Living Dangerously: Protective and Harmful ER Stress Responses in Pancreatic β-Cells

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Type 2 diabetes (T2D) is a growing cause of poor health, psychosocial burden, and economic costs worldwide. The pancreatic β-cell is a cornerstone of metabolic physiology. Insulin deficiency leads to hyperglycemia, which was fatal before the availability of therapeutic insulins; even partial deficiency of insulin leads to diabetes in the context of insulin resistance. Comprising only an estimated 1 g or <1/500th of a percent of the human body mass, pancreatic β-cells of the islets of Langerhans are a vulnerable link in metabolism. Proinsulin production constitutes a major load on β-cell endoplasmic reticulum (ER), and decompensated ER stress is a cause of β-cell failure and loss in both type 1 diabetes (T1D) and T2D. The unfolded protein response (UPR), the principal ER stress response system, is critical for maintenance of β-cell health. Successful UPR guides expansion of ER protein folding capacity and increased β-cell number through survival pathways and cell replication. However, in some cases the ER stress response can cause collateral β-cell damage and may even contribute to diabetes pathogenesis. Here we review the known beneficial and harmful effects of UPR pathways in pancreatic β-cells. Improved understanding of this stress response tipping point may lead to approaches to maintain β-cell health and function.

Pancreatic β-cells are the sole source of circulating insulin in the human body. Each cell is an insulin production powerhouse, packed with vesicles containing preformed insulin ready for secretion into the bloodstream in response to a meal. Distributed throughout the cytoplasm are strands of rough endoplasmic reticulum (ER), the initial site of synthesis, disulfide pairing, folding, and quality control of the proinsulin molecule (1). Under stimulatory conditions, one β-cell can synthesize many thousands of preproinsulin molecules per second, up to 50% of total protein synthesis, representing a high energetic and proteostatic burden (1). As such, the pancreatic β-cell relies heavily on the processes that reshape ER synthetic capacity in response to stress, known collectively as the unfolded protein response (UPR) (2).

Overview of the Cellular Response to ER Stress

UPR pathway roles in β-cell health and disease have been previously reviewed (2–5). The UPR comprises three signaling arms that sense ER stress and implement protective changes such as temporarily reducing new peptide folding load and increasing ER functional capacity through transcriptional upregulation of chaperones, oxidoreductases, and quality control proteins (1,2) (Fig. 1). The UPR pathways are named for the ER transmembrane proteins that initiate responses: protein kinase R-like ER kinase (PERK) (Fig. 2), inositol-requiring enzyme 1 (IRE1) (Fig. 3), and activating transcription factor 6 (ATF6) (Fig. 4). PERK, IRE1, and ATF6 activate when GRP78, an abundant ER chaperone that complexes with them under low stress conditions, is titrated away when ER stress increases (6). PERK phosphorylates eukaryotic translation initiation factor 2 subunit 1 (eIF2α), a key regulator of translation initiation, thus slowing translation of many transcripts and transiently reducing ER peptide load. Paradoxically, phosphorylation of eIF2α increases translation of other transcripts, including activating transcription factor 4 (ATF4) (7). The IRE1 and ATF6 arms have overlapping downstream effects. Upon ER stress activation, IRE1 oligomerizes and autophosphorylates, activating kinase and endoribonuclease activities in its cytoplasmic domain. A key downstream effector of IRE1 is the sXBP1 transcription factor, which is generated following splicing of *Xbp1* transcript at the ER

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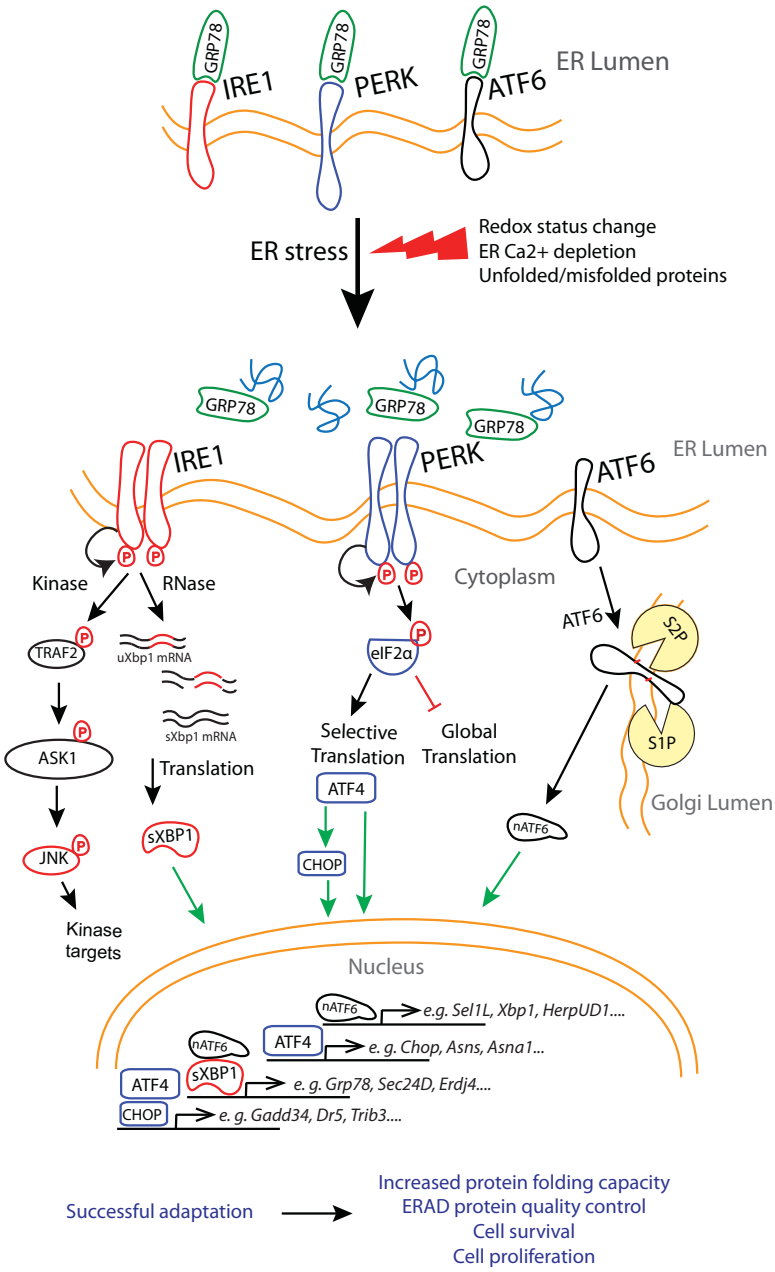

Figure 1—Overview of UPR stress response pathways. In unstressed conditions, the three UPR master regulators IRE1, PERK, and ATF6 are held in inactive monomeric form by the GRP78 chaperone. When ER stress increases, GRP78 complexes with misfolded proteins, allowing IRE1 and PERK to dimerize, autophosphorylate, and activate and releasing ATF6 to transit to Golgi to activate. Active IRE1 has both kinase and RNase activities, leading to a phosphorylation cascade culminating in phosphorylation of JNK, and *Xbp1* splicing to generate the sXBP1 transcription factor, respectively. Active PERK phosphorylates eIF2α leading to suppression of global translation but a selective increase in translation of transcripts such as *Atf4*, to generate the ATF4 transcription factor. ATF6 is cleaved in the Golgi in SREBP-like fashion by proteases S1P and S2P, releasing its cytoplasmic domain as the nATF6 transcription factor. Much of the adaptive response to ER stress is implemented by transcriptional upregulation of genes that increase capacity for protein folding and clearance of misfolded proteins. A few commonly examined transcriptional targets are included. Green arrows indicate transcriptional regulation. ASK1, apoptosis signal regulating kinase 1; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; CHOP, C/EBP homologous protein; eIF2α, eukaryotic translation initiation factor 2 subunit 1; GRP78, glucose regulated protein 78; IRE1, inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; nATF6, nuclear ATF6; P, phosphorylation; PERK, protein kinase R-like ER kinase; S1P, site 1 protease; S2P, site 2 protease; sXbp1, spliced Xbp1; TRAF2, TNF receptor–associated factor 2; uXbp1, unspliced Xbp1; XBP1, X-box binding protein 1.

membrane. ATF6α, in contrast, is activated in an SREBP1-like mechanism: ER stress frees ATF6α to proceed to the Golgi, where it is cleaved by S1P and S2P proteases to release the cytoplasmic domain, a transcription factor. In β-cells, as in other cell types, ATF6α and sXBP1 cooperatively regulate genes (8).

