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The Role for Endoplasmic Reticulum Stress in Diabetes Mellitus

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Accumulating evidence suggests that endoplasmic reticulum (ER) stress plays a role in the pathogenesis of diabetes, contributing to pancreatic β-cell loss and insulin resistance. Components of the unfolded protein response (UPR) play a dual role in β-cells, acting as beneficial regulators under physiological conditions or as triggers of β-cell dysfunction and apoptosis under situations of chronic stress. Novel findings suggest that "what makes a β-cell a β-cell", *i.e.*, its enormous capacity to synthesize and secrete insulin, is also its Achilles heel, rendering it vulnerable to chronic high glucose and fatty acid exposure, agents that contribute to β-cell failure in type 2 diabetes. In this review, we address the transition from physiology to pathology, namely how and why the physiological UPR evolves to a proapoptotic ER stress response and which defenses are triggered by β-cells against these challenges. ER stress may also link obesity and insulin resistance in type 2 diabetes. High fat feeding and obesity induce ER stress in liver, which suppresses insulin signaling via c-Jun N-terminal kinase activation. *In vitro* data suggest that ER stress may also contribute to cytokine-induced β-cell death. Thus, the cytokines IL-1β and interferon-γ, putative mediators of β-cell loss in type 1 diabetes, induce severe ER stress through, respectively, NO-mediated depletion of ER calcium and inhibition of ER chaperones, thus hampering β-cell defenses and amplifying the proapoptotic pathways. A better understanding of the pathways regulating ER stress in β-cells may be instrumental for the design of novel therapies to prevent β-cell loss in diabetes. (*Endocrine Reviews* 29: 42–61, 2008)

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Abbreviations: AP-1, Activator protein-1; ASK, apoptosis-signal-regulating kinase; ATF, activating transcription factor; BiP (also known as GRP78 or HSPA5), Ig heavy chain binding protein; C/EBP, CCAAT/enhancer binding protein; CHOP (also known as GADD153 or DDIT3), C/EBP homologous protein; CPA, cyclopiazonic acid; eIF, eukaryotic translation initiation factor; ER, endoplasmic reticulum; ERAD, ER-associated degradation pathway; FFA, free fatty acid; GADD, growth arrest and DNA damage inducible gene; IAPP, islet amyloid polypeptide; IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; IRE, inositol requiring ER-to-nucleus signal kinase; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κB; NO, nitric oxide; NOD, nonobese diabetic; ORF, open reading frame; ORP150 (also known as GRP170), oxygen-regulated protein 150; PDI, protein disulfide isomerase; PERK, PKR-like ER kinase; PKR, double-stranded RNA-activated kinase; PP, protein phosphatase; ROS, reactive oxygen species; SERCA, sarcoendoplasmic reticulum Ca^{2+} ATPase; S1P, site-1 protease; STAT, signal transducer and activator of transcription; TRAF, TNF receptor-associated factor; uORF, upstream ORF; UPR, unfolded protein response; SREBP, sterol-response element-binding protein; WFS1, Wolfram syndrome gene 1; XBP1, X-box binding protein-1; XBP1s, spliced XBP1.

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I. Introduction

THE TWO MAIN forms of diabetes mellitus are type 1 and type 2 diabetes (1). They are a major cause of morbidity and mortality, decreasing both life quality and life expectancy of millions of affected individuals. A reduction in β-cell mass, due to increased β-cell apoptosis and defective β-cell regeneration, is a key component of diabetes mellitus (2–4). In the case of type 2 diabetes, this is very often accompanied by insulin resistance in fat, muscle, and liver (5). The molecular mechanisms underlying decreased β-cell mass and insulin resistance remain to be clarified.

Type 1 diabetes is characterized by a severe lack of insulin production due to specific destruction of the pancreatic β-cells that typically develops over several years. Although some immune-related biomarkers (*i.e.*, autoantibodies to IA-2, GAD65, and insulin) can identify individuals at risk for type 1 diabetes, the process by which the β-cells are destroyed is not well understood. β-Cell loss in type 1 diabetes is the result of an autoimmune-mediated process, where a chronic inflammation called insulitis causes β-cell destruction. This is mediated by cytokines and other factors released

by and/or expressed on the surface of the immune cells invading the islets, which trigger secondary pathways of cell death in the target β-cells (2, 3, 6).

Type 2 diabetes results from a reduced ability of the pancreatic β-cells to secrete enough insulin to stimulate glucose utilization by peripheral tissues (7). As β-cell secretory capacity deteriorates, glucose tolerance worsens and fasting glucose levels progressively increase, eventually culminating in overt hyperglycemia (8–10). Defects in both insulin secretion and action contribute to the pathogenesis of type 2 diabetes, but it is now acknowledged that insulin deficiency is the crucial constituent, without which type 2 diabetes does not develop. This β-cell defect is present early in the disease process (7) and detectable as markedly reduced first phase or acute glucose-induced insulin secretion (11, 12). The genetic factors that predispose a subset of obese individuals to β-cell failure are now being identified (13–17), but the underlying biological mechanisms are not yet understood. From postmortem studies, it has been shown that type 2 diabetic patients have reduced β-cell mass (18–22) and increased β-cell apoptosis rates (21). It is unlikely that β-cell loss entirely accounts for reduced insulin secretion in type 2 diabetes, and the extent of its contribution to diabetes development will remain unclear until *in vivo* tools for imaging of β-cell mass become available. The loss of β-cell mass in type 2 diabetes may be due to chronic exposure to high glucose and free fatty acid (FFA) levels (gluco- and lipotoxicity) (5, 23, 24).

Accumulating evidence indicates that β-cell loss in both type 1 and type 2 diabetes results from stress responses regulated by key transcription factors and gene networks (25). Initial suggestions that β-cell apoptosis in both forms of diabetes is mediated by a common "up-stream" pathway, dependent on IL-1β production and activation of the Fas-FasL system (26), have not been confirmed (27, 28). Instead, it seems that there are diverging up-stream proapoptotic signals in both forms of diabetes, depending on the transcription factors nuclear factor (NF)-κB and signal transducer and activator of transcription (STAT)-1 in type 1 diabetes and on other signaling molecules, still to be discovered, in type 2 diabetes (Fig. 1). These early signaling pathways may converge downstream into common "execution" pathways, such as endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and production of reactive oxygen species (ROS) (Fig. 1) (25). ER stress may also act as a link between obesity and insulin resistance in liver and fat, raising the intriguing possibility that this cellular response is a common mechanism for both β-cell failure and defective insulin signaling in type 2 diabetes.

In the present review, we will focus on the role for ER stress in diabetes, with special emphasis on recent findings clarifying the transition between "normal" and "pathological" ER stress responses.

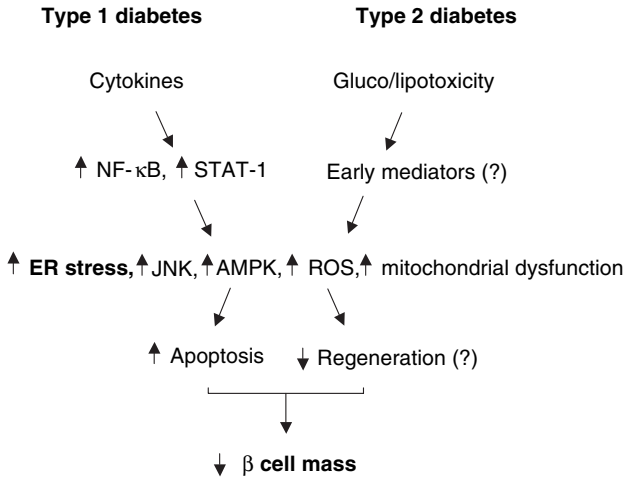

FIG. 1. β-Cell loss in type 1 and type 2 diabetes results from stress responses that may lead to apoptosis. There are diverging up-stream proapoptotic signals in both forms of diabetes, depending on the transcription factors NF-κB and STAT-1 in type 1 diabetes and on other signaling molecules, still to be discovered, in type 2 diabetes. These early signaling pathways may converge downstream into potentially common "execution" pathways, such as ER stress, activation of JNK and AMP-activated protein kinase (AMPK), mitochondrial dysfunction, and production of oxygen free radicals (ROS).

II. Endoplasmic Reticulum (ER) Stress and the Unfolded Protein Response (UPR)

The ER is a highly dynamic organelle with a central role in lipid and protein biosynthesis. The ER produces the transmembrane proteins and lipids for most cell organelles and is responsible for the synthesis of almost all secreted proteins. The ER also has an important role in Ca^{2+} storage and signaling. The resting intra-ER Ca^{2+} concentration is three to four orders of magnitude higher than cytosolic Ca^{2+}. This gradient is generated by the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) proteins, which pump Ca^{2+} into the ER, and the Ins(1,4,5)P_3 and ryanodine receptors that release Ca^{2+} from the ER (29). Due to its ability to store and secrete Ca^{2+}, the ER controls a wide range of cellular processes such as organogenesis, transcriptional activity, stress responses, and apoptosis (29).

