cell death resulting from either accumulation of misfolded mutant proinsulin or exposure to nitric oxide (66, 67). *Chop* deletion even promotes β -cell survival in multiple diabetic mouse models induced by a leptin receptor deficiency or a high-fat diet treatment with haplo-insufficiency of eIF2 α phosphorylation (68). The mechanism by which CHOP-mediated apoptosis occurs in response to ER stress is not well established. It was suggested that overexpression of CHOP decreases expression of the antiapoptotic Bcl-2 protein, depletes cellular glutathione, and exaggerates production of reactive oxygen species (ROS) (69, 70). However, a study of *Chop*-deleted cells revealed a different view of CHOP-mediated cell death. CHOP directly activates *Gadd34*, which promotes ER client protein biosynthesis by dephosphorylating eIF2 α in stressed cells, and causes further accumulation of high-molecular-weight detergent-resistant stress-associated ER complexes in the ER (Figure 2) (70). Therefore, impaired GADD34 expression reduces client protein load and ER stress in *Chop*-null cells exposed to ER stress. The *Chop*- and *Gadd34*-null mutant cells accumulate fewer high-molecular-weight protein complexes in their stressed ER than wild-type cells. Furthermore, ERO1 α (ER oxidoreductase a), a direct CHOP target gene, causes hyperoxidation of the ER to increase abnormal high-molecular-weight protein complexes. Thus, CHOP may disrupt ER function by promoting protein synthesis and oxidation.

Increasing evidence suggests that an accumulation of misfolded proteins in the ER lumen generates ROS (65, 71, 72). Formation of incorrect intermolecular and/or intramolecular disulfide bonds depletes glutathione that is required for their reduction. Disulfide bond formation in the ER is ushered in by the oxidative folding pathway, catalyzed by protein disulfide isomerase (PDI) and ERO1-mediated oxidation of substrate polypeptides, that produces ROS (Figure 3). For example, overexpression of a misfolded protein CPY (yeast vacuolar protein carboxypeptidase Y) activates the UPR, causes oxidative stress, and induces apoptosis. However, removal of all cysteine residues in CPY reduced the UPR, oxidative stress, and cell death (73). Furthermore, in macrophages, cholesterol and ER stress inducers oxidize inositol-1,4,5-trisphosphate (IP $_3$) receptors in the ER membrane to release Ca $^{2+}$ and activate Ca $^{2+}$ /calmodulin-dependent protein kinase II γ (CaMKII γ) (74). This study suggested also that there are three CaMKII γ -mediated apoptotic pathways: (a) CaMKII γ -mediated c-Jun N-terminal kinase (JNK) activation; (b) CaMKII γ -increased mitochondrial Ca $^{2+}$; and (c) CaMKII γ activation of STAT1, a proapoptotic signal transducer (74). These hypotheses are supported by the finding that either *Chop* or *Ero1a* deletion in yeast and mice reduces ROS accumulation and protects from ER stress-mediated cell death.

Recent studies demonstrated significant increases in ER stress marker proteins P58^{IPK}, BiP, and CHOP in islets in tissue sections from obese diabetic individuals (T2D patients) (75). Additionally, analysis of leptin receptor-deficient (*Lepr^{db/db}*) mice, as well as other murine models of T2D, revealed that insulin resistance increases proinsulin synthesis in β -cells beyond the capacity for folding of nascent polypeptides within the ER lumen, thereby disrupting ER homeostasis and triggering the UPR (68). The pancreatic β -cells in the *Lepr^{db/db}* mice displayed slightly increased expression of several UPR genes encoding adaptive functions to improve ER folding capacity, such as *BiP*, *Grp94*, *Fkbp11*, and *P58^{IPK}*, as well as accumulated oxidative damage. By contrast, the deletion of *Chop* gene

increased expression of UPR and oxidative stress response genes and reduced levels of oxidative damage, such as products of protein oxidation (carbonyls) and lipid peroxidation (hydroxyoctadecadienoic acid). These findings suggest that CHOP can be a fundamental factor linking protein mis-folding in the ER to oxidative stress and apoptosis in β -cells under conditions of increased insulin demand, such as in T2D. Therefore, small molecules that modulate the expression or transcriptional activity of CHOP may be useful to alleviate ER stress-mediated cell death.

IRE1 α -Mediated Cell Death Pathways

Studies have suggested that the IRE1 α / TRAF2/ASK1 complex promotes apoptosis (Figure 2) through JNK phosphorylation (76). Although polyglutamine aggregates are typically cytoplasmic, it was suggested that polyglutamine inhibits the proteasome and induces ER stress. It was demonstrated that *Ask1*-null primary neurons are resistant to ER stress-induced cell death (77). Activated ASK1 leads to JNK-mediated phosphorylation and activation of the proapoptotic protein Bim (78), but inhibits the antiapoptotic protein Bcl-2 (79). Furthermore, a cell-based chemical library screen identified compounds that enhance phosphorylation of serine 967 of ASK1, promoting 14-3-3 protein binding and thereby suppressing ASK1 function (80). The deficiency of ER-localized proapoptotic Bcl-2 family members BAX and BAK (81), ASK1-interacting protein 1 (AIP1) (82), or protein tyrosine phosphatase-1B (PTP-1B) (83) in cells and mice impairs IRE1 α activation, thereby attenuating *Xbp1* splicing, JNK phosphorylation, expression of XBP1 target genes, and ER stress-induced apoptosis. Conversely, BAX inhibitor-1 -deficient cells exhibit hyperactivation of IRE1 α associated with increased *Xbp1* mRNA splicing and upregulation of XBP1s-dependent genes, activation of JNK, and increased cell death (84). On the basis of these compelling data, we speculate that the IRE1 α UPRosome initiates multiple signaling responses through interaction with adaptors and modulators in a highly regulated manner. However, more studies are needed to define the physiological significance of these findings.

Recent reports suggest that during unresolvable ER stress, hyperactivation of IRE1 α 's RNase causes endonucleolytic decay of many ER-localized mRNAs, including those encoding chaperones, thereby culminating in cellular dysfunction and the death of β -cells as well as other cell types (85, 86). This process was termed regulated IRE1-dependent RNA degradation. It was proposed that the RNase of IRE1 α can yield different outputs in RNA cleavage depending on the conditions and/or the intensity of ER stress (85). Under conditions of low-level ER stress or artificial dimerization of IRE1 α without ER stress, IRE1 α mainly cleaves unspliced *Xbp1* mRNA to promote an adaptive response, whereas persistent and/or strong activation causes IRE1 α to cleave both unspliced *Xbp1* mRNA and ER-targeted mRNAs, including insulin. These findings suggest (a) that chronic exposure of β -cells to high glucose causes ER stress and hyperactivation of IRE1 α , leading to degradation of insulin mRNA; and (b) that IRE1 α kinase/endoribonuclease can function as an apoptotic switch in response to persistent ER stress.