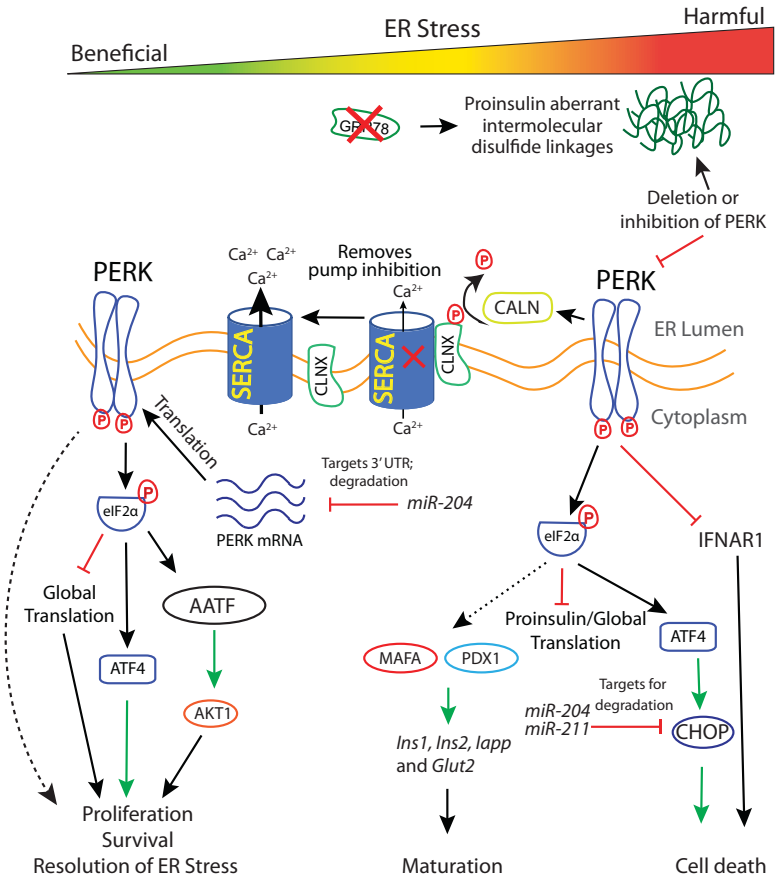

Figure 2—Beneficial and harmful effects of PERK activation. Normal PERK function is required for proinsulin folding, ER calcium balance, translation regulation, and cell survival, but PERK activation can also lead to cell death and loss of maturation markers. Activated by ER stress, low glucose, and low ER calcium, PERK phosphorylates eIF2α to simultaneously suppress global translation but selectively activate translation of some transcripts such as *Atf4* and *Aatf*. Beneficial effects of PERK activation include suppression of translation when the ER is overloaded with peptide inputs, promotion of cell survival through activation of ATF4 and AATF, improved SERCA pump function by dephosphorylation of CLNX to derepress calcium transport, and proliferation. Harmful effects of PERK activation include suppression of translation of required genes including maturation factors and insulin itself (although PERK deletion also caused loss of MAFA and PDX1), as well as increased cell death through CHOP and inflammatory pathways. Green arrows indicate transcriptional regulation. AATF, apoptosis antagonizing transcription factor; AKT1, protein kinase B; ATF4, activating transcription factor 4; CALN, calcineurin; CHOP, C/EBP homologous protein; CLNX, calnexin; eIF2α, eukaryotic translation initiation factor 2 subunit 1; GRP78, glucose-regulated protein 78; *Iapp*, islet amyloid polypeptide precursor; IFNAR1, interferon α and β subunit 1; *Ins1/2*, insulin 1/2; MAFA, Maf bZIP transcription factor A; circled P indicates phosphorylation; PDX1, pancreatic and duodenal homeobox 1; PERK, protein kinase R-like ER kinase; UTR, untranslated region.

Although all three arms of the UPR are important for acute cellular adaptation to ER load, they can also lead to paradoxical cellular harm (Figs. 2–4). Here we review current knowledge of the beneficial and harmful effects of UPR pathway activation in β-cells. We include studies in which UPR pathway activation was modulated but note that the observed impacts may be attributable to composite effects of the modulated pathway itself, the impact of the modification on cellular stress, and compensatory activation of other UPR pathways. We mostly exclude studies that simply quantified UPR pathway activation and studies only in cell lines. Given the breadth and depth of the topic and limitations of this article type, we apologize for work we were unable to include.

Disease Relevance: ER Stress and Stress Response Pathways Impact Diabetes Risk

Irremediable ER stress is harmful to β-cells. Misfolding proinsulin mutations such as the Akita variant cause diabetes in mice and humans (9,10), with insulin production failure due to a dominant negative proteotoxicity even when only one of four *Ins* alleles is affected in mice. β-Cell death by apoptosis ensues despite activation of all three stress response pathways (11). Wolfram syndrome is a rare monogenic cause of syndromic diabetes caused by pathogenic mutant forms of the ER-localized WFS1 protein (12). ER stress response pathways are also activated in islets from individuals with both type 1 diabetes (T1D) and type 2 diabetes (T2D) (e.g., 5,13); however, evidence supports a protective role for UPR pathways in the

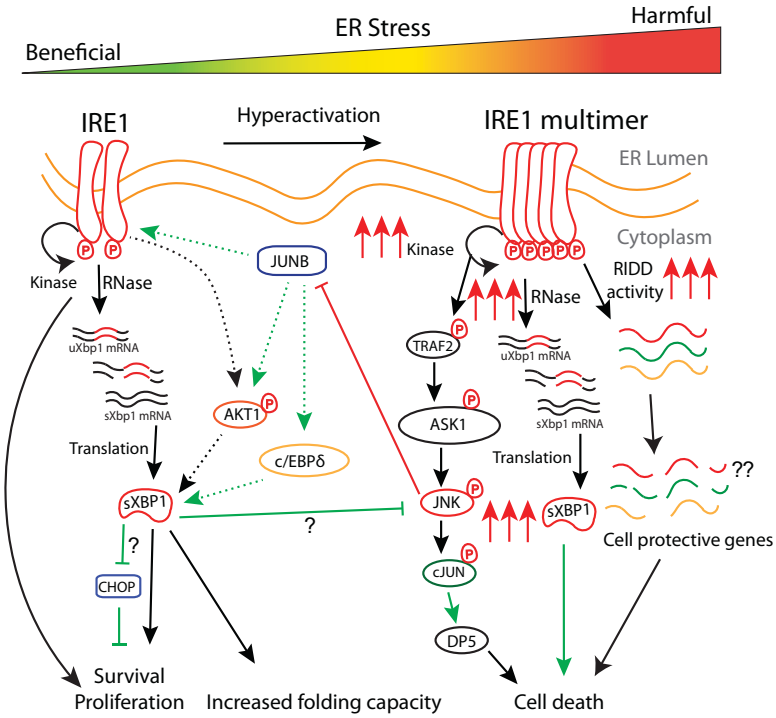

Figure 3—Downstream effects of IRE1 activation. Activation of IRE1α by dimerization and autophosphorylation leads to activities by the resulting dimer and by higher-order multimers, which are thought to have enhanced activity. Beneficial effects of IRE1 (increased folding capacity, survival and proliferation) are mostly due to RNase-induced splicing of *Xbp1* to generate the sXBP1 transcription factor. RNase activity can also be harmful in the setting of unregulated degradation of ER-associated RNAs in a process called RIDD, which cleaves a number of mRNAs including some related to β-cell maturation status and cell survival. IRE1α kinase activity results in a TRAF2-ASK1-JNK kinase cascade, leading to excess cell death by multiple mechanisms. Green arrows indicate transcriptional regulation. ASK1, apoptosis signal regulating kinase 1; CHOP, C/EBP homologous protein; DP5, death domain protein 5; IRE1, inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; circled P indicates phosphorylation; TRAF2, TNF receptor–associated factor 2; Xbp1, X-box binding protein 1; uXbp1, unspliced Xbp1; sXbp1, spliced Xbp1.

prevention of diabetes. Most conclusively, disruption of PERK pathway signaling leads to β-cell failure and diabetes. Loss-of-function mutations in *PERK* itself cause diabetes as part of Wolcott-Rallison syndrome; inheritance is recessive, and severity is proportionate to impairment in kinase function (14). Similarly, a mutation preventing phosphorylation at serine 51 of eIF2α, the immediate downstream target of PERK, causes glucose intolerance due to insulin deficiency even in the heterozygous state (15,16). Mutation in a PP1 phosphatase cofactor that reduces eIF2α phosphorylation is also linked to insulin-deficient diabetes in humans (17). Taken together, evidence supports an important role for the PERK pathway in diabetes prevention; roles of the IRE1 and ATF6 pathways in human β-cell health and disease are less well understood.