The translation of proteins is performed by ribosomes on the cytosolic surface of the ER (30), and the unfolded polypeptide chains are translocated into the ER lumen via the Sec61 complex (31). In the ER lumen these chains are often N-glycosylated and folded into secondary and tertiary structures that are stabilized by disulfide bonds (32). The unique oxidizing environment of the ER and the numerous protein chaperones present in the organelle are crucial for the proper folding of proteins and protein complexes (33). The disulfide bond formation is catalyzed by protein disulfide isomerase (PDI) (34). PDI is oxidized by Ero1α (35) and -β (36) into a disulfide donor, whereas reduced PDI can isomerize disulfide bonds in client proteins. Other ER-resident folding factors include amino acid cis-trans isomerases, the chaperones GRP94 and Ig heavy chain binding protein (BiP), N-glycosylation enzymes and the lectins calnexin and calreticulin that specifically chaperone N-glycans (37), all of which operate in complex multiprotein structures (38). While assisting with folding, these chaperones and foldases also retain client proteins in the ER until the maturely folded proteins meet all quality control standards and exit the ER (39–41).

The ER is exquisitely sensitive to alterations in homeostasis, and proteins formed in the ER may fail to attain correct

conformation due to: 1) lack of chaperones or cellular energy to promote chaperone-protein interactions; 2) Ca^{2+} depletion; 3) disruption of redox state; 4) protein mutations that hamper adequate folding; and 5) reduction of disulfide bonds. Accumulation of misfolded proteins that aggregate in the ER lumen causes ER stress and activation of a signal response termed the UPR (42–45). The aim of the UPR is to alleviate ER stress, restore ER homeostasis, and prevent cell death. To achieve these goals, the UPR induces several coordinated responses, including: 1) a decrease in the arrival of new proteins into the ER, thus preventing additional protein misfolding and overloading of the organelle; 2) an increase in the amount of ER chaperones, thus augmenting the folding capacity of the ER to deal with misfolded proteins; 3) an increase in the extrusion of irreversibly misfolded proteins from the ER and subsequently degradation of these proteins in the proteasome; and 4) in case the steps described above fail, apoptosis is triggered. Because these responses depend at least in part on *de novo* gene transcription, signals must be transmitted from the ER to the nucleus indicating the urgent need for the expression of relevant mRNAs and proteins. This signaling is mediated by three transmembrane ER proteins: inositol requiring ER-to-nucleus signal kinase (IRE) 1, activating transcription factor (ATF) 6, and double-stranded RNA-activated kinase (PKR)-like ER kinase (PERK) (Fig. 2). These proteins become active when unfolded proteins accumulate in the lumen of the ER and translate this information into signals that modulate expression of key genes and proteins.

A. The dialogue between the ER and the nucleus

The discovery of the signal transduction regulating the UPR is a telling example of the crucial role of basic research in model organisms such as yeast for the understanding of complex human diseases (42). Kozutsumi *et al.* (46) were the first to suggest that ER stress can activate a signal transduction pathway. They observed that the expression of a mutant influenza hemagglutinin, which is unable to fold correctly, induced expression of several ER resident proteins. The clarification of this signaling pathway was first described in the yeast *Saccharomyces cerevisiae*, where a 22-bp cis-acting ele-

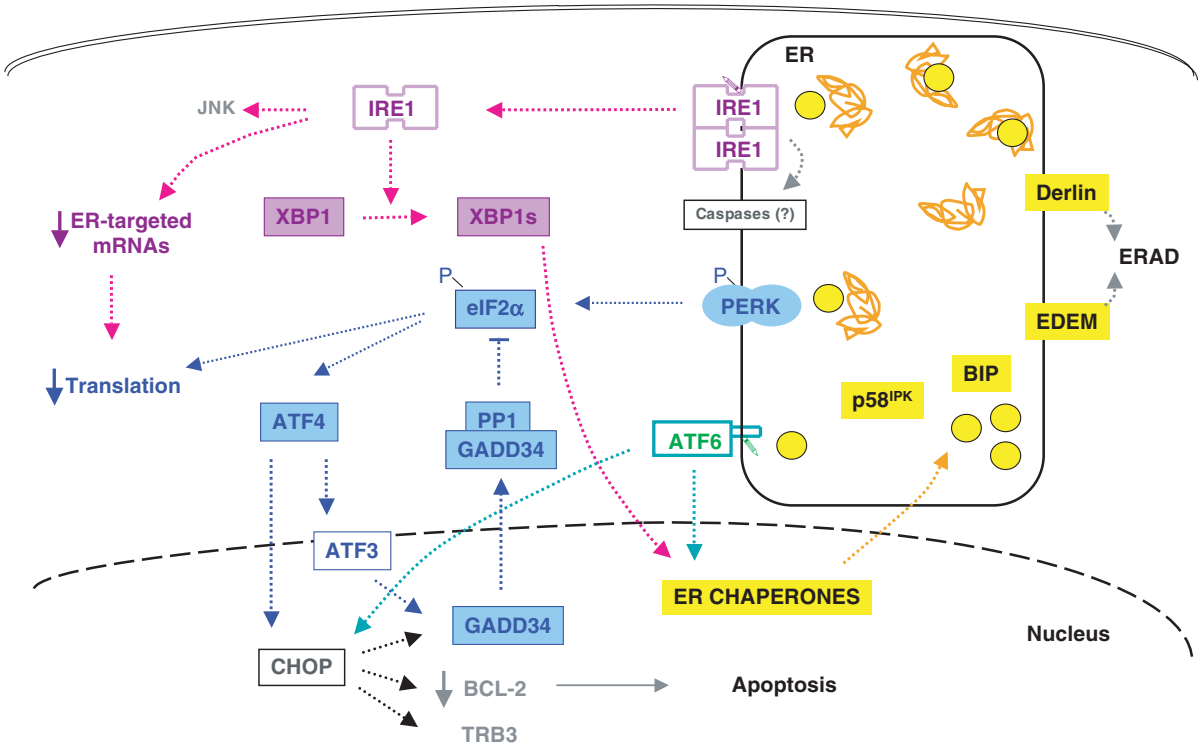

FIG. 2. Main components of the UPR. When misfolded proteins accumulate in the ER lumen, BiP (*yellow circles*) dissociates from the luminal side of the ER stress transducers IRE1α, ATF6, and PERK, thereby activating them. IRE1α activates XBP1 by its alternative splicing. XBP1s is a transcriptional transactivator of genes regulating protein maturation, folding, and export from the ER, as well as export and degradation of misfolded proteins. IRE1α also degrades ER-targeted mRNAs to decrease the production of new proteins in the organelle and activates JNK. ATF6 translocates to the Golgi and is cleaved by S1P and S2P proteases. ATF6 induces transcription of ER chaperones (for clarity, only one of these ER chaperones, BiP, is shown in the figure), XBP1 and CHOP. PERK phosphorylates eIF2α, thereby inhibiting global protein synthesis and decreasing the protein load in the ER. Translation of some proteins such as ATF4 is facilitated, and downstream CHOP and ATF3 expression is induced. The PERK-eIF2α branch undergoes negative feedback through GADD34-mediated PP1 activation and consequent eIF2α dephosphorylation, and perhaps through up-regulation of $p58^{IPK}$, which may suppress PERK activity. Misfolded ER proteins are disposed of by ERAD through dislocation to the cytosol via the retrotranslocon channel composed of derlins and degradation in the ubiquitin/proteasome pathway. EDEM (ER degradation enhancing α-mannosidase-like protein) contributes to this process by targeting misfolded mannose-trimmed glycoproteins for degradation. Prolonged and excessive ER stress may trigger apoptosis through JNK, CHOP, and ATF-3 and inhibition of Bcl-2 and/or activation of proapoptotic members of the Bcl-2 family. Execution of apoptosis may involve caspases whose nature remains unclear. Additional information and supporting references are provided in the text.

ment termed the UPR element was identified in the promoter of most genes up-regulated by UPR (47, 48). Subsequent screening of yeast mutants unable to induce UPR element led to the identification of an ER transmembrane protein, Ire1p (49–52). Ire1p is a bifunctional enzyme with Ser/Thr kinase and endoribonuclease activities in its carboxy-terminal domain and an ER stress-sensing domain in its N-terminal part. The endoribonuclease of Ire1p has as the main substrate the homologous to ATF/CREB1 mRNA that encodes the basic leucine zipper transcription factor Hac1. HAC1 is constitutively transcribed, but it is not translated due to the presence of a nonconventional intron of 252 bp at the 3′ end of the open reading frame (ORF), which base-pairs to the 5′ untranslated region and prevents translation. During ER stress, Ire1p is activated by dimerization and autophosphorylation, and its RNAse activity cleaves unspliced HAC1 mRNA, generating exon fragments that are then joined together by a tRNA ligase (53–55). This highly unique signal transduction pathway depends also on the transcriptional coactivator alteration/deficiency in activation (56) and will lead to removal of the inhibitory intron of HAC1, allowing translation of the transcription activator Hac1, which induces the transcription of ER stress-responsive genes (53, 54). There are two mammalian homologs of Ire1p: IRE1α, expressed in most cells and tissues with high levels of expression in pancreas, and IRE1β, which is mainly expressed in intestinal epithelial cells (57, 58). Once activated, the cytoplasmic domain of IRE1α gains endoribonuclease activity and cleaves 26 nucleotides from the mRNA encoding X-box binding protein (XBP) 1, generating a spliced variant (XBP1s) that functions as a potent transcriptional transactivator of genes involved in ER expansion, protein maturation, folding and export from the ER, as well as export and degradation of misfolded proteins (Fig. 2) (59–64). IRE1α may also degrade ER-targeted mRNAs, thus decreasing the production of new proteins in the organelle (65, 66).