ENDOPLASMIC RETICULUM STRESS STIMULI AND β -CELL DEATH IN TYPE 2 DIABETES

Obesity is nearly invariably associated with insulin resistance, but T2D only develops in genetically predisposed and insulin-resistant subjects with the onset of β -cell dysfunction (3). Pancreatic β -cell failure and loss of islet mass are the primary determinants in the pathogenesis of T2D. Many mechanisms for β -cell dysfunction and death in T2D have been proposed, including lipotoxicity, glucotoxicity, oxidative stress, amyloid deposition, and others. There is growing evidence that β -cell failure and death are caused by unresolvable ER stress, leading to chronic and/or strong activation of IRE1 α and/or PERK. Indeed, ER

stress alone can initiate and propagate all the characteristics of β -cell failure and death observed in T2D (28). Here, we review how insulin resistance and obesity may cause ER stress in the β -cell leading to T2D.

Endoplasmic Reticulum Stress by Lipotoxicity

Many studies suggest that high-fat diets and obesity are associated with elevated levels of plasma free fatty acids (FFAs) (2). FFAs are now considered as important mediators of β -cell dysfunction and apoptosis in T2D (3). Long saturated FFAs, such as palmitate, mediate apoptotic β -cell death in vivo and in vitro (87, 88), although both unsaturated and saturated FFAs eventually inhibit proinsulin synthesis and glucose-stimulated insulin secretion in β -cells (89). However, the precise mechanisms causing β -cell dysfunction and apoptosis by saturated FFAs (called lipotoxicity) are not fully understood. Accumulating evidence suggests that saturated long-chain FFAs induce ER stress and thereby cause β -cell failure and cell death, whereas unsaturated long-chain FFAs induce it to a lesser extent (6) and may even protect against these processes in some instances. The mechanisms by which saturated FFAs activate ER stress signaling pathways are also unclear. Several reports demonstrated that palmitate treatment of β -cells and/or islets activates the PERK pathway, including expression of ATF4 and CHOP by eIF2 α phosphorylation (Figure 4) (6). However, detection of IRE1 α activation and spliced *Xbp1* mRNAs' or XBP1s' protein was dependent on the palmitate preparation and/or the β -cell lines used in different laboratories (75, 90, 91). Further studies are required under physiologically relevant conditions. There is controversy about whether ATF6 α is activated by palmitate. Although some reports suggest that the ATF6 α pathway is activated by palmitate treatment (75, 92), other studies did not observe an increase in BiP expression induced by ATF6 α activation in palmitate-treated β -cells (90, 93). It is also unknown whether palmitate is a specific inducer for the ATF6 α branch because both palmitate and oleate treatment increased expression of known ATF6 α -target genes, total *Xbp1*, and *BiP/Gip78* (91). The protective effect of BiP overexpression is also controversial in palmitate-treated β -cells (90). These inconsistencies need reconciliation through direct evidence of ATF6 α cleavage and BiP expression in β -cells of palmitate-fed animals. The saturated fatty acid palmitate has multiple deleterious effects on pancreatic β -cells: (a) activation of PKC δ ; (b) accumulation of long-chain acyl-coenzyme As (CoAs) or lipid derivatives, such as diacylglycerol, lysophosphatic acid, and sphingolipids (ceramide and others); and (c) perturbation of ER Ca²⁺ to increase cytosolic Ca²⁺. Below, we discuss to what extent each of these mechanisms contributes to ER stress and β -cell apoptosis.

First, blocking of PKC δ translocation by the phospholipase C inhibitor, U-73122 (94), or direct inhibition of PKC δ by rottlerin (95) substantially reduced palmitate-induced apoptosis. Moreover, overexpression of dominant-negative PKC δ in pancreatic β -cells protected against high-fat diet-induced glucose intolerance and β -cell dysfunction in mice (96). Therefore, it is possible that FFA-induced activation of PKC δ may contribute to β -cell loss in T2D (97). It was recently shown that overexpression of dominant-negative PKC δ inhibited palmitate-induced nuclear accumulation of forkhead box protein O1 (FoxO1) in cultured β -cells and islets (96), although it remains to be confirmed whether apoptosis by activated PKC δ is caused by changes in FoxO1 nuclear localization. In pancreatic β -cells, the FoxO1 transcription factor is implicated in regulating differentiation, proliferation, and apoptosis (98). Furthermore, inhibition of FoxO1, which requires JNK inhibition, protects pancreatic β -cells against FFAs (99). In addition, FoxO1 activity was increased by an ER stress inducer, thapsigargin, and dominant-negative FoxO1 expression protected β -cells from thapsigargin-induced cell death (99). Recently, Qi & Mochly-Rosen (100) reported that during ER stress, PKC δ complexes with c-Abl (a protein tyrosine kinase involved in genotoxic and oxidative stresses) to cause JNK activation (101). Moreover, PKC δ inhibition reduced JNK activation and inhibited ER stress-mediated apoptosis. It is possible that FFAs

or ER stress may activate the PKC δ -JNK pathway to activate FoxO1 directly or through inhibition of AKT-mediated insulin signaling. Therefore, the implication of the PKC δ -JNK-FoxO1 pathway in FFA-induced β -cell apoptosis warrants further investigation. It is also important to address how PKC δ is activated in FFA-treated or ER stress-induced β -cells. It is interesting that hepatic XBP1s, or even DNA-binding-defective mutant XBP1s, bind FoxO1 and promote its degradation, and thereby improve glucose homeostasis in leptin-deficient *ob/ob* T2D model mice (102). This suggests that increased expression of XBP1s in FFA- or ER stress-exposed β -cells may provide a new therapeutic approach to modulate the activity of FoxO1 for the treatment of T2D. However, prolonged XBP1s overexpression interferes with β -cell function via inhibition of insulin, pancreatic duodenal homeobox 1 (PDX1), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) expression, eventually leading to β -cell apoptosis (103). Therefore, this issue requires further investigation.

Second, several studies suggest that the differential toxicity between saturated FFAs and unsaturated FFAs is directly related to their ability to promote triglyceride accumulation and fatty acid oxidation (Figure 4) (104, 105). In β -cells, oleate treatment leads to triglyceride accumulation and is well tolerated, whereas palmitate is poorly incorporated into triglyceride and causes ER stress and apoptosis (105). The lipotoxicity caused by excess oleate in acyl-CoA:diacylglycerol transferase 1 (DGAT1)-deficient fibroblasts (DGAT catalyzes the formation of triglycerides from diacylglycerol and acyl-CoA) (104) emphasizes the importance of a harmonious match between cellular lipid influx and lipid utilization. Moreover, other studies have revealed that overexpression of stearoyl-CoA desaturase 1 (SCD1) to desaturate excess saturated FFAs is sufficient to prevent lipotoxicity of palmitate (104, 106). By contrast, knockdown of SCD in INS-1 β -cells decreased desaturation of palmitate to monounsaturated fatty acid, lowered FFA partitioning into complex neutral lipids, and augmented palmitate-induced ER stress and apoptosis (107). The importance of lipid content in ER function was further suggested in diabetic murine models. Loss of SCD1 worsened diabetes in leptin-deficient obese mice (108). In addition, *Scd1* and *Scd2* mRNA expression was induced in islets from prediabetic hyperinsulinemic Zucker diabetic fatty (ZDF) rats, whereas several fatty acid desaturases, including *Scd1* mRNA levels, were markedly reduced in diabetic ZDF rat islets (107).