Beneficial Effects of UPR Pathways on β-Cells

The critical determinants of insulin secretion capacity are β-cell function and β-cell mass. β-Cell function is defined by maturation status, insulin production, glucose sensing, and insulin secretion, and β-cell number is determined by developmental accrual and the balance between β-cell death and new β-cell generation from proliferation and other sources (18). Reviewed in this section are studies defining the beneficial impact of UPR on β-cell function and number, such as improving insulin synthesis and secretion mechanisms, the adaptive increase in β-cell replication observed under conditions of moderate stress, and avoidance of cell death.

Beneficial Effects of UPR Pathways on β-Cell Differentiation Status

An important prerequisite to healthy insulin secretory function is an adequate complement of properly differentiated β-cells in pancreatic islets. To achieve this, robust embryonic and postnatal development and maintenance of differentiated characteristics in adult cells are required. Decompensated ER stress impairs postnatal β-cell maturation (19) and may also lead to dedifferentiation of adult β-cells (2,4). Surprisingly little direct evidence or molecular mechanisms are available, however. Healthy mouse islets engrafted into diabetic mice had reduced expression of UPR proteins and reduced mRNA levels of β-cell differentiation markers *Pdx1*, *Neurod1*, *Nkx6*0.1, and *Mafa*, suggesting indirectly that failure of the UPR may contribute to dedifferentiation (20). Loss of PERK signaling decreased expression of the maturation markers *Pdx1* and *Mafa*, as well as their target genes *Ins1*, *Ins2*, *Iapp*, and *Glut2*, in islets

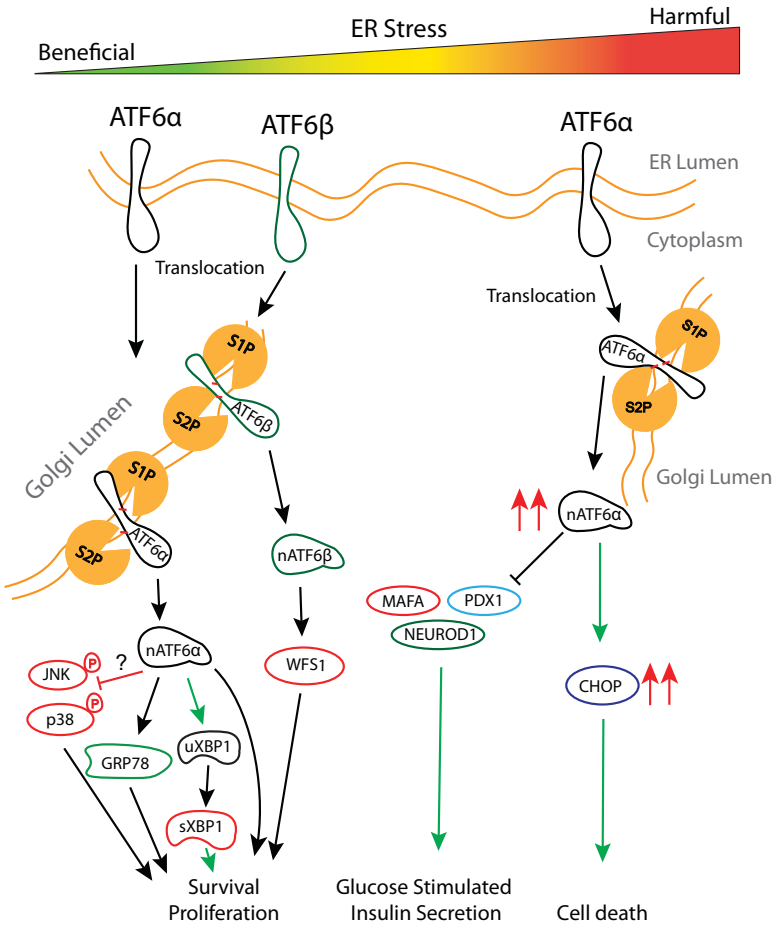

Figure 4—Cellular impact of downstream effects of ATF6 activation. Upon ER stress, ATF6α and related ATF6β are released from tethering in the ER membrane by ER luminal GRP78 and thus allowed to proceed to the Golgi, where they undergo regulated intramembrane proteolysis by S1P and S2P, releasing the respective cytoplasmic domains, nATF6α and nATF6β. The nATF6α transcription factor is more transcriptionally potent but also has a short half-life due to rapid degradation. nATF6α and nATF6β have overlapping transcriptional signatures; both contribute to cell survival signaling as well as chaperone, redox, and ERAD support. Atf6α promotes β-cell proliferation: overexpression increases and inhibition or knockdown decreases β-cell cycling in islet cell cultures. ATF6β induces WFS1, which has prosurvival activity. ATF6α also transcriptionally induces *Chop* and, as such, may contribute to cell death in some contexts. Green arrows indicate transcriptional regulation. ATF6, activating transcription factor 6; nATF6, nuclear ATF6; CHOP, C/EBP homologous protein; GRP78, glucose regulated protein 78; JNK, c-Jun N-terminal kinase; circled P indicates phosphorylation; S1P, site 1 protease; S2P, site 2 protease; WFS1, Wolfram syndrome 1.

of mice expressing the *Ser51Ala* mutant of eIF2α, suggesting that eIF2α phosphorylation helps maintain β-cell maturity (21). Chronic hyperglycemia may also have contributed in these settings. On the other hand, dedifferentiation has been proposed as a possible protective mechanism against autoimmunity in T1D (22). β-Cell deletion of IRE1α in NOD mice was reported to confer protection against autoimmunity by a mechanism involving β-cell dedifferentiation. Suggestively, β-cell autoantigens and MHC class 1 components were decreased, immune inhibitory markers were increased, and fewer $CD8^+$ cells were observed in the pancreas, although direct studies on cell-surface MHC class 1 and T cell repertoires have not yet been assessed (22).

UPR Pathways Support Proinsulin Synthesis and Folding

Proinsulin misfolding and aggregation contribute not only to rare monogenic mutant-insulin forms of diabetes but also to common T2D due to a "proinsulin-impacted-ER" phenotype caused by bulk misfolding of proinsulin during stress leading to toxic intermolecular disulfide linkages among proinsulin molecules (23,24). The GRP78 chaperone is required for proinsulin processing; cleaving GRP78 using the bacterial SubAB protease increased misfolded proinsulin disulfide-linked complexes in murine and human islets (23). All three UPR pathways contribute to the ER folding and oxidoreductase environment through regulation of translation and abundance of chaperones, oxidoreductases, and ER-associated protein degradation (ERAD) (2).

Intact PERK activity is important for proper proinsulin folding. Acute inhibition of PERK using GSK2606414, a high-affinity ligand that binds to the PERK kinase domain and competes for ATP, caused accumulation of misfolded proinsulin in the form of high–molecular weight disulfide-linked complexes (25). Aberrant disulfide-bonded proinsulin complexes after PERK inhibition have been confirmed in

human islets and INS1E cells (23,24). Since a major role of PERK is to slow peptide translation via phosphorylation of eIF2α, the misfolding caused by PERK inhibition was assumed to be due to excess proinsulin peptide entering the ER. However, although PERK inhibition modestly increased proinsulin synthesis across a population of β-cells in culture (24,25), when only ER-impacted cells were analyzed protein synthesis was lower compared with that of less impacted cells (24). The authors speculate that proinsulin aggregation after PERK inhibition may be more attributable to insufficient chaperone abundance and foldase activity by modulating calcium flux and redox state (24); alternatively, ER-impacted cells may represent a late, failing stage. PERK also supports ER chaperones and ERAD retrotranslocation of peptides out of the ER for proteasomal degradation (26). On the other hand, beneficial effects were observed after PERK inhibition in rat islets or in eIF2α phosphorylation-deficient β-cells (21,25). Differences may relate to methodology, experimental models, or timing.

PERK contributes to the beneficial reduction of insulin production occurring in low glucose conditions (25,27). Low glucose decreases ATP levels, inhibiting activity of the SERCA pump and lowering ER calcium levels, causing PERK activation (27). The decrease in both proinsulin and global protein synthesis observed in low glucose was partially reversed by an active form of GADD34, a scaffold that recruits the PP1 phosphatase to restore unphosphorylated eIF2α; cell death was also increased (27). In sum, ER calcium depletion–mediated PERK activation in low glucose conditions may be cytoprotective of β-cells and reduce potentially harmful insulin production during hypoglycemia.

IRE1α promotes ER protein folding capacity via its principal downstream transcriptional effector, sXBP1, by inducing genes responsible for ER peptide import, chaperones, glycosylation, disulfide redox status, quality control, and degradation (28). β-Cell–specific IRE1 deletion in mice reduced proinsulin translation, decreasing pancreatic proinsulin and insulin content and postprandial insulin secretion, with reduced mRNA levels of proteins involved in ribosome recruitment to the ER and in mRNA processing (29). Similarly, β-cell *Xbp1* deletion caused hyperglycemia due to a marked decrease in pancreatic insulin content (30). This phenotype was associated with downregulation of the *Ins1* gene along with genes involved in insulin secretion such as *PC1*, *PC2*, *CPE*, and Synaptophysin. XBP1 was not found at the promoters of these genes, and it was postulated that the mechanism involved compensatory IRE1 hyperactivation and unregulated endonuclease activity leading to mRNA degradation (30).