In addition to IRE1, higher eukaryotic cells have two additional UPR transducers: PERK and ATF6 (Fig. 2). These ER stress transducers are usually inactive due to binding to the ER chaperone BiP, but they are activated when BiP dissociates from their luminal side to assist in protein folding, signaling depletion of ER chaperone reserves (67–70). It has been proposed that IRE1 also directly binds to unfolded proteins (71), but this hypothesis was not confirmed by detailed studies of the x-ray crystal structure of the luminal domain of IRE1 and by functional studies indicating that IRE1 can dimerize independently of the presence of unfolded proteins (72). Recent findings in yeast indicate the presence of additional and potentially novel mechanisms of IRE1 regulation (73).

ATF6α is a 90-kDa bZIP protein that is activated by post-translational modifications. The disulfide and glycosylation-bound status of the ATF6α ER luminal domain probably participates in the sensing of ER stress (74, 75). ATF6 activation leads to its translocation to the Golgi and cleavage of the membrane by site-1 protease (S1P) and S2P. The 50-kDa cleaved ATF6α translocates to the cell nucleus where it binds to the ER stress response element CCAAT(N)9CCACG (76) in genes encoding ER chaperone proteins, increasing protein folding activity in the ER (76, 77). There are two main chaperone systems in the ER, namely the calnexin/calreticulin (lectin chaperones) and BiP/GRP94 (heat shock protein family); components of both systems are up-regulated by ATF6α in some cases in cooperation with XBP1s (61, 77, 78). ATF6 augments the expression of XBP1 mRNA, providing more substrate for IRE1-induced generation of XBP1s (61). Another important cochaperone in the ER lumen is $P58^{IPK}$, which is associated with BiP and favors protein maturation (79). ATF6α is ubiquitously expressed (80), but in recent years several tissue-specific ER transmembrane proteins that are similarly cleaved by S1P and S2P have been identified, including cAMP-responsive element binding protein, old astrocyte specifically induced substance (OASIS), luman, and TISP40 (81–84).

PERK phosphorylates eukaryotic translation initiation factor (eIF) 2α, thereby inhibiting *80S* ribosome assembly and protein synthesis and consequently decreasing the functional demand on the ER (85). eIF2α phosphorylation causes a general decrease in translation, but some selected proteins such as ATF4 are translated more efficiently (86, 87). The ATF4 mRNA has two upstream ORFs (uORF)s before the initiation codon. The 5′ proximal uORF1 is a positive-acting element that facilitates ribosome scanning and reinitiation at downstream coding regions, whereas the second (uORF2) is out of frame with the true ATF4 coding sequence. In nonstressed cells, which contain low levels of phosphorylated eIF2α, ribosomes scanning downstream of uORF1 reinitiate at the next coding region, namely uORF2, thus preventing effective ATF4 translation. During ER stress, however, the presence of high levels of eIF2α phosphorylation delays the capacitation of scanning ribosomes, favoring reinitiation at the ATF4 initiation codon and resulting in increased ATF4 translation (88, 89). ATF4 regulates genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress (90). ATF4 also induces the expression of the proapoptotic genes CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) and ATF3 (91–93). Recovery from the translational repression caused by eIF2α phosphorylation is mediated by growth arrest and DNA damage inducible gene (GADD) 34, which interacts with the catalytic subunit of protein phosphatase (PP) 1c and leads to eIF2α dephosphorylation (94–99). It was suggested that PERK function is also modulated by the cochaperone $p58^{IPK}$ (100, 101). A recent study, however, indicates that whereas a putative small cytosolic $p58^{IPK}$ fraction might mitigate translational attenuation via PERK inhibition, its predominant ER luminal localization suggests that $p58^{IPK}$ functions mainly as a chaperone (79). An additional mechanism exists to selectively decrease protein load in the ER, the "preemptive quality control." Thus, during ER stress, ER signal sequences in the protein will determine whether or not it gains access to the ER; for instance, while the chaperone BiP continues to translocate into the ER, proteins destined for the plasma membrane are denied access (102).

The phenotypes caused by targeted deletion of the PERK/eIF2α and IRE1/XBP1 pathways are not identical, indicating both overlapping and divergent functions for these pathways (103). Different secretory cells seem to utilize preferentially one or the other pathway *in vivo*. Thus, UPR-induced translational control via eIF2α phosphorylation is not re-

quired for B lymphocyte differentiation/maturation (104), whereas deficiency in the PERK-eIF2α pathway leads to progressive loss of pancreatic β-cells (105, 106). XBP1 deletion causes severe abnormalities in exocrine pancreas and salivary glands but does not affect embryonic development of islet cells (64, 103). These discrepancies underscore the need to evaluate the specific regulation and role of the UPR in differentiated secretory cells, such as β-cells, instead of relying on data obtained in genetically modified embryonic fibroblasts, a model widely used in the field.

Misfolded ER proteins are disposed of by the ER-associated degradation (ERAD) after a time lag of 30–90 min. Protein disposal requires retrotranslocation into the cytosol, via a presumptive retrotranslocon channel, and subsequent degradation by the ubiquitin/proteasome pathway (107). Recent evidence has implicated the mammalian translocon-associated protein complex, which associates with Sec61, binds preferentially to misfolded proteins, and accelerates their degradation (108). Other proteins that play an important role as translocon channels are the derlins (109–112). An alternative model has recently been presented in which lipid droplet formation from the ER membrane mediates misfolded protein dislocation from the ER (113).

Autophagy was recently described as a new alternative pathway that targets proteins for degradation during ER stress in yeast and mammalian cells (114–117). During autophagy, parts of the cytoplasm, including its organelles, are sequestered into membrane-bound compartments that then fuse with lysosomes where their content is degraded by acid hydrolases (118). An ER-selective UPR-induced form of autophagy, ER-phagy, is apparently required for cell survival under conditions of severe ER stress (114–116). Besides helping to clear misfolded proteins, the ER-phagy reduces the volume of the ER that is increased during the UPR. It remains to be clarified how ER stress and the UPR activate autophagy, but the IRE1 pathway seems to be involved (114, 115), and a recent study indicates that PERK/eIF2α phosphorylation mediates polyglutamine-induced LC3 conversion, an important step for autophagy formation (119).

B. ER stress and apoptosis

The integrated result of the UPR is attenuation of global protein translation paralleled by up-regulation of ER chaperones, thus increasing the folding capacity of the ER, and degradation of irreversibly misfolded proteins. In case the UPR fails to solve ER stress, the apoptosis pathway will be activated. ER stress can lead to apoptosis by various pathways, involving activation of some of the key regulators of the UPR described above. Thus, IRE1α was shown to recruit the adaptor molecule TNF receptor-associated factor 2 (TRAF2) and activate c-Jun N-terminal kinase (JNK) and the downstream proapoptotic kinase apoptosis-signal-regulating kinase (ASK1) (120, 121). Neurons from ASK1−/− mice are resistant to ER stress-mediated cell death, suggesting that ER stress-induced JNK and ASK is proapoptotic (121). The IRE1/TRAF2 complex can also lead to NF-κB activation (122, 123), which may have a pro- or antiapoptotic effect depending on the cell type and context (124). The IRE1α/TRAF2 association is also required for the activation of procaspase 12, which may contribute to execution of ER stress-triggered apoptosis (see below) (125).

Decreased protein translation via the PERK pathway has mostly an antiapoptotic role during the UPR, by decreasing the protein synthesis load on the ER and providing the cell with a "resting time" to recover from the ER stress (126). In line with this hypothesis, mice with deletion of different components of the PERK pathway have progressive β-cell loss and diabetes (see detailed discussion on these models in *Section IV.B*). Activation of the PERK pathway also contributes to an antioxidant response through the PERK target Nrf2 and via ATF4 (90). The Nrf2 transcription factor induces antioxidant response element-containing genes, including detoxification enzymes, chaperones, and components of the proteasome, but it may also mediate crosstalk with other pathways such as IRE1α (90, 127). On the other hand, prolonged activation of the PERK pathway may lead to cell death (128). Also, eIF2α phosphorylation via another pathway, namely the dsRNA-activated kinase PKR, is part of the proapoptotic responses of virally infected cells (129). The capacity of different cells to endure inhibition of protein synthesis is probably cell-type dependent. For instance, salubrinal, a selective inhibitor of eIF2α dephosphorylation (which consequently prolongs inhibition of translation), protects pheochromocytoma PC12 cells against ER stress-mediated apoptosis (126), whereas it triggers apoptosis in pancreatic β-cells (128).

