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The Pancreatic β -cell Response to Secretory Demands and Adaption to Stress

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Abstract

Pancreatic β cells dedicate much of their protein translation capacity to producing insulin to maintain glucose homeostasis. In response to increased secretory demand, β cells can compensate by increasing insulin production capability even in the face of

protracted peripheral insulin resistance. The ability to amplify insulin secretion in response to hyperglycemia is a critical facet of β -cell function, and the exact mechanisms by which this occurs have been studied for decades. To adapt to the constant and fast-changing demands for insulin production, β cells use the unfolded protein response of the endoplasmic reticulum. Failure of these compensatory mechanisms contributes to both type 1 and 2 diabetes. Additionally, studies in which β cells are “rested” by reducing endogenous insulin demand have shown promise as a therapeutic strategy that could be applied more broadly. Here, we review recent findings in β cells pertaining to the metabolic amplifying pathway, the unfolded protein response, and potential advances in therapeutics based on β -cell rest.

Keywords: pancreatic islet beta cell, insulin secretion, unfolded protein response, endoplasmic reticulum stress, beta cell rest

Recent research contributions in the islet biology field, particularly those based on experiments in human pancreatic islets, have dramatically improved our knowledge of the normal physiological mechanisms underlying pancreatic β -cell function, as well as what goes awry in these specialized cells during diabetes pathogenesis (see [\(1\)](#) for an extensive review). In this article, we consider advances in our understanding of the signal transduction mechanisms that fine tune stimulus–secretion coupling for optimal insulin output and glucose homeostasis. We next highlight new findings about the unfolded protein response in β cells and other relevant cell types that may have important implications in pancreatic islets. We expect these lines of research will lead to increased understanding of islet physiology as well as opportunities for therapeutic interventions. Additionally, treatment strategies that involve “resting” β cells have been suggested and pursued in the past and may warrant renewed effort considering the unique secretory demands placed on β cells.

Overview of Metabolic Triggering and Amplifying Pathways of Insulin Secretion

Secreted proteins are simultaneously translated and transported into the endoplasmic reticulum (ER) in the first step of an arduous biochemical journey that enables nascent polypeptides to be folded, posttranslationally modified, proteolytically processed, sorted, and packaged into vesicles that deliver the cargo to

the extracellular milieu via fusion with the plasma membrane (2, 3). Dedicated secretory cell types, in particular pancreatic islet β cells, devote much of their protein translation capacity to this process. The coupling of proinsulin synthesis to nutrient demand (4) and the energy-intensive production of insulin means β cells are potentially under repeated low-level stress every time nutrient levels rise in the circulation. Although β cells are normally adapted to handle this stress over long periods, these adaptive responses may fail or become maladaptive because of chronic stimulation and/or underlying genetic susceptibilities during diabetes pathogenesis. Underscoring the importance of this process, defects in proinsulin folding from mutations in translation factors, as well as within insulin itself, can cause or predispose individuals to different types of diabetes (5, 6).

The fundamental purpose of pancreatic islet β cells is to secrete insulin in response to elevated circulating nutrient secretagogues, largely glucose. Secreted insulin then signals to peripheral skeletal muscle and adipose tissue to increase glucose uptake. Circulating glucose enters the β cell through GLUT1/2 transporters and is metabolized, increasing the ATP/ADP ratio and causing the closure of ATP-sensitive potassium (K_{ATP}) channels (Fig. 1). Closure of K_{ATP} channels depolarizes the plasma membrane, leading to opening of voltage-dependent calcium channels and calcium influx. This calcium influx is the triggering signal for docked and primed insulin secretory vesicles to fuse with the plasma membrane and exocytose their cargo. In addition to the triggering signal, the metabolic amplifying pathway enhances insulin secretion without further increases in calcium influx; our current understanding of this process has been reviewed in detail (7-10). A major result of the metabolic amplifying pathway is an increase in the release competency of insulin granules. This pathway is critical because metabolic amplification is estimated to account for up to 50% of glucose-stimulated insulin secretion (11). Metabolic amplification of insulin secretion is also linked to β -cell maturation because adult β cells gain their functionally mature state during postnatal development (12). During embryonic development, lineage-determining transcription factors (LDTFs) control the formation of distinct endocrine islet cell types, whereas signal-dependent transcription factors (SDTFs) are guided by LDTFs to control actual β -cell function (13). For example, *Foxa2* is an LDTF that contributes to β -cell identity and eventual secretory function (14), whereas *NFATc1* and *NFATc2* are SDTFs activated by calcium influx and promote expression of genes involved in β -cell function, including *Gck* and *Slc2a2* (15, 16). In another example, as β cells mature, synaptotagmin 4 expression is increased because of a loss of repression from the SDTF *Myt1*;

synaptotagmin 4 is an insulin granule membrane protein that controls granule release competency (17). Given the number of metabolic coupling factors and signaling pathways involved, metabolic amplification of insulin secretion occurs as the complex summation of these many contributors (Fig. 1) (10). Even after nearly 30 years since its discovery (18, 19), new mechanisms related to the triggering and metabolic amplifying pathways continue to be discovered (see the following section).