The role of ATF6α in insulin production is less clear. Similar to XBP1, ATF6α targets include chaperones, disulfide redox enzymes, and some quality control and degradation factors (28). ATF6α whole-body deletion reduced pancreatic insulin content in high-fat diet–fed mice, and deletion of ATF6α in Akita mice ($Ins2^{WT/C96Y}$) accelerated the reduction in pancreatic insulin content (31). Knockdown of ATF6α significantly worsened the impact of PERK inhibition on proinsulin aggregation, by derepressing proinsulin synthesis and reducing chaperone levels such as GRP78 (24). These reports suggest that ATF6α helps sustain insulin production under ER stress conditions.

UPR Pathways Promote Insulin Secretory Function

PERK inhibition reduced glucose-stimulated insulin secretion (GSIS) in INS1 832/13 cells, rat islets, and human islets (32), by a mechanism involving PERK regulation of glucose-induced calcium influx. Inhibiting PERK did not reduce voltage-dependent calcium channel flux but, rather, reduced store-operated Ca^{2+} entry and SERCA activity. PERK was found to bind to and activate calcineurin, which in turn dephosphorylated (inactivated) calnexin, releasing SERCA inhibition (Fig. 2). Therefore, PERK positively impacts GSIS by maintaining healthy glucose-responsive calcium signaling. The IRE1 pathway also promotes β-cell ER calcium health. Monogenic diabetes gene hepatocyte nuclear factor 4α (HNF4α) is required for basal transcription of *Xbp1* (33,34). In HNF4α-deficient β-cells, reduced expression of *Xbp1* diminished *SERCA2b* levels, impairing ER Ca^{2+} homeostasis and GSIS (34). Less is known about roles of ATF6α in insulin secretion, but one study found ATF6α overexpression to improve insulin secretion in thapsigargin-treated Min6 cells (35).

UPR Pathways Increase β-Cell Number Through Proliferation

Several studies suggest a positive correlation between UPR activation and the proliferative state in human and mouse β-cells (36–38). In a mouse model of Wolcott-Rallison syndrome low β-cell mass due to reduced β-cell proliferation during a critical developmental window, e16.5 through P8, was reported. No differences in β-cell death or apoptotic genes were observed (39). In a follow-up study, the same group tested whether loss of PERK impaired islet tumorigenesis. Crossing PERK-deleted mice with a β-cell inducible SV40 large T-antigen strain revealed insulinomas in control mice but near-complete suppression of tumor formation in PERK-deleted mice, due to reduced proliferation without an increase in β-cell death. Similarly, CRISPR-generated PERK deletion, or chemical inhibition of PERK, in INS-1 cell-derived tumors markedly reduced the number of nuclei staining for KI67 (40). These studies link loss of PERK to loss of β-cell proliferation in both fetal and adult stages. The opposite result was obtained in a different study, however, in which acute deletion of PERK in adult mice resulted in rapid hyperglycemia and loss of islet mass, but histological analysis showed an increase in both death and proliferation, along with increased abundance of cyclin D1 (41). Reduction of PERK gene dosage by one-half also caused increased β-cell proliferation in a different study (42). Finally, inhibition of PERK using a small molecule neither

increased nor decreased glucose-induced β-cell proliferation (37). The apparently contradictory results of these studies may be explained by differences in the degree or duration of ER stress experienced under each condition, the extent of compensatory activation of other UPR pathways, other factors intrinsic to each model system, and the physiological and nutritional context. In sum, despite many excellent studies, it remains unclear whether the PERK pathway influences β-cell replication.

Evidence is stronger that the IRE1/XBP1 and ATF6 pathways support β-cell proliferation. Deletion of XBP1 in mice resulted in diabetes, due to impaired insulin production but also with loss of β-cell mass that was associated with reduced proliferation without a change in cell death (30). Similarly, deletion or inhibition of IRE1α reduced KI67 positivity in INS-1–derived tumors (40). In mouse islet cells ex vivo, both increasing XBP1 (by overexpression) and decreasing XBP1 (by chemical inhibition or genetic knockdown) reduced β-cell cycle entry, suggesting that XBP1 activity promotes proliferation but only within a narrow window (37). ATF6α, on the other hand, may positively regulate β-cell proliferation within a broader range. Inhibition or knockdown of ATF6α in mouse or human β-cells reduced cell cycle entry (37). Overexpression of ATF6α increased β-cell proliferation but only in conditions where glucose was in excess (37). Whole-body deletion of ATF6α caused subtle glucose intolerance in mice with diet-induced obesity, with insulin deficiency; β-cell mass and proliferation were not reported (31). Finally, derepression of ATF6α by deletion of C/EBPβ, a bZIP transcription factor that physically interacts with ATF6α and suppresses its transcriptional activity, led to markedly (15-fold) expanded β-cell mass in vivo in leptin receptor–deficient mice (43).

These studies suggest the possibility that β-cell intrinsic activation of UPR pathways can, in some contexts, trigger proliferation to adaptively increase β-cell mass. Some observations contradict this concept, however. Deletion of all four insulin alleles in adult mice resulted in hypoinsulinemic diabetes, as expected; PERK and IRE1 pathways were suppressed, but β-cell proliferation was increased (44). The authors carefully assessed the impact of insulin gene deletion on β-cell proliferation in various contexts in which exogenous glucose levels were normal and consistently observed higher proliferation in β-cells with insulin gene deletion (44), suggesting that insulin biosynthesis suppresses β-cell proliferation under euglycemic conditions. How this relates to the hyperglycemic environment, when both insulin biosynthesis and β-cell proliferation are increased, remains uncertain.

Cell Survival: Stress Response Pathways Keep β-Cells Alive in the Face of Stress

Since the primary function of UPR pathways is to enable cells to adapt to stress, and ER stress causes death if not remedied, it is not a surprise that all three UPR pathways play a cytoprotective role in β-cells. Here we review published evidence that individual UPR pathways reduce β-cell death.

PERK pathway loss of function leads to monogenic diabetes (14). Autopsy samples from pediatric Wolcott-Rallison patients confirmed that the diabetes is associated with a reduced number of β-cells, suggesting cell death, with dilated ER observed in surviving β-cells (45). Increased β-cell death was reported after deletion of PERK in mice; the exocrine pancreas was similarly affected (46). Inducible deletion of PERK in adulthood also caused hyperglycemia due to insulin insufficiency, with cell death, ER dilation, and compensatory activation of the other UPR pathways (41). Evidence for a protective role of PERK was also uncovered by miRNA studies. miR-204, which is increased in islets in T2D, directly targeted the 3′-untranslated region of PERK for degradation, inhibiting the PERK-eIF2α-ATF4 arm of the UPR (47). In INS1 cells, miR-204 overexpression, or pharmacologic PERK inhibition, reduced PERK signaling and increased ER stress–induced β-cell apoptosis (47).

Mechanisms by which PERK signaling protects against β-cell death likely include downstream ER peptide synthesis load reduction through phosphorylation of eIF2α, although as noted above there is some debate on the extent to which PERK activity suppresses global translation in β-cells (21,25,26). Additional mechanisms have been described. Apoptosis antagonizing transcription factor (AATF), downstream of PERK and eIF2α, transcriptionally upregulates *Akt1* to protect β-cells against ER stress (48); knockdown of AATF potentiated thapsigargin-induced cell death in INS1832/13 cells (48). PERK suppressed inflammatory cell death by reducing interferon signaling, as reviewed in the inflammation section below. Finally, PERK can induce a protective form of autophagy that switches β-cells from cell death to a cell survival mode; induction of autophagy was protective against β-cell death caused by intermittent hypoxia (49).

The IRE1α multifunctional enzyme exerts both protective and harmful downstream effects on β-cell survival (Fig. 3). Beneficial impacts, which are achieved mostly through the splicing of *Xbp1*, are reviewed in this section. Reduction in sXBP1 increased cell death in NOD and *ob/ob* islets, possibly related to increased *Chop* expression (50). Intriguingly, inhibition of JNK phosphorylation decreased cell death in *db/db* islets, placing IRE1α at a tipping point between cytoprotective responses via sXBP1 and cytotoxic responses via JNK phosphorylation (50). However, the JunB transcription factor reduced palmitate-induced β-cell death in part through upregulation of *Xbp1* expression via a mechanism requiring c/EBPδ (51). IRE1α and XBP1 activity were required to increase AKT phosphorylation in response to cAMP (51). In INS-1 cells, exendin-4 protection of cell survival during palmitate treatment was associated with increased phosphorylation of AKT; knockdown of IRE1α decreased p-AKT and increased cell death, and both toxicities were rescued by overexpression of sXBP1 (52).