Studies using inhibitors (e.g., etomoxir or bromopalmitate) or activators (e.g., T090217) of fatty acid mitochondrial β -oxidation suggest that increased fatty acid oxidation is important to prevent lipotoxicity and ER stress (92, 109). For example, overexpression of the mitochondrial fatty acid transporter carnitine palmitoyltransferase 1 alleviated palmitate-induced apoptosis and decreased expression of ER stress markers, eIF2 α -P and CHOP, in β -cells (110). Fatty acid oxidation and triglyceride formation were most pronounced in oleate-treated β -cells when compared to palmitate (92, 105), although the responsible genes are not known.

Several saturated lipid intermediates (e.g., lysophosphatidic acid, phosphatidic acid, and diacylglycerols) of the saturated FFA esterification pathway (3) and sphingolipids (such as ceramide) de novo synthesized from saturated FFAs (111, 112) were proposed as toxic molecules that can induce β -cell dysfunction and apoptosis. Among these toxic lipid intermediates, studies using proximal inhibitors (such as myriocin or fumonisins B1) of de novo ceramide synthesis suggested that ceramide induces ER stress and cell death in several cell lines, including β -cells (92, 111, 112). Recently, Boslem et al. (113) employed mass spectrometry in a comprehensive lipidomic screen of MIN6 β -cells treated with palmitate. They observed that the major alterations following palmitate exposure were in the sphingolipid class [glucosylceramide (GlcCer), lactosylceramide, trihexosylceramide] without any significant alteration in the amounts of either ceramide or sphingomyelin.

Among the sphingolipid class, the amount of GlcCer changed most significantly, and increased conversion of ceramide to GlcCer by overexpression of GlcCer synthase reduced ER stress and apoptosis and also ameliorated the palmitate-mediated ER-to-Golgi protein trafficking defect. Although these results support ceramide as a causative factor in palmitate-induced β -cell death and possibly in β -cell dysfunction in T2D, it remains unknown how ceramide causes ER stress and cell death without an increase in the steady-state level of ceramide, as other accumulated sphingolipids are not toxic to β -cells.

Third, studies have revealed that ER Ca^{2+} homeostasis is important for β -cell function. ER Ca^{2+} measurement using an ER-targeted cameleon showed that the MIN6 β -cell has about 250 μM resting ER Ca^{2+} (56). The high intraluminal Ca^{2+} concentration in the ER is important to maintain Ca^{2+} -dependent ER chaperone functions, and Ca^{2+} released into the cytosol is an important signaling molecule (114, 115). In response to a variety of external stimuli, Ca^{2+} is released from the lumen of the ER via two Ca^{2+} channels; the IP_3 receptor and the ryanodine receptor. The concentration of Ca^{2+} in the cytosol is maintained at a low level by active Ca^{2+} transport into the ER via SERCA, creating a large $[\text{Ca}^{2+}]$ gradient between the cytosol and the ER lumen (0.1 μM versus 400 μM). Ca^{2+} is buffered in the ER by proteins (e.g., calnexin, calreticulin, ORP 150, ERp57, and others) that bear multiple low-affinity Ca^{2+} -binding sites. More importantly, Ca^{2+} binding to molecular chaperones regulates their activity, and therefore ER Ca^{2+} directly affects posttranslational protein folding, modification, and trafficking. For example, the interaction between calreticulin and other ER chaperones (PDI and ERp57) depends on the ER Ca^{2+} concentration. Therefore, blocking the SERCA pump by thapsigargin, a selective inhibitor of SERCA, depletes ER Ca^{2+} , inhibits ER functions, and thereby causes cell death through hyperactivation of the IRE1 α and PERK pathways (116). ER stress and cell death could occur if saturated FFAs perturb ER Ca^{2+} homeostasis through reduction of SERCA activity or by activation of IP_3 or ryanodine receptors. A previous study demonstrated that islets from *db/db* mice, an animal model of typical T2D, lack the initial reduction of intracellular Ca^{2+} and subsequent intracellular Ca^{2+} oscillations following stimulation with high glucose, possibly caused by a defect in cytosolic Ca^{2+} sequestration secondary to a reduction in SERCA activity (117). Several recent studies have indicated that palmitate triggers ER stress in pancreatic β -cells through perturbation of ER Ca^{2+} levels (Figure 4) (6). Long-term palmitate treatment depleted ER Ca^{2+} (by 40%) to a greater extent than oleate (by 24%) (91). The reduced ER Ca^{2+} content is explained by impaired ER Ca^{2+} uptake, which may reflect inhibition of the SERCA pump in palmitate-treated β -cells. The inhibition of ER Ca^{2+} uptake was observed at an early time (3 h) after palmitate treatment and was followed by UPR activation. Furthermore, CHOP depletion by siRNA partially protected against palmitate-induced β -cell apoptosis, suggesting that ER stress is involved in palmitate-induced apoptosis through ER Ca^{2+} depletion. However, this model is challenged by evidence described below. In the absence of extracellular Ca^{2+} , the palmitate-induced Ca^{2+} signal was mostly inhibited, except for the initiating Ca^{2+} signal from the ER (118). Several plasma membrane L-type Ca^{2+} channel blockers (nifedipine, nimodipine, and verapamil) and the K_{ATP} channel opener (diazoxide) efficiently inhibited the palmitate-induced Ca^{2+} signals and significantly reduced CHOP expression and cell death in pancreatic β -cells and islets, whereas the IP_3 receptor Ca^{2+} channel blocker (xestospongine *c*) and ryanodine receptor Ca^{2+} channel blocker (dantrolin) did not significantly alter the palmitate-induced Ca^{2+} signal and did not protect from palmitate-induced apoptosis (95). This suggests that a Ca^{2+} influx through the voltage-sensitive Ca^{2+} channel of the L-type coupled to membrane depolarization through closure of the K_{ATP} channel is crucial for cytosolic Ca^{2+} increase in response to palmitate. Moreover, several mono-/polyunsaturated FFAs (oleate, linoleic acid, and α -linolenic acid) in addition to palmitate also elicited intracellular Ca^{2+} signals (119) and reduced ER Ca^{2+} content (118). This suggests that the increase in cytosolic Ca^{2+} alone is not sufficient to induce cell death in palmitate-treated pancreatic β -cells. However, the unsaturated FFAs

were less toxic than palmitate to β -cells. Furthermore, palmitate immediately induced PERK activation in 5 min and subsequently caused a significant increase in both XBP1s and CHOP in β -cells. Therefore, it is possible that reduced ER Ca^{2+} content is a prerequisite for activation of palmitate-mediated UPR signaling or apoptosis in pancreatic β -cells. Clearly, further studies are required to unravel the relative contribution of reduced ER Ca^{2+} content in palmitate-induced ER stress and apoptosis in β -cells.