Activation of the PERK pathway can also induce apoptosis via ATF4 overexpression and consequent CHOP and ATF3 induction (91, 92, 130). CHOP, also known as GADD153, has attracted special attention as a putative mediator of apoptosis in ER stress (131). CHOP is a transcription factor of the C/EBP family (131, 132). Its expression is low under non-stressed conditions, but it increases markedly in response to ER stress and other cellular stresses, such as nuclear DNA damage by alkylating agents (131, 133); it is thus not a specific marker for ER stress. Several studies point to a proapoptotic effect of CHOP downstream of irremediable ER stress (131, 134, 135), but this effect may depend on the parallel expression of other components of the UPR (136). Possible mechanisms for CHOP-induced apoptosis include: 1) translocation of Bax from the cytosol to the mitochondria (137); 2) down-regulation of Bcl-2 expression and perturbation of the cellular redox state by depletion of cellular glutathione (138) and sensitization to a subsequent oxidative stress (139); 3) up-regulation of the death receptor 5 (140) and of Bim (141) (see below); and 4) induction of TRB3, an Akt inhibitor shown to contribute to ER stress-mediated death (142).

Members of the Bcl-2 family, usually considered as regulators of the mitochondrial pathway of cell death (143), are also involved in the regulation of cell death induced by ER stress (144). The Bcl-2 protein has been found to localize at the ER, besides mitochondria and nucleus, where it modulates the permeability of the ER membrane to Ca^{2+} (145, 146). Overexpression of Bcl-2 protects against lethal ER stress (145, 147), whereas fibroblasts deficient in Bax and Bak, proapoptotic members of the Bcl-2 family, are resistant to ER stress-induced cell death (148, 149). Bax and Bak regulate the outcome of ER stress by acting at both the mitochondrial and ER membranes and by regulating ER luminal Ca^{2+} (150, 151).

The BH3 only proteins PUMA, Noxa, Bik, and Bim (all proapoptotic members of the Bcl-2 family) have also been reported to contribute to cell death during ER stress (152–155). Recent observations indicate that ER stress induced by thapsigargin, tunicamycin, or viral infection requires Bim activation for the induction of apoptosis in macrophages, breast, and kidney epithelial cells (141). This Bim activation depends on both transcriptional induction by CHOP/C/EBPα and posttranslational modifications via PP2A-mediated dephosphorylation and consequent inhibition of Bim degradation by the ubiquitin-proteasome pathway (141).

Most proapoptotic signals ultimately lead to caspase activation, but it remains to be defined whether there is a specific caspase pathway responsible for ER stress-mediated apoptosis. The involvement of initiator caspases 2, 8, and 9 and effector caspases 3, 4, and 7 has been reported (123, 150, 156–161), and caspase 12 has been proposed as a specific mediator of ER stress-induced apoptosis in rodent cells (162, 163). The human homolog of caspase 12, however, is not catalytically active (164), and recent data indicate that ER stress-induced death in rodent cells can take place in the absence of caspase 12 (165, 166).

The precise mechanisms by which ER stress leads to apoptosis remain to be clarified, and this domain is perhaps the least well understood in the field of ER stress/UPR. Many of the mechanisms proposed for triggering apoptosis are cell and context dependent, which may explain some of the apparently contradictory results.

III. The UPR and β-Cell Adaptation to Physiological Demand

World life expectancy has increased by 2-fold in the past two centuries (167). Dietary composition has changed profoundly in the last 25 yr, favoring energy-dense and saturated fat-enriched diets (168), and physical activity is infrequent if not rare in the Western lifestyle. The increased lifespan and high prevalence of obesity and insulin resistance poses physiological challenges to long-living cells such as pancreatic β-cells. β-Cells confronted with child and then adulthood obesity will face decades of increased demand on insulin synthesis and secretion.

The main role of pancreatic β-cells is the adequate synthesis and release of insulin in response to glucose and other nutrients (169). The cell is geared to this task: proinsulin mRNA represents 20% of the total mRNA expression (170), whereas (pro)insulin biosynthesis approaches 50% of the total protein production in stimulated β-cells (171). Insulin mRNA is translational quiescent at low ($<$3 mM) glucose concentrations, but after stimulation by higher glucose concentrations there is a greater than 10-fold increase in biosynthesis (172–174). Synthesis of the endo- and exoproteases involved in proinsulin conversion augments in parallel, contributing to a nearly 5-fold glucose-induced increase in total protein synthesis (175). Translation of insulin and other secretory or cell membrane proteins takes place on ribosomes on the cytosolic surface of the ER. The newly formed proinsulin is directed into the ER as a single molecule of 110 amino acids; in the specialized ER environment the proinsulin molecule will form disulfide bonds and fold into its correct three-dimensional structure (169, 176). The marked nutrient-induced protein synthesis poses a burden on the ER, and minor changes in the insulin molecule that favor protein misfolding are sufficient to induce ER stress, progressive β-cell dysfunction and death, and early onset diabetes in mice (177, 178).

Like many other proteins targeted for secretion or expression on the cell surface, proinsulin and its converting enzymes require special maturation steps in the ER. Because synthesis of proinsulin may vary severalfold under physiological conditions, β-cells utilize the UPR homeostatic mechanism to balance the load posed by newly synthesized proteins against the ER capacity to properly fold them. Transducers, such as IRE1α and PERK, total XBP1, and the chaperones BiP, GRP94, and oxygen-regulated protein (ORP) 150 are highly expressed in β-cells (105, 179–181). Compared with glucose-responsive insulin-producing MIN-6 cells, MIN-6 cells that lose the glucose-responsiveness of insulin synthesis have a marked decrease in the expression of ER chaperones, including GRP94, BiP, ERp29, and PDI (182). Similarly, in cultured primary rat islets, lowering glucose from 30 to 5 mM causes a rapid ($>$50% by 2 h) and parallel decline in insulin secretion and XBP1 mRNA splicing (181). Furthermore, deficiency in key UPR pathways, such as the PERK-eIF2α branch, suffices to trigger β-cell dysfunction and death (105, 183) (see *Section IV.B*).

The steady-state eIF2α phosphorylation results from a balance between phosphorylation, induced by PERK in response to the ER protein load, and dephosphorylation induced by GADD34/PP1 (Fig. 2). Glucose-induced protein translation in β-cells results from a rapid (within 15 min) dephosphorylation of eIF2α by PP1 (184). On the other hand, prolonged (12–24 h) phosphorylation of eIF2α causes β-cell dysfunction and death (128).

Transient (1–3 h) exposure of β-cells to high glucose (10–25 mM) induces phosphorylation and activation of IRE1α (185). This effect requires glucose metabolism, but it is not accompanied by BiP dissociation from IRE1α or by XBP1 splicing. Prevention of IRE1α signaling by small interfering RNA decreases glucose-induced insulin biosynthesis, indicating that acute IRE1α activation is required for proinsulin biosynthesis (185). Prolongation of the time of exposure to high glucose to 3–7 d, however, induces a protracted IRE1α phosphorylation, which is accompanied by XBP1 splicing and progressive inhibition of insulin mRNA and protein expression (185). This decrease in insulin mRNA expression may also be secondary to mRNA degradation (66) (see *Section VI*).

These observations suggest that early activation of some components of the UPR, such as the PERK-eIF2α and IRE1α pathways, plays a physiological role in supporting proinsulin and total protein biosynthesis and in adapting the ER chaperone capacity to increased protein synthesis (181, 185). On the other hand, prolonged UPR activation may impair β-cell function and lead to apoptosis. The mechanisms for this transition from physiology to pathology remain to be clarified, but it may be related to XBP1 splicing (185) and to an IRE1-mediated degradation of insulin mRNA (66). The study of β-cell behavior after cessation of ER stress may shed

light on the mechanisms of recovery from an acute UPR (see *Section VI*).

IV. ER Stress and β-Cell Death in Monogenic and Type 2 Diabetes

Autopsy data suggest that the early and progressive decline in insulin secretion in type 2 diabetes is accompanied by a decrease in β-cell mass and that this is secondary to increased β-cell apoptosis (21). The putative mechanisms involved in β-cell failure and death in type 2 diabetes have been reviewed extensively (4, 23–25). Among the potential factors contributing to progressive β-cell loss, glucose, FFA, and islet amyloid polypeptide (IAPP) have been implicated as triggers of β-cell ER stress. The most convincing evidence for the role of ER stress in β-cell failure and diabetes comes from rare genetic disorders.

A. ER stress and human diabetes

A mutation in EIF2AK3, encoding the human eIF2α kinase (equivalent to rodent PERK), causes monogenic diabetes in Wolcott-Rallison syndrome, a rare disorder characterized by neonatal or early-infancy insulin-dependent diabetes (186). Epiphyseal dysplasia and developmental delay become apparent at later ages. The EIF2AK3 mutations identified so far result in a truncated or missense eIF2α kinase with little or no activity (187). Autopsy findings include pancreatic hypoplasia and β-cell loss (188). This disease highlights the importance of the PERK-mediated ER stress response in the regulation of normal β-cell function and survival, at least in neonatal life; its importance for β-cells in adult life has been questioned (189).

In Wolfram syndrome, mutations in Wolfram syndrome gene 1 (WFS1), which encodes an ER Ca^{2+} channel, lead to young-onset diabetes associated with selective β-cell loss, optic atrophy, sensorineural deafness, diabetes insipidus, and neurological manifestations (190).