ATF6α is also believed to provide protection against β-cell death (Fig. 4), although there are fewer published studies on this arm of the UPR, especially in live animals or islets. ATF6α expression was reduced in islets from mice in two different T1D models, NOD mice and an RIP-LCMV-GP transgenic model, as well as in human samples from subjects with T1D. Systemic treatment of mice with tauroursodeoxycholic acid (TUDCA), a chemical chaperone, rescued ATF6α expression, decreased cell death, and reduced diabetes incidence. Although the site of action of the chaperone between immune cells and islets or another tissue was uncertain, the rescue was dependent on β-cell ATF6α, and in Min6 cells, ATF6α overexpression and TUDCA treatment synergistically reduced β-cell death (35). In INS-1 832/13 cells, ATF6 knockdown increased cell death even under unstressed conditions, possibly related to activation of the PERK, JNK, and p38 stress pathways (53). The role of related family member ATF6β is even less studied in β-cells. ATF6β was not required for acute UPR pathway activation in INS1 cells, but knockdown of ATF6β increased susceptibility to ER stress–induced cell death (54). Of note, ATF6α transcriptionally upregulates Xbp1 (8,28,55). In sum, all three UPR pathways protect β-cells against cell death under some conditions.

ER Stress Response Suppresses Inflammatory Pathways

Cellular and molecular inflammation contribute to diabetes both by worsening insulin resistance in insulin-responsive tissues (56) and through direct damage to β-cells (57,58). ER stress response pathways in some cases activate inflammatory pathways and participate in harm, as reviewed in sections below, but in other cases UPR pathways suppress inflammation. For example, deletion of IRE1α in adult mice increased islet abundance of transcripts associated with inflammation and oxidative stress, and treatment with the BHA antioxidant restored glucose tolerance (29). Similarly, *Perk* ablation in the mouse pancreas increased interferon signaling in islets, associated with increased β-cell death. The mechanism was due to accumulation of the type 1 interferon receptor *Ifnar1* (Fig. 2); deletion of *Ifnar1* protected against cell death and prevented diabetes (59).

UPR Pathways Suppress Damaging Oxidative Stress

ER stress and oxidative stress exert overlapping and related effects on β-cell health and function. ER calcium depletion, a key driver of ER stress, also leads to reactive oxygen species production and oxidative stress (60). Coexistence of oxidative stress was found to tip the balance toward decompensated ER stress in human islets and MIN6 cells (61). Similarly, hypoxia, which paradoxically increases oxidative stress, suppressed adaptive UPR genes and increased decompensated ER stress genes (62). Both the IRE1 and PERK UPR pathways protect against oxidative stress. IRE1α deletion increased oxidative stress genes including reactive oxygen species–generating enzymes, lysyl-oxidases, and glutathione peroxidases, along with a detectable increase in actual oxidation, e.g., lipid peroxides and nitrotyrosines. Feeding the IRE1α-null mice an antioxidant diet decreased islet fibrosis and improved glucose tolerance (29). Deletion of Xbp1 resulted in mitochondrial swelling that could be consistent with oxidative stress (30). The PERK pathway protects β-cells against oxidative stress both through translation attenuation (21) and by inducing a protective autophagy observed during intermittent hypoxia conditions (49). In contrast to the beneficial effects of adaptive UPR on oxidative stress, the CHOP decompensation factor promotes harmful effects. Deletion of *Chop* reduced oxidative stress and improved β-cell function in *db/db* mice by increasing expression of antioxidant stress response genes (63). Knockdown of *Chop* protected against oxidative cell death (61); inhibition of JNK or CHOP reversed hypoxia-induced decompensation (62).

Harmful Effects of UPR Pathways on β-Cells

UPR pathways clearly benefit many aspects of β-cell health and function, including promoting robust insulin production and secretion, β-cell survival in the face of stress, and even expansion of β-cell number through proliferation. However, numerous reports also indicate that the same pathways can exert harmful effects in some contexts. In most cases, the proximate cause of tipping from beneficial to harmful signaling is not clear. This is an important area of future research.

Harmful Effects of UPR Pathways on Insulin Production and the β-Cell Differentiated State

The PERK/eIF2α pathway exerts tonic negative pressure on proinsulin translation (64). PERK is constitutively activated in insulin-producing cells under physiologic conditions, but eIF2α phosphorylation is kept low by abundant GADD34 and CReP phosphatases, illustrating a regulatory balance under tension that can tip in either direction depending on conditions (64). Consistent with excess PERK activity reducing insulin production, PERK overexpression in mice decreased pancreatic insulin content and in vivo GSIS (26). In further support of this concept, arsenic (As_2O_3)-treated mice were found to have increased PERK activation, and PERK inhibition rescued the insulin secretion defect in islets isolated from arsenic-treated mice (65). These observations must be placed in context, however, given the abundant evidence reviewed in sections above showing that disruption of normal PERK pathway activation also causes insulin-deficient diabetes, illustrating the delicate balance required for optimal function.

A similar careful balance of IRE1α signaling is required to preserve β-cell health and function. IRE1α activation leads to divergent downstream activities including kinase activity, specific *Xbp1* splicing activity, and a less specific regulated IRE1-dependent decay (RIDD) activity that degrades ER-associated mRNAs (Fig. 3) (66,67). The RIDD activity of IRE1 has been suggested to degrade *INS* mRNA itself; interestingly, the protein disulfide isomerase A6 (PDIA6) may counteract this activity (22). Although

the sXbp1 transcription factor is generally considered to exert positive effects, prolonged activation in rat islet cells led to β-cell dedifferentiation, with reduced mRNA expression of *Ins2*, *Mafa*, *Pdx1*, and *Glut2*, along with loss of insulin secretion (68). Related, deletion of IRE1α protected against diabetes in NOD mice by preserving β-cell mass and reduced autoimmunity (22).

ATF6α may have detrimental effects on insulin gene expression. In INS-1 cells, overexpression of full-length ATF6α reduced activity of a human *INS* promoter luciferase construct, and knockdown of ATF6α prevented glucotoxicity-induced loss of *Ins* mRNA (69). In this transformed cell model, ATF6α overexpression suppressed not only RNA but also protein levels of MAFA, PDX1, and NEUROD1 and completely eliminated GSIS (69).

Decompensated ER Stress Leads to Loss of Adaptive Proliferation

Although studies show that modest levels of ER stress trigger an adaptive response that can lead to proliferative expansion of β-cell number, as reviewed above, multiple lines of evidence suggest that decompensated ER stress suppresses β-cell proliferation. Our own data confirm this observation. In mouse islet cells, chemical stressors thapsigargin and tunicamycin, given in high enough doses to increase cell death, decreased PCNA abundance and markers of S-phase entry (37). Human β-cells also showed a dose-dependent proliferation response to thapsigargin and tunicamycin, with BrdU incorporation at low doses but not at higher doses (37). One mechanism suppressing proliferation at a higher level of stress may be the CHOP protein, principally induced downstream of the PERK/ATF4 pathway. CHOP was induced in high-stress conditions in our study, when proliferation was suppressed (37). Deletion of CHOP resulted in a higher frequency of β-cell proliferation in *db/db* mice and also promoted expansion of β-cell mass in *eIF2α-S51A* mutant mice (63). CHOP may exert negative feedback on adaptive UPR-induced proliferation. Intriguingly, deletion of a related bZIP factor, C/EBPβ, also markedly increased proliferation in *db/db* mice, resulting in a sixfold increase in β-cell mass, and C/EBPβ was found to bind to ATF6α and inhibit its transcriptional activity (43). One isoform of C/EBPβ is instrumental in nuclear translocation of CHOP, further connecting these two family members (70).

Whether the PERK arm of the UPR is proproliferative or antiproliferative is disputed, with conflicting reports in the literature that may indicate context-dependent differences. Evidence that PERK suppresses β-cell proliferation comes from in vivo deletion studies. PERK knockdown in the adult pancreas resulted in activation of the IRE1 and ATF6 pathways, increased β-cell proliferation, and increased protein levels of cyclin D1 and phosphorylated Rb (41). Similarly, mice heterozygous for PERK deletion, resulting in a modest reduction in PERK and phosphorylated eIF2α protein levels, were reported to have increased β-cell number, although a nonhistological methodology was used to make this estimation. Although β-cell proliferation was statistically increased at some ages, the effect size was small. Together, these studies provide evidence that PERK signaling exerts a negative effect on β-cell proliferation; decompensated ER stress due to loss of translation regulatory capacity, and induction of CHOP, may be the root causes.