Endoplasmic Reticulum Stress by Glucotoxicity

In T2D, absolute or relative insulin deficiency associated with insulin resistance causes blood glucose levels to remain high, called hyper-glycemia (120). In chronic hyperglycemia, consistently overstimulated β -cells show a gradual decrease of glucose-induced insulin secretion and insulin gene expression and eventually impaired β -cell function and survival, a process called glucotoxicity. As diabetic hyperglycemia becomes chronic, the glucose that normally serves as fuel or substrate is used to generate detrimental metabolites for β -cells. Glucotoxicity is mediated at least in part by accumulation of excess ROS generated by several metabolic pathways, including mitochondrial oxidative phosphorylation and other alternative metabolic pathways, such as glucose autooxidation, hexosamine metabolism, sorbitol metabolism, and increased protein glycation (120, 121). The pancreatic β -cell is vulnerable to ROS because it has a low antioxidative stress response (122). β -Cells do not express catalase and only low levels of glutathione peroxidase. Furthermore, oxidative stress by elevated ROS reduced proinsulin synthesis by decreasing mRNA expression through inactivation of the β -cell-specific transcription factors, PDX1 and MafA, that regulate expression of proinsulin genes and multiple downstream genes required for β -cell differentiation, proliferation, and survival (123). Therefore, it is thought that oxidative stress is an important factor in β -cell failure.

Another possible mechanism of ROS generation from hyperglycemia-exposed pancreatic β -cells has emerged in recent years. Protein folding pathways in the ER and ROS production are closely linked events (Figure 3) (65, 71, 73). Prolonged UPR activation leads to the accumulation of ROS via two sources: the UPR-regulated oxidative protein folding machinery in the ER and oxidative phosphorylation in mitochondria. First, in hyperglycemia-exposed β -cells, the increased demand for insulin requires increased disulfide bond formation, which generates ROS during the process (73). Moreover, the increased amount of proinsulin synthesis further depletes glutathione, used for reducing nonnative disulfide bonds in misfolded proinsulin molecules. It has been estimated that approximately 25% of the ROS generated in a cell may result from formation of disulfide bonds in the ER during oxidative protein folding (124). Second, ER Ca^{2+} uptake to mitochondria mediates mitochondrial ROS production through disruption of mitochondrial electron transport and eventually induces mitochondrial apoptotic pathways (Figure 3). Specifically, more than 50% of the protein synthesized in the ER during glucose stimulation is proinsulin that requires delicate intermolecular disulfide bond formation and exchange (9). If chronic hyperglycemia leads to an increased rate of proinsulin synthesis, it would overcrowd the ER and increase the rate of oxidative protein folding. This would place an enormous burden on the ER and would enhance the probability of protein misfolding. Thus, during hyperglycemia, the accumulation of misfolded proinsulin in the ER lumen can generate ROS by the UPR-regulated oxidative protein folding machinery and by functional perturbation of mitochondria by a Ca^{2+} leak from the ER. In this manner, glucotoxicity may cause ER stress-regulated ROS accumulation, leading to diminished insulin gene expression, β -cell failure, and apoptosis. However, additional studies need to test the notion whether the hyperglycemia-increased proinsulin synthesis-ER stress-ROS pathway is a main cause for the onset and/or the progression of T2D.

Under conditions of chronic hyperglycemia, increased proinsulin biosynthesis may overwhelm the ER protein folding capacity, leading to UPR activation. Chronic high-glucose exposure (24 h) causes hyper-activation of IRE1 α to splice *Xbp1* mRNA, whereas acute exposure (1–3 h) to high glucose activates IRE1 α without *Xbp1* mRNA splicing (Figure 4) (86). During chronically high glucose exposure over several days, hyperactivated IRE1 α , which may have a different activation states (85), degrades ER proinsulin mRNA, possibly including ER-localized mRNAs, contributing to the reduction of proinsulin biosynthesis and further β -cell demise (85, 125). Further studies under physiological conditions are necessary to elucidate the validity of these in vitro systems that use prolonged states of high glucose exposure, which are rarely observed in vivo.

It appears that β -cell death in T2D is not associated with hyperglycemia or hyperlipidemia alone but is a combination of hyperglycemia and hyperlipidemia, which is called glucolipotoxicity (3). FFA-mediated UPR signaling pathways are potentiated by high-glucose cosupplementation of β -cells as high glucose exacerbates β -cell lipotoxicity (Figure 5) (126, 127). Although high glucose activates PERK and IRE1 α phosphorylation in palmitate-treated β -cells, it remains to be determined how high glucose synergizes with palmitate to alter ER stress signaling, especially the PERK pathway. In general, acute high glucose, leading to increased proinsulin synthesis and folding, represses PERK activation, which limits excessive proinsulin synthesis and causes expression of the integrated stress response genes (such as *Atf4*, *Chop*, *Gadd34*, and others) (33, 128). The potential role of high glucose as an enhancer of FFA-mediated ER stress now warrants further investigation to fully understand glucolipotoxicity in T2D.

Endoplasmic Reticulum Stress by Islet Amyloid

In addition to β -cell failure and insulin resistance, islet hyalinosis (hyaline deposits in β -cells), reported for the first time by Opie in 1901 (129), also plays a role in T2D. The hyaline deposits in pancreatic β -cells occur in approximately 90% of individuals with T2D and are associated with reduced β -cell volume (130). The hyaline deposits are known as amyloids (131), insoluble fibrous protein aggregates sharing specific structural traits. A number of human neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, Huntington's disease) are now thought to be associated with the formation of amyloids or amyloid-like fibrils (132). In 1987, the islet amyloid in T2D was shown to be an islet amyloid polypeptide (IAPP) (133). It is coex-pressed with insulin in pancreatic β -cells, traffics through the insulin secretory pathway and is secreted with insulin after food ingestion (134). The mature IAPP is a 37-amino acid polypeptide derived from an 89-amino acid precursor by proteolytic processing (135). The primary sequence of processed IAPP in humans, non-human primates, and cats is highly conserved, but rodent IAPPs show sequence differences between the twentieth and twenty-ninth amino acids. In vitro IAPP in humans, nonhuman primates, and cats, but not rodents, forms amyloid fibrils and conveys cellular toxicity to pancreatic β -cells (136). It is believed that the sequence differences in IAPP conveys its propensity to form a fibrillar amyloid in aqueous environments and confers toxicity to β -cells (7). This amyloid hypothesis for T2D was further tested by β -cell-specific overexpression of human IAPP (hIAPP) in transgenic mice. The hIAPP-transgenic mice recapitulated the metabolic characteristics of T2D, i.e., hyperglycemia, impaired insulin secretion, and insulin resistance (7). Recent examination of amyloid toxicity found that the toxic form of amyloidogenic proteins appears not to be the extracellular or intracellular large amyloid deposits detected by Congo red dye under light microscopy but rather smaller intracellular nonfibrillar oligomers that can be detected by a specific antibody against amyloid β protein toxic oligomers (7). Through use of the toxic oligomer-specific antibody, Lin et al. (137) were able to show that toxic oligomers of hIAPP in hIAPP-