Monogenic forms of diabetes may serve as "caricature" models for the identification of small effects of common gene variants in type 2 diabetes that have a polygenic and environmental cause (191–194). Genetic variation in the WFS1 gene has been associated with type 2 diabetes (195, 196). ATF6 has also been identified as a minor type 2 diabetes susceptibility gene in Pima Indians (197). In some cohorts, an association with insulin secretion was observed for a DNA variant that extends into the 5′ end of the ATF6 gene (198). Further studies are needed on the genetic variation in key UPR genes in type 2 diabetes and on possible gene–environment interactions.

More direct evidence for a role of ER stress in type 2 diabetes comes from a recent study by Laybutt *et al.* (199). Thus, a higher staining intensity was observed for BiP, CHOP, and $p58^{IPK}$ in β-cells from pancreatic sections of type 2 diabetic patients compared with nondiabetic pancreatic tissue (199), whereas another study demonstrated a 2-fold increase in ER size in β-cells from type 2 diabetic patients compared with nondiabetic patients (200). Increased staining for ATF3, downstream of eIF2α-ATF4 (92), was also shown in insulin-positive cells in pancreatic sections of type 2 diabetic patients (201). Huang *et al.* (202) recently reported increased CHOP expression in β-cells from obese individuals, whether diabetic or not. In the pancreatic sections of diabetic patients, CHOP was found to be more frequently localized in the nucleus as opposed to the cytoplasmic presence of CHOP in obesity (202). Based on these findings, it was speculated that CHOP nuclear translocation is a discrete and necessary step for apoptosis induction, although there are no published data in other models of ER stress to support this hypothesis. The factor triggering nuclear CHOP translocation was suggested to be IAPP because adenovirus-mediated human IAPP expression in INS-1 cells increased CHOP expression and nuclear translocation and DNA fragmentation (202). Unfortunately, no additional ER stress markers were examined in this study. CHOP can be induced by a variety of cellular stresses, and amyloid precursor protein was shown to induce CHOP expression in neurons in the absence of an UPR (203). Extracellular IAPP oligomers induced heat shock protein 90 in MIN-6 cells and human islets, and mild XBP1 splicing in MIN-6 cells. Inhibition of the ubiquitin-proteasome pathway was implicated in the IAPP-induced β-cell apoptosis (204). A recent study of the expression of ER stress markers in islets isolated from type 2 diabetic organ donors showed no marked differences compared with control islets after 3- to 6-d culture at 5 mM glucose. The islets from diabetic donors, however, induced BiP and XBP1 expression markedly after an increase to 11 mM glucose, whereas this response was absent in islets from nondiabetic donors (200).

B. Animal models of ER stress and diabetes

In the Akita mouse, the C96Y mutation in insulin-2 prevents formation of one of the disulfide bonds between the A and B chains (177). The misfolded proinsulin accumulates in the ER, is complexed to BiP, and is eventually degraded. Despite the presence of normal insulin-1 and of one normal insulin-2 allele in the heterozygous Akita mouse, the animals develop diabetes due to progressive β-cell loss caused by ER stress. In heterozygous, but not homozygous, Akita mice, the homozygous disruption of CHOP delayed diabetes development by 8–10 wk (205), suggesting that the cell death mechanism is partially CHOP-dependent. A recent report describes the C95S mutation in insulin-2 in the Munich mouse, causing loss of the intra-A chain disulfide bond (178). This leads to insulinopenic glucose intolerance and severe diabetes in heterozygous and homozygous mice, respectively (178). A mouse model of Wolfram syndrome has also been established. Deletion of the WFS1 gene leads to diabetes as a result of β-cell ER stress and apoptosis (206, 207). The WFS1 gene product is an ER Ca^{2+} channel induced during ER stress. It exerts an inhibitory effect on IRE1α, PERK, and ATF6 (208), thereby constituting another feedback loop to tone down the UPR. These observations indicate that both deficient and exaggerated responses to ER stress result in β-cell loss and diabetes.

Mice with a PERK deletion cannot phosphorylate eIF2α and attenuate insulin translation, and they develop diabetes within a few weeks after birth due to progressive β-cell loss (105, 106). As in Wolcott-Rallison syndrome, the

mice exhibit skeletal dysplasia, postnatal growth retardation, and exocrine pancreas insufficiency. To identify the cause of diabetes in PERK-deficient mice, Zhang *et al.* (189) generated diverse tissue- and cell-specific PERK-knockout mice and observed that PERK is of particular relevance for the fetal and early neonatal development of β-cell mass and function. Mice homozygous for a Ser51Ala substitution in eIF2α, precluding its phosphorylation by PERK or other kinases, die of hypoglycemia within hours after birth because of defective gluconeogenesis. The pancreas from these mice exhibits β-cell defects and insulin depletion at late embryonic and neonatal stages (209). These animal models show that lack of PERK-eIF2α signaling in β-cells is detrimental by hampering their ability to down-regulate insulin synthesis and thus adapt it to the prevailing ER protein handling capacity. The PERK-eIF2α pathway undergoes feedback inhibition via GADD34/PP1-mediated eIF2α dephosphorylation (95), and perhaps also by up-regulation of $p58^{IPK}$, an ER-resident cochaperone (100, 101, 79). $p58^{IPK}$-null mice develop diabetes as they reach adult age due to increased β-cell apoptosis (210). Although mice homozygous for a Ser51Ala substitution in eIF2α die shortly after birth, the heterozygotes are phenotypically normal. Challenged with a high-fat diet, however, the heterozygous Ser51Ala mice develop glucose intolerance (183). This is due to decreased islet insulin content and lesser nutrient-stimulated insulin secretion. In the β-cells, the ER appeared dilated, and increased amounts of proinsulin were bound to BiP, suggesting delayed proinsulin processing (183). In islets obtained from 10- to 12-wk-old diabetic db/db mice (which have a defective leptin receptor), ER stress markers were increased compared with control islets (199). There was more eIF2α phosphorylation; ATF4, CHOP, and $p58^{IPK}$ expression; and increased XBP1 splicing and BiP, GRP94, and ERp72 expression (199). Increased XBP1s expression may indicate "pathological" β-cell ER stress (185), reinforcing the idea that ER dysfunction contributes to the β-cell loss. It is not known whether ER stress is present in db/db islets in the prediabetic phase. A 4-wk treatment of db/db mice with exendin-4, a glucagon-like peptide 1 receptor agonist, led to a significant decrease in nuclear CHOP expression in β-cells and whole pancreas XBP1s levels, in parallel to a reduction in hyperglycemia (211). Interestingly, exendin-4 also protected β-cells *in vitro* against the synthetic ER stressors thapsigargin and tunicamycin (211). In these *in vitro* studies, however, this protection occurred in parallel to increased PERK and IRE1α signaling and with enhanced expression of ATF4, CHOP, and XBP1s. It was suggested that the resulting GADD34 induction (GADD34 is regulated by CHOP) and PP1c activation mediated the eIF2α dephosphorylation observed with exendin-4 and forskolin, thereby attenuating the ER stress response and allowing recovery of translational repression (211). Exendin-4 treatment also improved the glycemia of β-cell-specific calmodulin-overexpressing mice, which lose β-cells by apoptosis induced by nitric oxide (NO) production and ER stress (212, 213). In these animals, exendin-4 reduced BiP and CHOP expression and augmented islet insulin content (213).

C. Lipotoxicity and glucotoxicity as triggers of ER stress—when physiology turns into pathology

A high-fat diet and/or obesity may contribute to the development of type 2 diabetes by causing β-cell lipotoxicity and insulin resistance. FFAs activate an ER stress response in β-cells, with palmitate being more potent than oleate (128, 199, 214, 215). Palmitate leads to phosphorylation of PERK and eIF2α, inhibition of protein synthesis, and induction of ATF4 and CHOP (128, 215) (Fig. 3). CHOP induction by FFA is mediated by ATF4 binding to the C/EBP-ATF binding site in the CHOP promoter, as well as by c-Fos and Jun-B dimer binding to the activator protein-1 (AP-1) binding site (93). Palmitate also activates IRE1 (as evidenced by XBP1 splicing) and ATF6 (214) and up-regulates ER chaperones including BiP, GRP94, $p58^{IPK}$, ORP150, ERp72, Dnajb9, Herp, and Edem (128, 199, 214), although BiP induction was not observed in one study (215). Oleate does not activate the PERK pathway and is less effective in activating IRE1α, but it does induce ER chaperone expression (128, 214). That ER stress contributes to palmitate-induced β-cell apoptosis is supported by the observation that MIN-6 cells overexpressing BiP have a milder ER stress response and are partially protected against palmitate-induced apoptosis (199). The molecular mechanism by which FFA-induced ER stress causes β-cell apoptosis is not well understood. Because previous observations suggested that defective PERK-eIF2α activation contributes to β-cell death (209, 216–218), attempts were made to protect β-cells against FFA with salubrinal (128), a selective inhibitor of eIF2α dephosphorylation (126) (Fig. 3). Unexpectedly, salubrinal-induced eIF2α phosphorylation was proapoptotic in β-cells, and it specifically potentiated the deleterious effects of oleate and palmitate, but not of other ER stressors, through a synergistic activation of the PERK-eIF2α branch (128). The percentage of apoptosis in β-cells exposed to FFA increased by 3- to 6-fold in the presence of salubrinal, whereas no such potentiation was seen with cytokines, which also trigger ER stress (128) (see *Section V*), or the synthetic ER stressors cyclopiazonic acid (CPA) and thapsigargin (128). ER stress-mediated apoptosis in β-cells therefore seems to depend on both the ER stressor and the magnitude and duration of eIF2α phosphorylation and activation of downstream events. Both deficient and chronic excessive eIF2α phosphorylation are poorly tolerated by β-cells and trigger the apoptotic program.