Activation of ER Stress Response Pathways Can Paradoxically Lead to Increased β-Cell Death

Reported examples of UPR pathways leading to cell death are mostly limited to downstream mediators in the IRE1 and PERK pathways. IRE1α hyperactivation can lead to cellular demise in other cell types (66). As discussed above, IRE1α is a bifunctional enzyme with both kinase and endonuclease activities, the latter resulting in specific *Xbp1* splicing or nonspecific RIDD degradation of ER-associated RNAs. All three of these activities have been linked to cell death in some cases.

The kinase activity of IRE1α is linked to ER stress–related cell death through phosphorylation and activation of JNK. When IRE1α is activated by ER stress it forms homodimers, which autophosphorylate and then phosphorylate TRAF2, which phosphorylates ASK1, which subsequently phosphorylates JNK (2) (Fig. 3). JNK activation is a known cause of β-cell death in response to other stresses and conditions. Several observations link IRE1α activation to JNK-mediated β-cell death. A microarray approach identified death domain protein 5 (DP5) as a cause of cell death during lipotoxicity-induced ER stress. DP5 induction was dependent on IRE1/JNK and PERK activation. Knockdown of DP5 protected INS-1 cells and rat primary β-cells from apoptosis (60), and mice null for DP5 were resistant to diabetes due to expanded β-cell mass (71). JNK target JunB may also play a role in lipotoxic cell death. Active JNK phosphorylates JunB, leading to its degradation in the proteasome. JunB protein levels decline after palmitate exposure; JunB knockdown increased cell death and overexpression decreased cell death (51). Intriguingly, JunB also transcriptionally upregulated *Xbp1*, and increased AKT phosphorylation, suggesting a multifactorial mechanism of protection against cell death. Finally, in Min6 cells and mouse islets palmitate treatment and cytokine exposure both increased phosphorylation of JNK (50). Inhibiting IRE1α RNase activity or deletion of *Xbp1* increased cell death, but inhibiting JNK protected against cell death (50). Based on experiments in islets from leptin-deficient *ob/ob* mice, intensity of IRE1/JNK signaling was postulated to be an important tipping point between β-cell adaptation and failure (50).

IRE1α RNase activity can also increase β-cell death. IRE1α splicing of *Xbp1* is generally protective against glucotoxic, lipotoxic, and cytokine-induced cell death, as reviewed above. However, sustained overexpression of XBP1 in rat islet cells resulted in increased β-cell apoptosis, with BAX translocation to mitochondria, diffuse cytochrome C staining, and fragmentation of mitochondria (68). This result emphasizes the important concept in

stress response biology that the final impact of any type of response ultimately depends on the duration and intensity of that response.

IRE1α RIDD activity leading to general degradation of ER-associated mRNAs can cause extensive cellular toxicity including death (72). Kinase inhibiting RNase attenuators, or KIRAs, a new class of allosteric IRE1α inhibitors, stabilize the monomeric form of IRE1α, suppressing RNase activity (73). KIRA6 reduced ER stress–induced cell death in INS-1 cells and mouse islets and preserved islet mass in Akita mice (74). KIRA8 also had antidiabetes activity in NOD mice (75). IRE1α oligomerization, which is dependent on IRE1α kinase activity, is also supported by ABL kinases that localize to the ER membrane during stress (75). Imatinib, a tyrosine kinase inhibitor cancer therapeutic linked to improved glycemic control in cancer patients with diabetes, also led to β-cell protection and diabetes prevention in NOD mice. Intriguingly, nicotine also has IRE1α inhibitory properties, rescuing β-cells from ER stress–mediated cell death by activating the nicotinic acetylcholine receptor (76). Taken together with the new evidence that deletion of IRE1α reduced incidence of diabetes through avoidance of autoantigen production (22), IRE1α seems to be a promising target for T1D prevention.

The PERK pathway contributes to cell death via downstream activation of CHOP (Fig. 2). Although CHOP is most prominently activated by ATF4, *Chop* mRNA is also induced by ATF6 and XBP1. CHOP contributes to ER stress–induced β-cell death in a number of mouse models: CHOP-null mice were protected against diabetes and β-cell death in *eIF2α-S51A* knock-in mice, in mice injected with the β-cell toxin streptozotocin, and in *db/db* mice (63). Deletion of CHOP restored adaptive UPR and decreased proapoptotic gene expression, while increasing antioxidative stress genes and protecting β-cells from oxidative damage (63). CHOP knockdown was also protective against lipotoxic cell death (60) and cytokine-induced cell death (77). Proinflammatory cytokines interleukin-1β (IL-1β) and interferon γ (IFNγ) are released during insulitis and trigger ER stress and expression of proapoptotic members of the BCL2 protein family in β-cells (77). In human islets and the human fetal β-cell line EndoC-β^1H, cytokine treatment downregulated miR-211-5p, which were predicted to suppress CHOP expression. Inhibiting miRNA-211-5p and miR-204-5p increased human β-cell apoptosis, an effect that was partially rescued by CHOP inhibition (77).

Although there is some evidence in other fields suggesting that excessive activation of ATF6α leads to cell death, data to this effect are currently lacking in β-cells. ATF6α transcriptionally induces *Chop* expression (78,79) (Fig. 4), although the physiologic relevance of this has not yet been tested in genetic models. In vitro and in vivo models have been generated in our laboratory and others for study of this pathway in more detail.

The Future: Therapeutic Opportunities Harnessing UPR

Promoting beneficial and suppressing harmful β-cell ER stress responses has exciting potential to preserve or expand β-cell capacity. A long-standing concept relevant to decreasing stress is "β-cell rest," achieved by interventions that reduce the physiologic requirement for insulin production and secretion. Remarkably, most established effective therapies for T2D could be considered to provide β-cell rest. For example, reduction in caloric intake (dietary restriction, glucagon-like peptide 1 receptor agonists [GLP1RA]), increased caloric expenditure (exercise), engineered caloric loss (gut malabsorptive procedures, acarbose, sodium–glucose cotransporter 2 inhibitors), reduced whole-body insulin requirement (weight loss, insulin sensitizers), or exogenous insulin therapy would all be expected to reduce β-cell load. Conversely, therapies that increase insulin secretion and might increase β-cell stress (sulfonylureas) accelerate β-cell failure and worsen the T2D disease trajectory (80). Some experts have even advocated for counterintuitive approaches that decrease insulin secretion, such as glucokinase inhibitors or diazoxide, as a long-term T2D preventive strategy (81,82). Thus, β-cell ER stress reduction may already play a large, if mostly unrecognized, role in T2D therapy. However, to our knowledge, molecular data confirming the impact of systemic insulin load–reducing interventions on β-cell ER stress level, UPR activation, or survival in the human pancreas are lacking.

In contrast to the systemic physiology-driven interventions described above, human therapies that improve β-cell stress level through molecular actions directly in the islet are still largely aspirational. Although many existing or novel agents have β-cell ER stress protective effects in cell culture (including GLP1RA [supporting literature is beyond the scope of this review]), evidence that these observations are relevant to the in vivo human pancreas is lacking. Perhaps surprisingly, direct β-cell stress–targeting therapies have moved forward more quickly for T1D than T2D. Developments include the use of verapamil in early T1D (83), dantrolene in Wolfram syndrome (84), and clinical trials for GLP1RA in Wolfram syndrome and TUDCA chemical chaperone in T1D. Preclinical studies in mice suggest that an inhibitor of IRE1 RNAse activity may offer protection in T1D (75). In theory, multiple additional opportunities exist for both T1D and T2D. Modulation of the IRE1/XBP1 and ATF6 pathways under the correct circumstances has the potential to trigger β-cell proliferation to expand β-cell mass (37). Optimizing ER function could improve insulin production and secretion in cases where glucose intolerance is related to bystander misfolding proinsulin (23). Prevention of β-cell apoptosis by therapeutic stress reduction could also be a valuable approach. Inhibiting the integrated stress response with ISRIB has generated much excitement in neurobiology (85); whether this compound has a use in diabetes remains uncertain.

Critical Knowledge and Technology Gaps

Many knowledge gaps exist, largely due to technical gaps. Most importantly, the field lacks tools to quantify ER stress level in human β-cells in the in vivo environment. The proinsulin–to–C-peptide ratio has been touted as a circulating β-cell ER stress marker, but since most processing of proinsulin to insulin occurs in compartments distal to the ER (1), the utility of this measurement to determine ER health is uncertain. The field lacks biochemical or imaging methodologies to estimate total β-cell mass. Circulating demethylated insulin gene to quantify ongoing β-cell death is promising but currently lacks sensitivity to be a robust tool in routine settings (86). Technologies like these are required for identification of subjects who would benefit from stress-lowering therapies, determining efficacy of those therapies, and developing and testing new therapies.