IRE1	inositol-requiring protein 1
ATF6	activating transcription factor 6
PERK	PKR (double-stranded RNA-dependent kinase)-like endoplasmic reticulum kinase
Eukaryotic translation initiation factor 2α(eIF2α)	a subunit of heterotrimeric eIF2 complex, which mediates the binding of methionyl-tRNA to ribosome
Wolcott-Rallison syndrome	a rare autosomal recessive disease, characterized by neonatal/early-onset nonautoimmune insulin-requiring diabetes associated with skeletal dysplasia and growth retardation
eIF2αA/A	a homozygous mutant mouse model harboring an alanine mutation at the phosphorylation site (serine 51 amino acid) in eIF2 α
SERCA	sarco/ endoplasmic reticulum Ca ²⁺ -ATPase
Preemptive quality control	a cotranslational rerouting pathway reducing the burden of misfolded substrates entering the ER by cytosolic degradation
ROS	reactive oxygen species
<i>Lepr^{db/db}</i> mouse	a mouse model of obesity, diabetes, and dyslipidemia wherein leptin receptor activity is deficient
UPRosome	a complex of IRE1 α and multiple signaling molecules, which can modulate the amplitude and duration of IRE1 α signaling
FFAs	free fatty acids
Integrated stress response	eIF2 α phosphorylation-dependent, stress-inducible signaling pathways that can be activated by eIF2 kinases (PERK, PKR, HRI, GCN2)

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SUMMARY POINTS

1. When ER homeostasis is altered, signaling pathways mediated by three ER-proximal sensors: IRE1, PERK and ATF6 are activated, eliciting an adaptive response that is collectively called the unfolded protein response (UPR). However, when the adaptive mechanisms fail to restore ER homeostasis, several death signaling pathways are activated.
2. During the death response, the PERK-mediated cell death pathway is mainly initiated by CHOP expression, which causes an increase in misfolded protein accumulation and oxidative stress, activates the mitochondrial death signal through ER Ca²⁺ release, and regulates expression of pro- and antiapoptotic genes.
3. During the death response, IRE1 α may propagate death by signaling activation of JNK and by regulating IRE1-dependent RNA degradation.
4. The UPR pathway is activated by chronic exposure to high glucose or saturated long-chain FFAs, and activation of the UPR pathways is amplified by cosupplementation of both high glucose and saturated long-chain FFAs.
5. The activation of the UPR in T2D β -cells is connected with saturated long-chain fatty acid-mediated proapoptotic mechanisms that include PKC δ activation, accumulation of lipid derivatives, and ER Ca²⁺ release.
6. Under chronic hyperglycemia in T2D, increased proinsulin synthesis induces oxidative stress by ROS production and ER Ca²⁺ release, leading to mitochondria-dependent cell death.
7. Under chronic hyperglycemia conditions, hyperactivated IRE1 α contributes to β -cell failure by degrading proinsulin mRNA, possibly including ER-localized mRNAs. 8. Activation of the UPR induced by ER accumulation of the toxic hIAPP oligomer may be an important mechanism in β -cell death in humans.

FUTURE ISSUES

1. Is hyperglycemia and/or hyperlipidemia essential for β -cell failure in T2D?
2. What is the molecular mechanism of glucose level-dependent differential activation of PERK and IREa in pancreatic β -cells?
3. How does ceramide activate the UPR? What other lipid intermediates activate or inhibit the UPR?
4. How does the UPR activate PKC δ in palmitate-treated β -cells?
5. How does palmitate induce ER calcium release?
6. How does high glucose activate the UPR in β -cells? Is there any direct evidence of ER stress?
7. What are the molecular mechanisms involved in the formation of the toxic hIAPP oligomers and UPR induction by hIAPP oligomers?
8. Further investigation is needed to identify effective chemical chaperones that can improve ER function to prevent hyperactivation of the UPR.

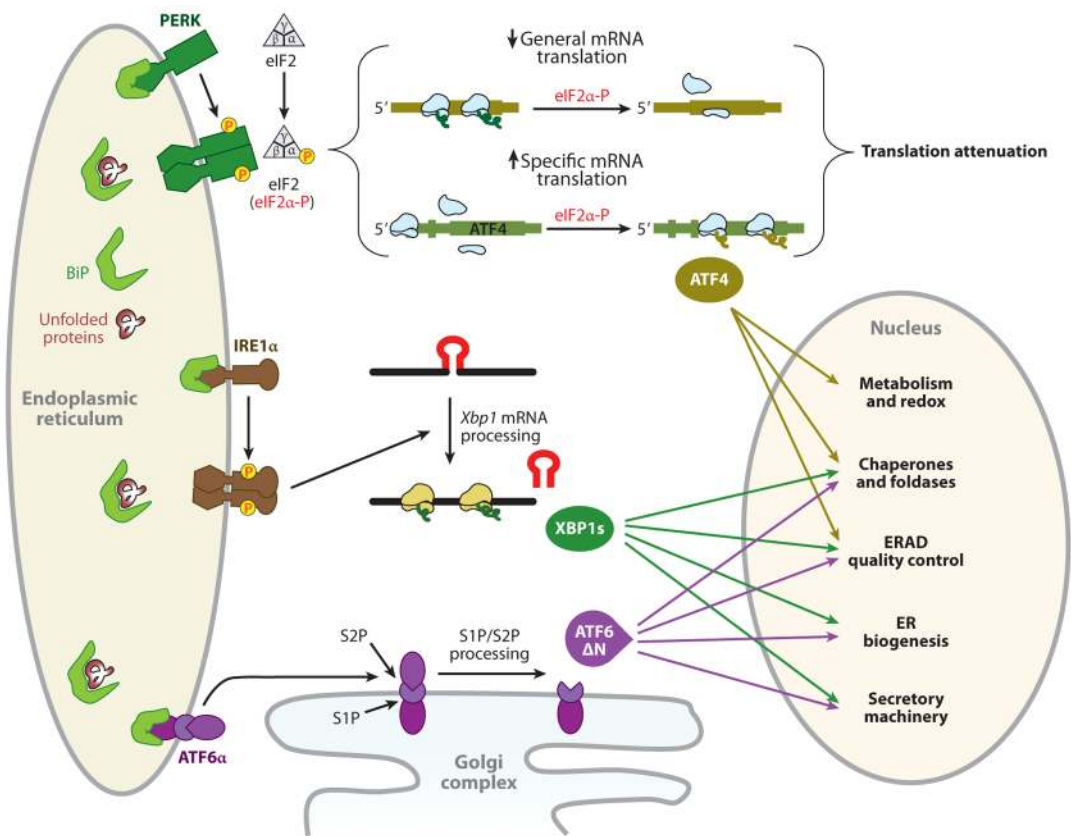


Figure 1.