High glucose (30 mM) also induced a modest (around 2-fold) activation of the UPR in cultured rat islets, triggering XBP1 splicing, expression of the ER chaperones BiP, GRP94, and Edem, and of PERK-dependent ATF3, CHOP, and GADD34 (181). This did not depend on Ca^{2+} influx or insulin release by the β-cells because this response was not affected by diazoxide (which opens the β-cell's ATP-dependent K^+ channels and thereby reduces Ca^{2+} influx and insulin release) or by clonidine (which inhibits Ca^{2+} influx) (181). The regulation of CHOP expression by glucose in β-cells is mediated by the MAPK ERK1 and 2 (219). It remains to be determined whether high glucose potentiates FFA-induced β-cell ER stress.

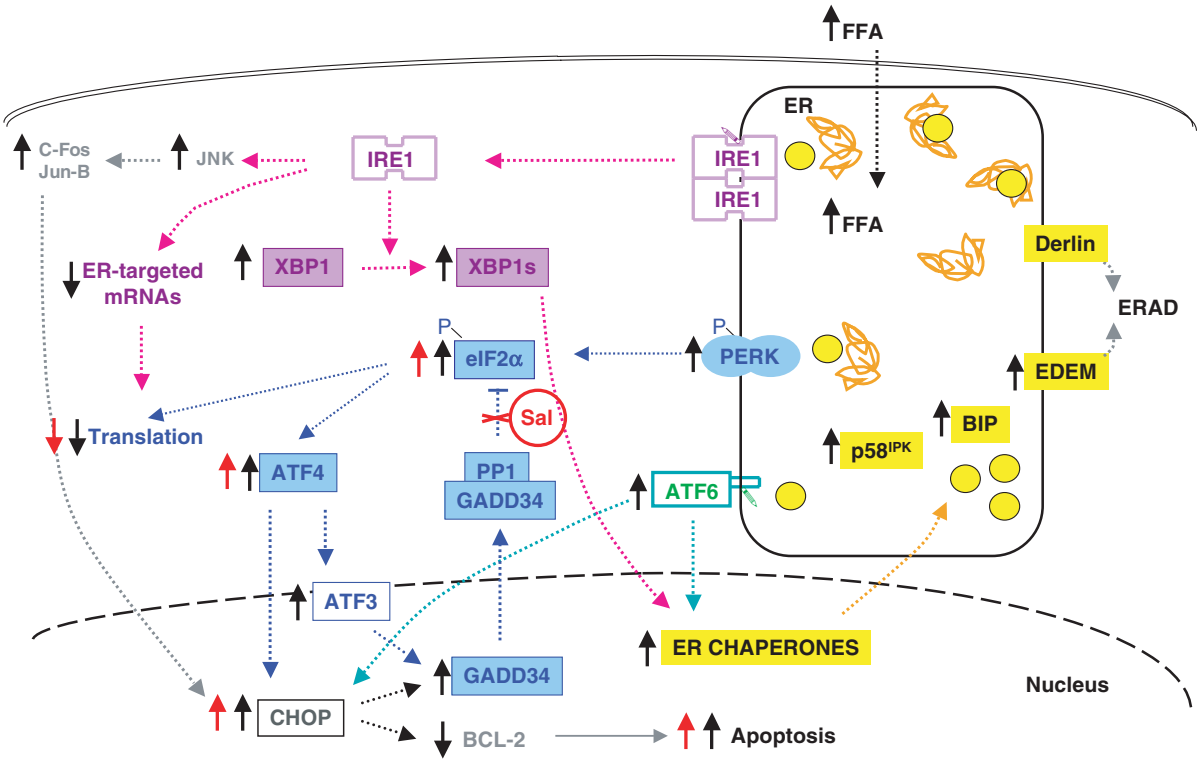

FIG. 3. FFA-induced ER stress response in β-cells. FFA effects are indicated by *short black arrows* and salubrinal potentiation by *short red arrows*. Palmitate and, to a lesser extent, oleate activate IRE1α, and both FFAs activate ATF6. The activation of PERK is observed only with palmitate. ATF6 and XBP1s increase ER chaperone expression, which may be beneficial for cell survival. IRE1 activates JNK and leads to formation of c-Fos/Jun-B AP-1 dimers, which will induce CHOP together with ATF4. The induction of CHOP or other downstream effectors of the PERK-eIF2α branch is probably proapoptotic because the potentiated activation of this pathway by salubrinal (sal), a selective inhibitor of eIF2α dephosphorylation, much increases FFA-induced β-cell apoptosis (128). Additional information and supporting references are provided in the text. EDEM, ER degradation enhancing α-mannosidase-like protein.

V. ER Stress and β-Cell Death in Type 1 Diabetes—the Role of Cytokines and Nitric Oxide (NO)

In type 1 diabetes, β-cell death precedes massive T cell infiltration in nonobese diabetic (NOD) mice (220) and insulin-dependent diabetes mellitus rats (221). In both animal models and human type 1 diabetes, β-cell apoptosis coincides with expression of cytokines such as IL-1β, interferon (IFN)-γ, and TNF-α by the infiltrating immune cells, and inducible NO synthase (iNOS) by both β-cells and immune cells (2, 221, 222), suggesting that these are early mediators of β-cell death. Under *in vitro* conditions, IL-1β and/or TNF-α, in combination with IFN-γ, induce NO production, severe functional suppression, and death of β-cells (2, 223–225). Cytokine-induced death in human, rat, and mouse β-cells, and in insulin-producing cell lines, occurs mostly by apoptosis (2, 25, 226), but there is also a minor NO-dependent necrotic component in rodent β-cells (227). Cytokine-triggered β-cell apoptosis is regulated by complex gene networks under the control of the key transcription factors NF-κB and STAT-1 (25, 228–233).

One of the cytokine-induced and NF-κB-regulated genes in β-cells is iNOS, leading to massive NO formation (2, 234). The chemical NO donor SNAP depletes ER Ca^{2+} in MIN-6 cells (180). Because Ca^{2+} is required for the protein binding and chaperoning ability of ER chaperones, severe ER Ca^{2+} depletion will impair the quality of ER protein folding and assembly (78) and trigger CHOP expression and apoptosis (180). IL-1β plus IFN-γ, via NO synthesis, decrease the expression of SERCA in primary β-cells and insulin-producing INS-1E cells, depleting ER Ca^{2+} stores (235). Inhibition of SERCA by the chemicals thapsigargin and CPA also triggers ER stress and apoptosis in β-cells, and these cells are more sensitive than fibroblasts to the proapoptotic effects of SERCA inhibition (235, 236). IL-1β and IFN-γ induce diverse components of the ER stress response, including activation of IRE1α, as observed by XBP1 splicing, and of eIF2α/ATF4/CHOP/Bim, but not ATF6 (93, 229, 230, 235) (Fig. 4). In line with the deficient ATF6 activation by IL-1β and IFN-γ, the cytokines failed to increase BiP expression (235). It is conceivable that this deprives the β-cells of an important mechanism for cell survival during ER stress, which could contribute to their susceptibility to cytokine- and NO-mediated apoptosis. That ER stress contributes to cytokine-induced cell death is supported by the recent finding that insulin-producing NIT-1 cells overexpressing BiP have a decreased CHOP induction and are partially protected against apoptosis induced by IL-1β and IFN-γ or cytotoxic T lymphocytes (237). In neuronal cells, NO induces s-nitrosylation and inhibition of PDI (238), thereby hampering proper protein folding and aggravating the ER stress (239). It remains to be tested whether NO has similar effects on PDI in β-cells. Interestingly, low concentrations of NO, as induced by ac-