Knowledge gaps are many as well, especially a molecular understanding of adaptive and decompensated stress responses in the in vivo human pancreas. Critical questions include those of the timing and intensity of stress response activation across the spectrum of diabetes pathogenesis, with a particular need to understand the switch from adaptive to maladaptive responses. For example, the degree to which progression from prediabetes to T2D represents failure of adaptive UPR to adapt β-cell mass and function is unknown. Similarly, since autoimmunity is known to trigger β-cell ER stress, progression from antibody-positive to active T1D may represent inadequate adaptive UPR or early susceptibility to decompensated ER stress. Therapeutically, interventions that promote adaptive UPR to increase β-cell number through proliferation or cell survival, or increase function by streamlining insulin synthesis, processing, and secretion, could potentially prevent or delay diabetes onset in a variety of disease settings. Once T1D or T2D is established, if sufficient β-cell mass remains, then therapeutic approaches to bring some β-cells back from the brink of stress-related decompensation and death might meaningfully restore metabolic health. Much work remains to improve understanding of the biology of ER stress in the human endocrine pancreas in health and disease.

Conclusion

The pancreatic β-cell, an essential pillar supporting whole-body metabolism, is vulnerable both to ER stress itself and to collateral damage caused by overactivation of stress response pathways. While all three UPR pathways play important roles in cellular adaptation to ER stress, downstream signaling can cause harm through transcriptional, translational, and signaling mechanisms. Most of the stress response impacts reviewed here are not unique to β-cells and may represent therapeutic opportunities for other disease processes as well. Through better understanding of the tipping point between beneficial and harmful outcomes it may be possible to develop tools to maintain β-cell stress in an adaptive range to maintain, or even improve, functional β-cell mass and metabolic outcomes.

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References

1. Liu M, Weiss MA, Arunagiri A, et al. Biosynthesis, structure, and folding of the insulin precursor protein. Diabetes Obes Metab 2018;20(Suppl. 2):28–50
2. Back SH, Kaufman RJ. Endoplasmic reticulum stress and type 2 diabetes. Annu Rev Biochem 2012;81:767–793
3. Eizirik DL, Cnop M. ER stress in pancreatic beta cells: the thin red line between adaptation and failure. Sci Signal 2010;3:pe7
4. Evans-Molina C, Hatanaka M, Mirmira RG. Lost in translation: endoplasmic reticulum stress and the decline of β-cell health in diabetes mellitus. Diabetes Obes Metab 2013;15(Suppl. 3):159–169
5. Cnop M, Toivonen S, Igoillo-Esteve M, Salpea P. Endoplasmic reticulum stress and eIF2α phosphorylation: the Achilles heel of pancreatic β cells. Mol Metab 2017;6:1024–1039
6. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2000;2:326–332
7. Baird TD, Wek RC. Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. Adv Nutr 2012;3:307–321
8. Sharma RB, Darko C, Alonso LC. Intersection of the ATF6 and XBP1 ER stress pathways in mouse islet cells. J Biol Chem 2020;295:14164–14177
9. Wang J, Takeuchi T, Tanaka S, et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. J Clin Invest 1999;103:27–37
10. Støy J, Edghill EL, Flanagan SE, et al.; Neonatal Diabetes International Collaborative Group. Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci U S A 2007;104:15040–15044
11. Liu M, Sun J, Cui J, et al. INS-gene mutations: from genetics and beta cell biology to clinical disease. Mol Aspects Med 2015;42:3–18
12. Stone SI, Abreu D, McGill JB, Urano F. Monogenic and syndromic diabetes due to endoplasmic reticulum stress. J Diabetes Complications 2021;35:107618
13. Lombardi A, Tomer Y. Interferon alpha impairs insulin production in human beta cells via endoplasmic reticulum stress. J Autoimmun 2017;80:48–55
14. Delépine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 2000;25:406–409
15. Scheuner D, Song B, McEwen E, et al. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell 2001;7:1165–1176
16. Scheuner D, Vander Mierde D, Song B, et al. Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. Nat Med 2005;11:757–764
17. Abdulkarim B, Nicolino M, Igoillo-Esteve M, et al. A missense mutation in PPP1R15B causes a syndrome including diabetes, short stature, and microcephaly. Diabetes 2015;64:3951–3962
18. Weir GC, Bonner-Weir S. Islet β cell mass in diabetes and how it relates to function, birth, and death. Ann N Y Acad Sci 2013;1281:92–105
19. Riahi Y, Israeli T, Yeroslaviz R, et al. Inhibition of mTORC1 by ER stress impairs neonatal β-cell expansion and predisposes to diabetes in the *Akita* mouse. eLife 2018;7:e38472

20. Walters SN, Luzuriaga J, Chan JY, Grey ST, Laybutt DR. Influence of chronic hyperglycemia on the loss of the unfolded protein response in transplanted islets. J Mol Endocrinol 2013;51:225–232
21. Back SH, Scheuner D, Han J, et al. Translation attenuation through eIF2α phosphorylation prevents oxidative stress and maintains the differentiated state in β cells. Cell Metab 2009;10:13–26
22. Lee H, Lee Y-S, Harenda Q, et al. Beta cell dedifferentiation induced by IRE1α deletion prevents type 1 diabetes. Cell Metab 2020;31:822–836.e5
23. Arunagiri A, Haataja L, Pottekat A, et al. Proinsulin misfolding is an early event in the progression to type 2 diabetes. eLife 2019;8:e44532
24. Sowers CR, Wang R, Bourne RA, et al. The protein kinase PERK/EIF2AK3 regulates proinsulin processing not via protein synthesis but by controlling endoplasmic reticulum chaperones. J Biol Chem 2018;293:5134–5149
25. Harding HP, Zyryanova AF, Ron D. Uncoupling proteostasis and development in vitro with a small molecule inhibitor of the pancreatic endoplasmic reticulum kinase, PERK. J Biol Chem 2012;287:44338–44344
26. Gupta S, McGrath B, Cavener DR. PERK (EIF2AK3) regulates proinsulin trafficking and quality control in the secretory pathway. Diabetes 2010; 59:1937–1947
27. Moore CE, Omikorede O, Gomez E, Willars GB, Herbert TP. PERK activation at low glucose concentration is mediated by SERCA pump inhibition and confers preemptive cytoprotection to pancreatic β-cells. Mol Endocrinol 2011;25:315–326
28. Shoulders MD, Ryno LM, Genereux JC, et al. Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. Cell Rep 2013;3:1279–1292
29. Hassler JR, Scheuner DL, Wang S, et al. The IRE1α/XBP1s pathway is essential for the glucose response and protection of β cells. PLoS Biol 2015;13:e1002277
30. Lee A-H, Heidtman K, Hotamisligil GS, Glimcher LH. Dual and opposing roles of the unfolded protein response regulated by IRE1alpha and XBP1 in proinsulin processing and insulin secretion. Proc Natl Acad Sci U S A 2011;108:8885–8890
31. Usui M, Yamaguchi S, Tanji Y, et al. Atf6α-null mice are glucose intolerant due to pancreatic β-cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. Metabolism 2012;61:1118–1128
32. Wang R, McGrath BC, Kopp RF, et al. Insulin secretion and Ca2+ dynamics in β-cells are regulated by PERK (EIF2AK3) in concert with calcineurin. J Biol Chem 2013;288:33824–33836
33. Kirkpatrick CL, Wiederkehr A, Baquié M, et al. Hepatic nuclear factor 1alpha (HNF1alpha) dysfunction down-regulates X-box-binding protein 1 (XBP1) and sensitizes beta-cells to endoplasmic reticulum stress. J Biol Chem 2011;286:32300–32312
34. Moore BD, Jin RU, Lo H, et al. Transcriptional regulation of X-box-binding protein one (XBP1) by hepatocyte nuclear factor 4α (HNF4A) is vital to beta-cell function. J Biol Chem 2016;291:6146–6157. DOI: 10.1074/jbc.M115.685750
35. Engin F, Yermalovich A, Nguyen T, et al. Restoration of the unfolded protein response in pancreatic β cells protects mice against type 1 diabetes. Sci Transl Med 2013;5:211ra156
36. Xin Y, Dominguez Gutierrez G, Okamoto H, et al. Pseudotime ordering of single human β-cells reveals states of insulin production and unfolded protein response. Diabetes 2018;67:1783–1794
37. Sharma RB, O'Donnell AC, Stamateris RE, et al. Insulin demand regulates β cell number via the unfolded protein response. J Clin Invest 2015;125:3831–3846
38. Zeng C, Mulas F, Sui Y, et al. Pseudotemporal ordering of single cells reveals metabolic control of postnatal β cell proliferation. Cell Metab 2017;25:1160–1175.e11
39. Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. Cell Metab 2006;4:491–497
40. Moore PC, Qi JY, Thamsen M, et al. Parallel signaling through IRE1α and PERK regulates pancreatic neuroendocrine tumor growth and survival. Cancer Res 2019;79:6190–6203
41. Gao Y, Sartori DJ, Li C, et al. PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Mol Cell Biol 2012;32:5129–5139
42. Wang R, Munoz EE, Zhu S, McGrath BC, Cavener DR. Perk gene dosage regulates glucose homeostasis by modulating pancreatic β-cell functions. PLoS One 2014;9:e99684
43. Matsuda T, Kido Y, Asahara S, et al. Ablation of C/EBPbeta alleviates ER stress and pancreatic β cell failure through the GRP78 chaperone in mice. J Clin Invest 2010;120:115–126
44. Szabat M, Page MM, Panzhinskiy E, et al. Reduced insulin production relieves endoplasmic reticulum stress and induces β cell proliferation. Cell Metab 2016;23:179–193
45. Collardeau-Frachon S, Vasiljevic A, Jouvet A, Bouvier R, Senée V, Nicolino M. Microscopic and ultrastructural features in Wolcott-Rallison syndrome, a permanent neonatal diabetes mellitus: about two autopsy cases. Pediatr Diabetes 2015;16:510–520
46. Harding HP, Zeng H, Zhang Y, et al. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. Mol Cell 2001;7:1153–1163
47. Xu G, Chen J, Jing G, Grayson TB, Shalev A. miR-204 targets PERK and regulates UPR signaling and β-cell apoptosis. Mol Endocrinol 2016;30:917–924
48. Ishigaki S, Fonseca SG, Oslowski CM, et al. AATF mediates an antiapoptotic effect of the unfolded protein response through transcriptional regulation of AKT1. Cell Death Differ 2010;17:774–786
49. Song S, Tan J, Miao Y, Sun Z, Zhang Q. Intermittent-hypoxia-induced autophagy activation through the ER-stress-related PERK/eIF2α/ATF4 pathway is a protective response to pancreatic β-cell apoptosis. Cell Physiol Biochem 2018;51:2955–2971
50. Chan JY, Luzuriaga J, Maxwell EL, West PK, Bensellam M, Laybutt DR. The balance between adaptive and apoptotic unfolded protein responses regulates β-cell death under ER stress conditions through XBP1, CHOP and JNK. Mol Cell Endocrinol 2015;413:189–201
51. Cunha DA, Gurzov EN, Naamane N, et al. JunB protects β-cells from lipotoxicity via the XBP1-AKT pathway. Cell Death Differ 2014;21:1313–1324
52. Jiang D, Wan F. Exendin-4 protects INS-1 cells against palmitate-induced apoptosis through the IRE1α-Xbp1 signaling pathway. Exp Ther Med 2018;16:1029–1035
53. Teodoro T, Odisho T, Sidorova E, Volchuk A. Pancreatic β-cells depend on basal expression of active ATF6α-p50 for cell survival even under nonstress conditions. Am J Physiol Cell Physiol 2012;302:C992–C1003
54. Odisho T, Zhang L, Volchuk A. ATF6β regulates the Wfs1 gene and has a cell survival role in the ER stress response in pancreatic β-cells. Exp Cell Res 2015;330:111–122
55. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 2001;107:881–891
56. Lee YS, Olefsky J. Chronic tissue inflammation and metabolic disease. Genes Dev 2021;35:307–328
57. Ying W, Fu W, Lee YS, Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities. Nat Rev Endocrinol 2020;16:81–90
58. Clark M, Kroger CJ, Tisch RM. Type 1 diabetes: a chronic anti-self-inflammatory response. Front Immunol 2017;8:1898
59. Yu Q, Zhao B, Gui J, et al. Type I interferons mediate pancreatic toxicities of PERK inhibition. Proc Natl Acad Sci U S A 2015;112:15420–15425
60. Cunha DA, Hekerman P, Ladrière L, et al. Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. J Cell Sci 2008;121:2308–2318