The adaptive unfolded protein response (UPR). Activation of three UPR pathways initiates the adaptive endoplasmic reticulum (ER) stress response. During activation of the UPR in mammals, BiP (immunoglobulin heavy chain binding protein, also known as GRP78) is sequestered through binding to unfolded or misfolded polypeptide chains, thereby leading to BiP release from the ER stress sensors for their activation. Unconventional cytoplasmic splicing, mediated by IRE1 α , removes a 26-nucleotide intron from unspliced *X-box-binding protein 1* (*Xbp1*) mRNA (encoding 267 amino acids) to produce a translational frameshift, yielding a fusion protein encoded from two evolutionarily conserved open reading frames (16). The fusion protein, XBP1s, acts as a potent transcription factor for expression of UPR target genes involved in protein folding and export from the ER, export and degradation of misfolded proteins, and lipid biosynthesis, to resolve ER stress (16). Upon accumulation of unfolded protein in the ER lumen, oligomerization of the PKR-like ER kinase (PERK) in ER membranes induces its autophosphorylation and kinase domain activation (141, 142). Activated PERK phosphorylates serine 51 on the α -subunit of heterotrimeric eIF2 (143). When eukaryotic translation initiation factor 2 α (eIF2 α) is phosphorylated, the eIF2 complex shows increased affinity for its guanine nucleotide exchange factor eIF2B and sequesters all available eIF2B. Because the cellular level of eIF2B is 10- to 20-fold lower than the level of eIF2, very small changes in eIF2 α phosphorylation can dramatically change the rate of translation initiation (144). Inhibition of general mRNA translation by the phosphorylation of eIF2 α reduces accumulation of misfolded protein in the ER lumen (22), thereby protecting the cell from diverse stimuli that perturb the ER homeostasis. In contrast to inhibition of general mRNA translation, the PERK/eIF2 α pathway stimulates the translation of several specific mRNAs containing multiple 5³-upstream open reading frames, such as *Atf4* and *Atf5*, *Chop*, *Gadd34*, and the cationic amino acid transporter 1 (*Cat-1*, an

Na⁺-independent transporter of L-arginine and L-lysine) (28, 145). Among them, ATF4 activates transcription of the adaptive genes that encode functions in ER protein folding, endoplasmic reticulum-associated degradation (ERAD), amino acid biosynthesis and transportation, and the antioxidative stress response (24). Under ER stress, ATF6 α and ATF6 β are released from BiP and translocate to the Golgi complex, where they are cleaved by Golgi-resident proteases, first by S1P (site 1 protease) and then in the intramembrane region by S2P (site 2 protease), to release the N-terminal basic leucine zipper protein (bZIP) transcription factor domain (16). The bZIP domain of ATF6 α then translocates into the nucleus, where it activates the transcription of genes encoding ER-localized molecular chaperones and folding enzymes, ERAD, protein secretion machineries, and ER biogenesis (146), in some cases in cooperation with XBP1s (42).

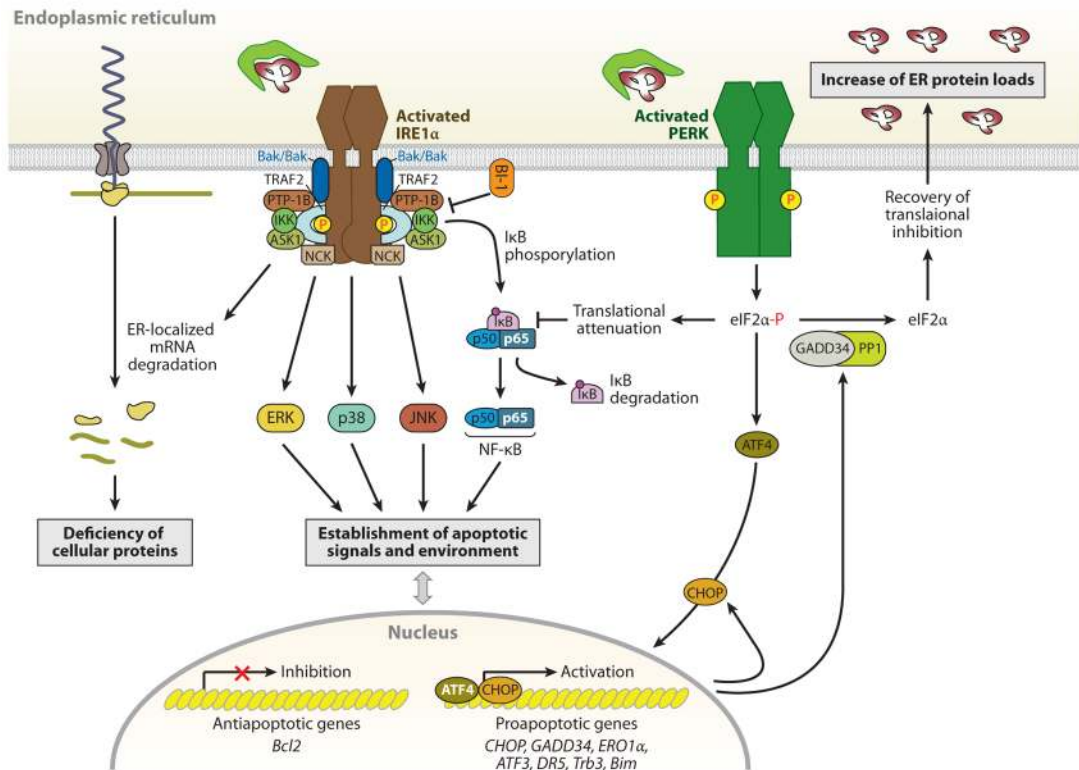


Figure 2.