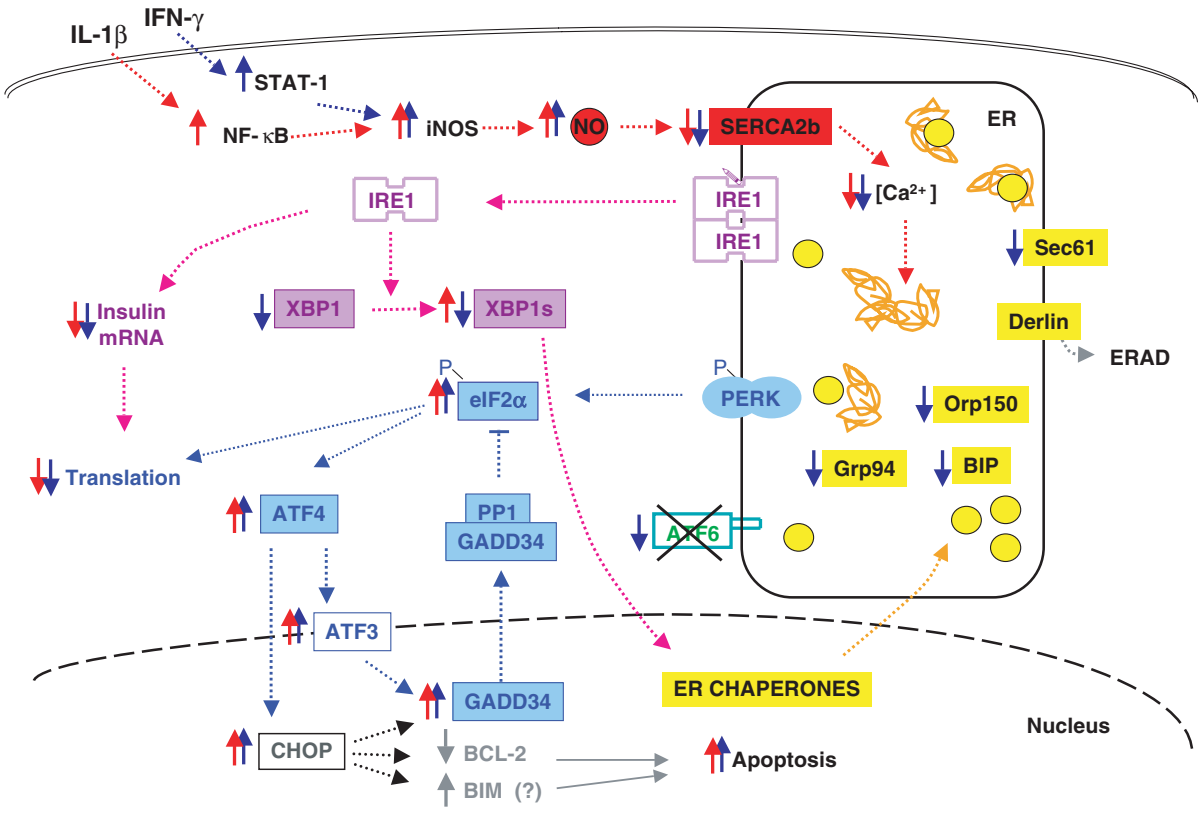

FIG. 4. Cytokine-induced ER stress response in β-cells. Effects of IL-1β are indicated by *short red arrows*, and those of IFN-γ by *short blue arrows* (IFN-γ alone does not up-regulate iNOS or ER stress markers, but it potentiates the effects of IL-1β). IL-1β and IFN-γ up-regulate iNOS in an NF-κB- and STAT-1-dependent manner, leading to NO accumulation. NO decreases SERCA2b expression and depletes ER Ca^{2+} stores. This leads to ER stress and activation of IRE1α and PERK, but not ATF6. The lack of ATF6 activation by IL-1β and the down-regulation of some components of the UPR and XBP1 splicing by IFN-γ result in unchanged or decreased ER chaperone expression. IRE1α degrades insulin-1 and -2 mRNAs, decreasing the ER protein load. eIF2α activation and ATF4 up-regulation contribute to CHOP induction. A prolonged cytokine-induced ER stress response may impair β-cell function and defense mechanisms, thereby tilting the balance between protective and detrimental effects of the UPR in the direction of apoptosis. Additional information and supporting references are provided in the text.

tivation of constitutive NOS by glucose, protect against ER stress by dissipating ROS (240), suggesting that the effects of NO on β-cell ER stress are concentration- and time-dependent.

IL-1β alone induces ER stress but fails to induce β-cell death, whereas IFN-γ by itself causes neither but it potentiates IL-1β- or CPA-induced cell death (2, 25, 235, 241). Treatment with IFN-γ decreases expression of XBP1s mRNA, several ER chaperones (BiP, GRP94, and ORP150, but not calnexin/calreticulin), and Sec61α, while augmenting CHOP and ATF-4 expression (241, 242). By decreasing ER chaperones, and thus protein folding and Ca^{2+} storage capacity (78), IFN-γ decreases β-cell defense against ER stress and favors the proapoptotic signals, such as CHOP and other ATF4-dependent genes. Other potential mechanisms by which IFN-γ synergizes with IL-1β to induce β-cell apoptosis are augmentation of IL-1β-induced iNOS expression (2, 234) and stimulation of expression of major histocompatibility complex classes I and II and of other components of the antigen processing machinery (242); because the MHC complex is assembled in the ER, this may contribute to the ER overload (243).

Activation of CHOP transcription after β-cell exposure to palmitate or cytokines depends on the binding of ATF4 and AP-1 to the CHOP promoter, but these two treatments induce formation of different AP-1 dimers (c-Fos and c-Jun and/or Jun-B in the case of cytokines, and c-Fos and Jun-B for palmitate) at different time points (93). In line with this, cytokines, but not palmitate, induce Ser-63 phosphorylation of c-Jun. CPA, but not cytokines or palmitate, activates the CHOP promoter via ER stress response element (93). These observations suggest that different pathways of the UPR are triggered in β-cells depending on the source and intensity of the ER stressor. The fate of the β-cells, death or survival, will depend on the balance between the ER stress and the UPR pathway(s) activated, their time course and intensity, and the adequacy of other β-cell defense mechanisms, such as the scavenging of ROS. Once the balance tilts for apoptosis, JNK, ATF3, and CHOP are potential mediators of β-cell death (180, 201).

Evidence for human islet ER stress exists for type 2 diabetes (199, 202), but this remains to be proven for type 1 diabetes. Compared with rat β-cells, NO is of less importance for cytokine-induced human β-cell death (244), and islet cells from iNOS−/− mice are only partially protected against cytokines (227, 245), pointing to non-NO (and putatively non-ER stress) cell death mechanisms (246) in human type 1 diabetes. Histological exam of autopsy material from eight type 1 diabetic patients (five with recent onset of diabetes and

three with long-standing disease) failed to show increased nuclear or cytosolic CHOP expression (202). These data should, however, be interpreted with caution. CHOP activation is only one among several markers of ER stress; on one hand, this protein can be activated in β-cells by toxic stimuli unrelated to ER stress, *e.g.*, after exposure to toxic doses of streptozotocin or other alkylating agents (133), and on the other hand cell populations adapted to chronic ER stress can maintain an activated UPR without displaying up-regulation of downstream genes such as CHOP (247). Furthermore, the pattern of islet infiltration and destruction is nonuniform in type 1 diabetes, suggesting that expression of β-cell ER stress markers should be evaluated in correlation with local infiltration by T cells and macrophages (which was not done in this study). For instance, expression of ATF3, another potentially proapoptotic gene regulated by ATF4, is present in β-cells adjacent to infiltrating lymphocytes in NOD mice, but not in β-cells away from the lymphocytes (201). Increased expression of ATF3 was also detected in β-cells from type 1 diabetic patients, but not in islets from nondiabetic individuals (201). ER stress may contribute to β-cell death in the early stages of the immune assault—which are probably cytokine-dependent (2)—but be less relevant after clinical diabetes onset, when other mechanisms predominate and there remain only "surviving" β-cells, which may be a selected cell population. Additional studies are required to define the extent of the ER stress and non-ER stress contributions to β-cell death in experimental models and human type 1 diabetes. These studies should ideally utilize more specific markers for ER stress, such as IRE1 activation, XBP1 splicing, BiP overexpression, and ATF6 activation and examine material from both prediabetic and diabetic patients, correlating local immune infiltration with expression of β-cell ER stress markers.

VI. β-Cell Recovery from ER Stress

As discussed above, β-cells are sensitive to ER stress, but this vulnerability is relative. Assuming that increased functional load *in vivo* augments the UPR in human β-cells, as suggested by increased CHOP expression in β-cells from obese nondiabetic patients (autopsy material) (202), it is remarkable that most obese individuals cope with decades of insulin resistance without developing β-cell failure and diabetes. Furthermore, islet cells surviving a 48-h exposure to IL-1β *in vitro* (248) or exposure to the autoimmune assault in NOD mice *in vivo* (249, 250) are able to recover function after an additional 6-d in culture without the cytokine (248) or the T cells (249, 250), and β-cells exposed for up to 12 h to a severe CPA-induced ER stress do not reach "the point of no return" for cell death (66). A time course microarray analysis in INS-1E cells exposed to CPA for up to 12 h, including an additional group of cells treated for 6 h and then allowed to recover without CPA for 3 h (66), indicated that the two groups of genes most affected by CPA were those related to cellular responses to ER stress, which were up-regulated, and those related to differentiated β-cell functions, which were down-regulated. After a 3-h recovery period, most genes returned to control levels, as for instance the proapoptotic transcription factors ATF3 and CHOP, whereas expression of the ER chaperones BiP and GRP94 remained elevated. This pattern of gene expression is probably due to the longer half-life of chaperones such as BiP, compared with proapoptotic genes such as CHOP (247), and may explain why β-cells can endure 12 h of severe ER stress without reaching the point of no return for cell death. The most marked inhibitory effect of CPA was on the expression of mRNAs for insulin-1 and -2. Similar findings were observed in INS-1E and primary β-cells exposed to thapsigargin or IFN-γ plus IL-1β (66). ER stress induces a rapid degradation of mRNAs targeted for translation at the ER in *Drosophila* cells (65). This degradation is mediated by IRE1α and complements other UPR mechanisms by decreasing production of nonvital proteins at the ER. The CPA-induced early degradation of insulin-1 and -2 occurred in parallel to IRE1 activation and in the absence of altered insulin promoter activity (66). It is conceivable that degradation of insulin mRNA, the most prevalent ER-targeted mRNA in β-cells, alleviates functional demand on the ER. This, together with an up-regulation of ER chaperones and down-regulation of proapoptotic genes, may contribute to β-cell survival once the source of ER stress is removed.