61. Plaisance V, Brajkovic S, Tenenbaum M, et al. Endoplasmic reticulum stress links oxidative stress to impaired pancreatic beta-cell function caused by human oxidized LDL. PLoS One 2016;11:e0163046
62. Bensellam M, Maxwell EL, Chan JY, et al. Hypoxia reduces ER-to-Golgi protein trafficking and increases cell death by inhibiting the adaptive unfolded protein response in mouse beta cells. Diabetologia 2016;59:1492–1502
63. Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest 2008;118:3378–3389
64. Akai R, Hosoda A, Yoshino M, Iwawaki T. Constitutive role of GADD34 and CReP in cancellation of phospho-eIF2α-dependent translational attenuation and insulin biosynthesis in pancreatic β cells. Genes Cells 2015;20:871–886
65. Wu W, Yao X, Jiang L, et al. Pancreatic islet-autonomous effect of arsenic on insulin secretion through endoplasmic reticulum stress-autophagy pathway. Food Chem Toxicol 2018;111:19–26
66. Ferri E, Le Thomas A, Wallweber HA, et al. Activation of the IRE1 RNase through remodeling of the kinase front pocket by ATP-competitive ligands. Nat Commun 2020;11:6387
67. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol 2009;186:323–331
68. Allagnat F, Christulia F, Ortis F, et al. Sustained production of spliced X-box binding protein 1 (XBP1) induces pancreatic beta cell dysfunction and apoptosis. Diabetologia 2010;53:1120–1130
69. Seo H-Y, Kim YD, Lee K-M, et al. Endoplasmic reticulum stress-induced activation of activating transcription factor 6 decreases insulin gene expression via up-regulation of orphan nuclear receptor small heterodimer partner. Endocrinology 2008;149:3832–3841
70. Chiribau C-B, Gaccioli F, Huang CC, Yuan CL, Hatzoglou M. Molecular symbiosis of CHOP and C/EBP beta isoform LIP contributes to endoplasmic reticulum stress-induced apoptosis. Mol Cell Biol 2010;30:3722–3731
71. Cunha DA, Igoillo-Esteve M, Gurzov EN, et al. Death protein 5 and p53-upregulated modulator of apoptosis mediate the endoplasmic reticulum stress-mitochondrial dialog triggering lipotoxic rodent and human β-cell apoptosis. Diabetes 2012;61:2763–2775
72. Han D, Lerner AG, Vande Walle L, et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 2009;138:562–575
73. Carlesso A, Chintha C, Gorman AM, Samali A, Eriksson LA. Effect of kinase inhibiting RNase attenuator (KIRA) compounds on the formation of face-to-face dimers of inositol-requiring enzyme 1: insights from computational modeling. Int J Mol Sci 2019;20:E5538
74. Ghosh R, Wang L, Wang ES, et al. Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. Cell 2014;158:534–548
75. Morita S, Villalta SA, Feldman HC, et al. Targeting ABL-IRE1α signaling spares ER-stressed pancreatic β cells to reverse autoimmune diabetes. Cell Metab 2017;25:883–897.e8
76. Ishibashi T, Morita S, Kishimoto S, et al. Nicotinic acetylcholine receptor signaling regulates inositol-requiring enzyme 1α activation to protect β-cells against terminal unfolded protein response under irremediable endoplasmic reticulum stress. J Diabetes Investig 2020;11:801–813
77. Grieco FA, Schiavo AA, Brozzi F, et al. The miRNAs miR-211-5p and miR-204-5p modulate ER stress in human beta cells. J Mol Endocrinol 2019;63:139–149
78. Yoshida H, Okada T, Haze K, et al. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol 2000;20:6755–6767
79. Okada T, Yoshida H, Akazawa R, Negishi M, Mori K. Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. Biochem J 2002;366:585–594
80. Kahn SE, Haffner SM, Heise MA, et al.; ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med 2006;355:2427–2443
81. Wajchenberg BL. beta-cell failure in diabetes and preservation by clinical treatment. Endocr Rev 2007;28:187–218
82. Whitticar NB, Nunemaker CS. Reducing glucokinase activity to enhance insulin secretion: a counterintuitive theory to preserve cellular function and glucose homeostasis. Front Endocrinol (Lausanne) 2020;11:378
83. Ovalle F, Grimes T, Xu G, et al. Verapamil and beta cell function in adults with recent-onset type 1 diabetes. Nat Med 2018;24:1108–1112
84. Abreu D, Stone SI, Pearson TS, et al. A phase Ib/IIa clinical trial of dantrolene sodium in patients with Wolfram syndrome. JCI Insight 2021;6:145188
85. Krukowski K, Nolan A, Frias ES, et al. Small molecule cognitive enhancer reverses age-related memory decline in mice. eLife 2020;9:e62048
86. Speake C, Ylescupidez A, Neiman D, et al. Circulating unmethylated insulin DNA as a biomarker of human beta cell death: a multi-laboratory assay comparison. J Clin Endocrinol Metab 2020;105:dgaa008