IRE1 α - and PERK-mediated cell death pathways. During endoplasmic reticulum (ER) stress, inositol-requiring protein 1 α (IRE1 α) forms a hetero-oligomeric complex with TNF receptor-associated factor 2 (TRAF2) (76) and apoptosis signal-regulating kinase 1 (ASK1) (77) and then recruits the protein kinase JNK, leading to the activation of JNK (76). The IRE1 α -TRAF2 complex recruits I κ B kinase (IKK), which phosphorylates inhibitor of κ B (I κ B), leading to the degradation of I κ B and the nuclear translocation of nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) (147). IRE1 α also modulates the activation of other “alarm genes,” such as p38 and ERK (80), possibly by the binding of the Src homology (SH) 2/3 -containing adaptor proteins Nck and TRAF2, respectively. Furthermore, several proapoptotic (i.e., BAX/BAK, AIP1, and maybe PTP-1B) or antiapoptotic proteins (i.e., BI-1) interact with IRE1 α , regulating its activation state (81–84). Thus, the formation of a macromolecular signaling complex of IRE1 α with several proapoptotic proteins can generate apoptotic signals and establish an apoptotic environment. In addition, the endoribonuclease activity of IRE1 α , aside from specific cleavage of *Xbp1* mRNA, degrades ER-targeted mRNAs that can decrease cellular functions, such as proinsulin synthesis in β -cells. In contrast to the adaptive response by the PERK-phosphorylated eIF2 α -ATF4 pathway, this pathway also contributes to stress-induced cell death by ATF4-mediated induction of proapoptotic genes, including CHOP, ATF3, and GADD34 (16). The induced transcription factor CHOP contributes to increased expression of the proapoptotic factors, such as death receptor 5 (DR5) (148), *tribbles*-related protein 3 (Trb3) (149), and binding to microtubule (Bim) (150), and it can suppress B cell lymphoma 2 (Bcl2) expression. Bim is also activated through protein phosphatase 2A-mediated dephosphorylation, which prevents its ubiquitination and proteasomal degradation (150). PERK-mediated translational attenuation upregulates NF- κ B-dependent transcription because I κ B has a shorter half-life than NF- κ B, so NF- κ B is released to translocate to the nucleus (151). Recovery from translational repression is mediated by eIF2 α .

dephosphorylation by the two regulatory subunits of protein phosphatase 1 (PP1), GADD34 and CREP (constitutive repressor of eIF2 α phosphorylation) (16). GADD34 is induced transcriptionally during ER stress by ATF4, whereas *CreP* is a constitutive activator of PP1. The premature dephosphorylation of eIF2 α by the GADD34-PP1 complex restores translation of general mRNAs, which may be detrimental if the ER protein-folding defect is not resolved.

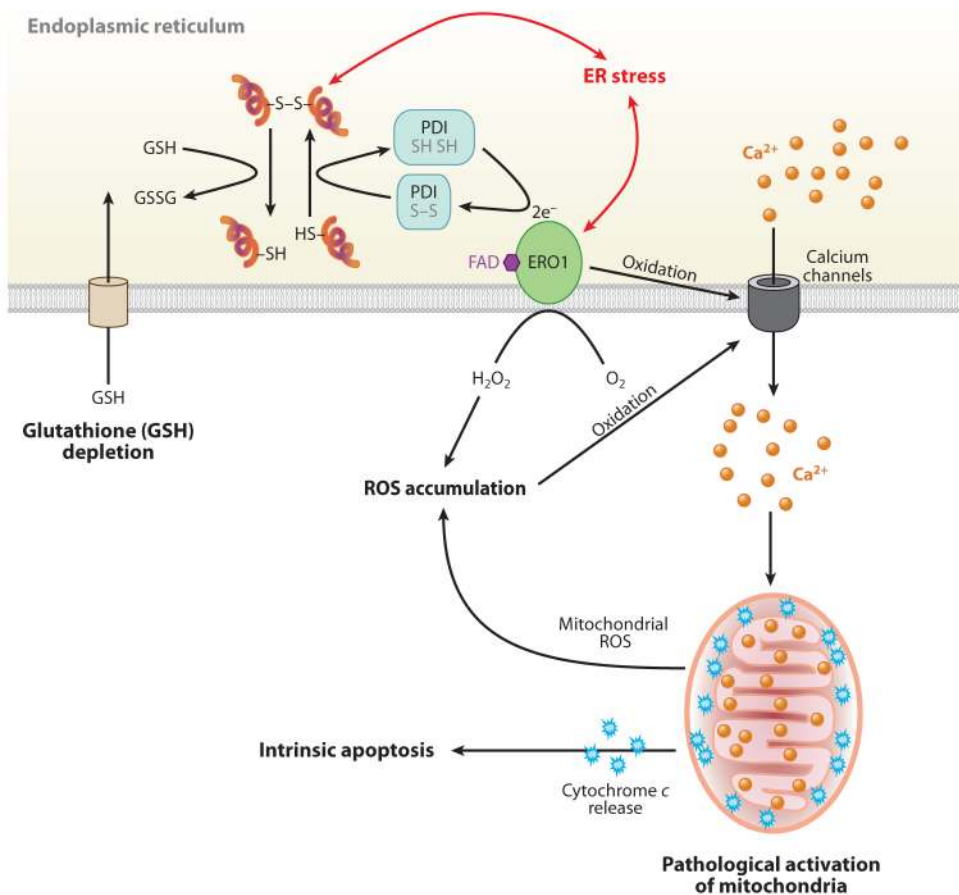


Figure 3.

The role of calcium and reactive oxygen species (ROS) in endoplasmic reticulum (ER) stress-mediated cell death. In the ER lumen, oxidative protein folding is catalyzed by protein disulfide isomerase (PDI) and ER oxidoreductase (ERO1). In this reaction, an oxidant flavin adenine dinucleotide (FAD)-bound ERO1 oxidizes PDI, which subsequently oxidizes folding proteins directly. FAD-bound ERO1 then passes two electrons to molecular oxygen, resulting in the production of hydrogen peroxide (73). During unfolded protein response activation, CHOP-mediated induction of ERO1 α hyperoxidizes the ER lumen and causes oxidation-induced activation of the ER Ca²⁺ release channel inositol 1,4,5-trisphosphate receptor (152), causing a large and transient release of Ca²⁺ from the ER. Increased cytosolic Ca²⁺ is taken up into the mitochondrial matrix, and this stimulates mitochondrial ROS production through disruption of mitochondrial electron transport (153). High levels of ROS generated from mitochondria, in turn, further increase Ca²⁺ release from the ER. The increase in mitochondrial Ca²⁺ eventually dissociates cytochrome *c* from the inner membrane cardiolipin, which triggers permeability transition pore opening and cytochrome *c* release across the outer membrane. Now the vicious cycle of ER calcium release and mitochondrial ROS production activates cytochrome *c*-mediated apoptosis. In addition, ER stress may cause consumption of excessive cellular glutathione (GSH) because reduced GSH may also assist in reducing nonnative disulfide bonds in misfolded proteins, resulting in the production of oxidized glutathione (GSSG).