VII. ER Stress as a Putative Link between Obesity and Insulin Resistance

ER stress has been proposed as one of the molecular mechanisms linking obesity with insulin resistance and might thus be a common molecular pathway for the two main causes of type 2 diabetes, namely insulin resistance and β-cell loss. Since 2004, several papers were published on ER stress and insulin signaling, mostly in rodent liver, and the subject has been discussed in a series of reviews (251–255).

A. Liver

In high-fat-fed and ob/ob (leptin-deficient) mice, markers for ER stress (PERK and eIF2α phosphorylation and BiP expression) are increased in liver and fat (256) but not in muscle, which is the main site of glucose disposal. Synthetic ER stressors impaired proximal insulin signaling in hepatoma cells, increasing serine and decreasing tyrosine phosphorylation of insulin receptor substrate (IRS) 1 and reducing Akt phosphorylation (256). The alterations in the insulin signaling cascade were dependent on JNK activation, previously shown to mediate insulin resistance (257). ER stress and JNK activation were shown to increase glucose-6-phosphatase activity and glucose output in primary hepatocytes (258). IRE1 activation mediated this suppression of insulin signaling, probably through TRAF2 recruitment and JNK activation (120), but increasing cellular XBP1s levels had the inverse effect and favored insulin signaling (256). Conversely, XBP1-deficient fibroblasts, which are more sensitive to ER stress, exhibited impaired insulin signaling. $XBP1^{+/-}$ mice placed on a high-fat diet developed greater insulin resistance and glucose intolerance compared with high-fat-fed $XBP1^{+/+}$ mice, and this correlated with increased PERK, c-Jun, and IRS-1 serine phosphorylation and decreased tyrosine phosphorylation of the insulin receptor, IRS-1 and

IRS-2, in liver and fat (256). ER stress can also lead to NF-κB activation (122, 123, 259, 260). It is possible that ER stress activates IKK-β and NF-κB signaling in the liver (261), thereby inducing proinflammatory cytokines and consequently insulin resistance (262, 263). The study by Ozcan *et al.* (256) did not address how obesity induces ER stress signaling, but it can be speculated that it is lipid mediated. Thus, ER stress and insulin resistance were detected in high-fat-fed XBP1$^{+/-}$ mice but not in those fed normal chow. Saturated FFA were shown to induce sustained JNK activation and insulin resistance in hepatocytes both *in vitro* and when perfused into the liver *in vivo*, whereas hyperglycemia had no such effect (264).

In rats, sucrose- and saturated fat-enriched diets induced steatosis, characterized by increased liver content in saturated fatty acids, hepatic ER stress marker expression, and caspase 3 activation. In contrast, polyunsaturated fat diets that induce steatosis without increased liver accumulation of saturated fat, did not induce hepatic XBP1 splicing, BiP expression, or liver injury (265). The induction of ER stress and steatosis in rats fed the sucrose- and saturated fat-enriched diets occurred early (after 1 wk on the diet), before obesity and independently of changes in insulin action. The accumulation of saturated fat probably triggers the ER stress response, and ER stress may reciprocally contribute to steatosis through the activation of sterol-response element-binding proteins (SREBP). Under normal circumstances, cholesterol deprivation leads to SREBP migration from the ER to the Golgi apparatus where SREBP are proteolytically activated by S1P and S2P (266), similar to the mechanism of ATF6 activation (267), and induce lipogenic genes. ER stress also activates SREBP in hepatocytes exposed to homocysteine and thereby induces cholesterol and triglyceride synthesis and steatosis (268), whereas BiP overexpression inhibits SREBP activation and expression of its downstream genes (268). In rat islets, synthetic ER stressors also activate SREBP, and this was suggested to contribute to β-cell glucolipotoxicity (269).

Increased BiP expression was also detected in the liver of obese db/db (leptin receptor-deficient) mice (270), although low ER chaperone expression levels were observed in these mice in another study (271). In the db/db mice, hepatic overexpression of the ER chaperone ORP150 was induced with a sense ORP150-encoding adenovirus (270). The glycemia of these mice decreased already 2 d after viral injection. Two weeks later, their insulin sensitivity and glucose tolerance had improved and hepatic glucose output was suppressed as a result of improved insulin signaling and decreased expression of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Conversely, antisense ORP150 virus administration in wild-type mice resulted in loss of IRS-1 tyrosine phosphorylation and increased gluconeogenesis and insulin resistance (270). Further evidence for the role of ORP150 in regulating insulin sensitivity comes from a study in which ORP150$^{+/-}$ or systemic ORP150 transgenic mice were crossed with heterozygous Akita mice. Heterozygous ORP150 deficiency in Akita mice impaired glucose tolerance, which was related to decreased insulin action possibly due to increased sensitivity to hyperglycemia-induced oxidative stress (272). Akita mice overexpressing ORP150 (around 2-fold increase in pancreas and fat, 3-fold in liver, and 8-fold in muscle) had improved glucose tolerance, due to improved insulin sensitivity, but β-cell-specific ORP150 overexpression (under the rat insulin promoter) did not improve glucose levels (272). It is conceivable that overexpression of specific ER chaperone(s) may be beneficial in one cell type and without effect, or perhaps even detrimental, in another.

Another approach to ameliorate insulin resistance by targeting ER stress has been the use of chemical chaperones, low molecular weight osmolytes that stabilize proteins and improve their folding in and export from the ER (273). Treatment of ob/ob mice with the chemical chaperones 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid improved insulin sensitivity and glycemia within 10 d of treatment through decreased hepatic glucose production and greater glucose disposal in muscle and fat (274). The chaperones alleviated ER stress, decreasing PERK and IRE1 phosphorylation, reduced c-Jun phosphorylation by JNK, and improved insulin signaling as shown by increased tyrosine phosphorylation of the insulin receptor, IRS-1 and IRS-2, in liver and fat (274).

B. Adipose tissue

Recent data suggest that hypoxia in adipose tissue of obese mice contributes to the induction of ER stress and thereby affects adipokine production (275). CHOP and BiP were increased and adiponectin expression was decreased in adipose tissue from high-fat-fed and KKAy mice. This was replicated *in vitro* in hypoxia-exposed adipocytes, and interference with CHOP expression partially reversed the decrease in adiponectin mRNA levels (275). Evidence for a role of ER stress in human tissue is presently not available, but increased expression and phosphorylation of stress-activated kinases such as p38 and JNK were detected in omental, but not sc, fat from obese women compared with lean controls (276). Although there was no change in serine or insulin-stimulated tyrosine phosphorylation of IRS-1 in the omental fat tissue, the activation of the stress kinases correlated with the patients' glucose levels and insulin resistance.

VIII. Future Areas of Research

The UPR was discovered nearly 20 yr ago, the first indications that ER stress might contribute to diabetes were published 6–7 yr ago, and there has been an exponential growth in the field since then. As presently reviewed, it seems possible that ER stress and the UPR have physiological and pathophysiological roles in β-cells and insulin signaling. We should keep in mind, however, that the mechanisms causing β-cell dysfunction and death in diabetes are complex (25), and ER stress is probably only one of several factors contributing to β-cell loss in diabetes. ER stress also seems to play a role in high fat- and obesity-induced insulin resistance in liver, at least in rodent models. Its role in adipose tissue is not well documented yet, and, from currently available data, ER stress does not seem to play a role in muscle insulin resistance. In conclusion, time and the accumulation of novel experimental data will confirm or disprove the hypothesis

that ER stress contributes to the pathogenesis of diabetes and other chronic degenerative diseases.

We outline below some of the areas where future research may contribute to clarify the role for ER stress in diabetes:

1) Are there additional components of the UPR/ER stress response with an important role for the regulation/dysregulation of insulin biosynthesis and release?

2) Which are the mechanisms by which chronic exposure to FFA and/or high glucose trigger an ER stress response?

3) Is ER stress relevant for β-cell death in human type 1 diabetes? Studies on human and mouse islets, and on histological preparations from type 1 diabetic patients and rodent models of autoimmune diabetes are required to clarify whether there is indeed a role for ER stress in type 1 diabetes.

4) How does IFN-γ inhibit ER chaperones? Are there common and novel binding sites in the promoter region of these genes for IFN-γ-induced transcription factors?

5) Which are the downstream mediators of ER stress-induced β-cell death? Are these mediators different for FFA and cytokines, or is there a "final common" pathway for β-cell death?

6) Is apoptosis the only form of ER stress-induced β-cell death, or is there a role for autophagy?

7) Is it possible to boost β-cell defenses against ER stress without affecting their exquisite ability to sense glucose and release insulin?

8) Is there a role for the UPR in the physiology of adipocytes and hepatocytes, as recently suggested for the β-cells?

9) Does ER stress play a role for insulin resistance in human type 2 diabetes? If yes, which are the tissues involved?

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