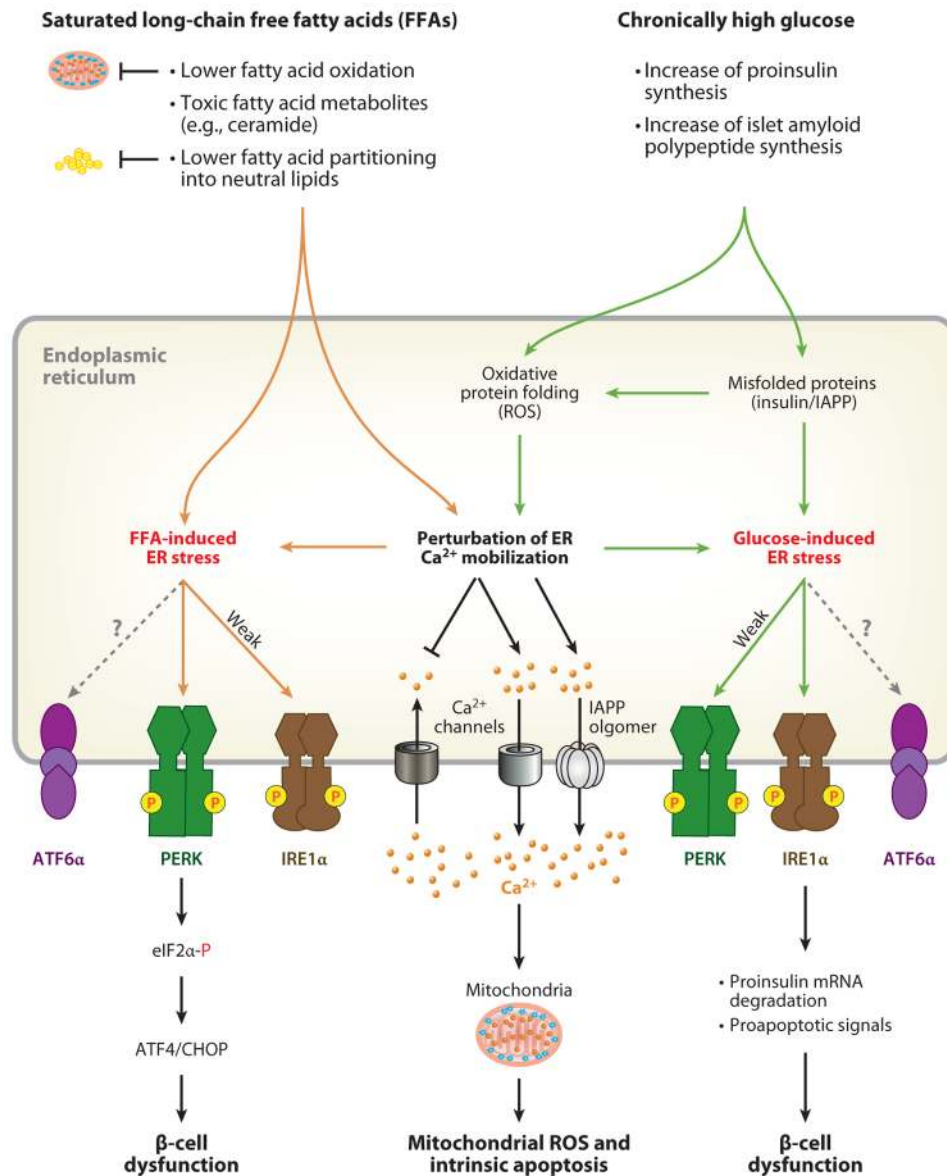


Figure 4.

Apoptotic unfolded protein response (UPR) pathways induced by free fatty acids (FFAs) and chronically high glucose in β -cells. In contrast to unsaturated FFAs, saturated FFAs serve as poor substrates for mitochondrial fatty acid oxidation and de novo triglyceride synthesis. However, saturated FFAs serve as intermediates in ceramide biosynthesis. The saturated FFAs activate UPR pathways (primarily PKR-like ER kinase, PERK) by perturbation of ER Ca²⁺ mobilization through inhibition of sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA), or activation of inositol-1,4,5-trisphosphate (IP₃) receptors, and/or direct impairment of endoplasmic reticulum (ER) homeostasis. In addition, chronically high glucose increases biosynthesis of proinsulin and islet amyloid polypeptide in β -cells, which increases accumulation of misfolded proteins [insulin and islet amyloid polypeptide (IAPP)] and oxidative protein folding-mediated reactive oxygen species (ROS) production. The oxidative stress created by ROS and toxic IAPP oligomers perturb ER Ca²⁺ mobilization through activation of IP₃ receptors to release ER Ca²⁺. Perturbation of ER Ca²⁺ causes protein misfolding in the ER and activates the UPR pathways (primarily inositol-requiring

protein 1a, IRE1 α) that induce proapoptotic signals, including proinsulin mRNA degradation as described in Figure 2. Abbreviations: ATF4: activating transcription factor 4, ATF6 α : activating transcription factor 6 α , CHOP: CCAAT-enhancer-binding protein (C/EBP) homology protein, eIF2 α -P: phosphorylated form of eukaryotic translation initiation factor 2 α .

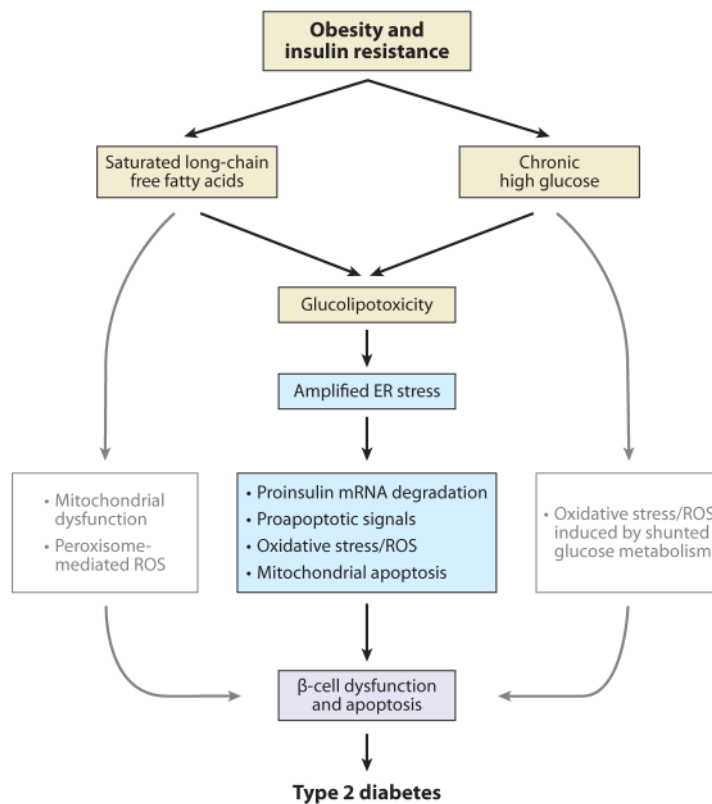


Figure 5. Amplified endoplasmic reticulum (ER) stress and β -cell death in type 2 diabetes (T2D). Conditions of insulin resistance and obesity cause hyperglycemia and hyperlipidemia, which result in glucolipototoxicity for the β -cell. Studies suggest that free fatty acid-mediated unfolded protein response signaling pathways are potentiated by high-glucose cosupplementation to β -cells as high glucose exacerbates β -cell lipotoxicity (126, 127). The amplified ER stress response leads to β -cell dysfunction and apoptosis through proinsulin mRNA degradation, oxidative stress, proapoptotic signals, and mitochondrial apoptosis, eventually culminating in T2D. Abbreviation: ROS, reactive oxygen species.