Several new insights into the mechanisms governing metabolic triggering and amplification are derived from studies of β -cell energetic pathways and their linkage to insulin secretion (Fig. 1). Pyruvate kinase was recently demonstrated to control K_{ATP} channel closure and insulin secretion via plasma membrane-localized production of ATP from phosphoenolpyruvate (20). However, a small molecule activator of pyruvate kinase also increased glucose-induced metabolic amplification under conditions in which the K_{ATP} channel was pharmacologically held in an open state with diazoxide. This finding indicates that pyruvate kinase has a dual role in the metabolic control of both the triggering and amplifying pathways.

Phosphoenolpyruvate may be provided to pyruvate kinase via upstream succinyl-CoA synthetase. The β cell expresses 2 isoforms of succinyl-CoA synthetase, 1 that produces ATP (SCS-ATP) and another that produces GTP (SCS-GTP). In rat islets (and INS-1 832/13 cells), knockdown of SCS-ATP increased glucose-stimulated insulin secretion, whereas suppression of SCS-GTP reduced insulin secretion (21). When SCS-ATP is absent, SCS-GTP generates increased amounts of mitochondrial GTP and enhances downstream Ca^{2+} influx. GTP generation within the mitochondrial matrix is not a major contributor to the cytosolic pool, so it is unlikely that this source of GTP influences insulin secretion via cytosolic- or plasma membrane-localized small GTPase activity. Accordingly, mitochondrial GTP generated by SCS-GTP drives metabolic amplification of insulin secretion through mitochondrial phosphoenolpyruvate carboxykinase via the phosphoenolpyruvate cycle (22). Furthermore, a reductive tricarboxylic acid (TCA) cycle flux, operating in a “counterclockwise” manner, can influence the amplifying pathway stimulated by glucose and glutamine (23). This process occurs through mitochondrial isocitrate dehydrogenase 2 converting α -ketoglutarate to isocitrate, which is then transported to the cytosol and converted back to α -ketoglutarate by cytosolic isocitrate dehydrogenase 1, generating NADPH, a key factor for metabolic amplification of insulin secretion (9). Interestingly, although the citrate-isocitrate carrier is not required for glucose-stimulated insulin secretion in mouse β cells, cytosolic isocitrate

dehydrogenase 1 is indeed required (24).

The mitochondrial enzyme glutamate dehydrogenase (GDH; *GLUD1*) is also a major contributor to insulin secretion because mutations in this enzyme or in its regulators cause defects in insulin secretion (25-30). GDH converts glutamine-derived glutamate into α -ketoglutarate, which can feed the reductive TCA cycle, increasing downstream generation of cytosolic NADPH, as well as fuel succinyl coenzyme A (CoA) synthetase for mitochondrial GTP production. This key role of GDH has been confirmed using clonal knockout β cells (31) and in β -cell-specific GDH knockout mice (27, 28). Downstream, NADPH is critical for regenerating reduced glutathione and promoting insulin secretion through the removal of inhibitory SUMO modifications on exocytotic proteins (32). Another metabolite, adenylosuccinate within the de novo purine synthesis pathway, has also been linked to the deSUMOylating enzyme SENP1 and promoting metabolic amplification of insulin secretion through a yet-undefined mechanism (33).

Lipids also influence the metabolic amplifying pathway both intracellularly and extracellularly by serving as a source of metabolic coupling factors and signaling via certain G protein-coupled receptors (GPCRs), respectively (34). Free fatty acids (FFAs), or nonesterified fatty acids, including short-, medium-, and long-chain forms, can signal through the lipid GPCRs GPR40 and GPR120, activating either G_i/o or G_q to modulate insulin secretion (35). Of note, recent work showed that deletion of both GPR40 and GPR120 (*FFAR1* and *FFAR4*, respectively) had a minimal effect on β -cell function and glucose homeostasis (36). Nevertheless, FFAs support and are required for normal nutrient-induced insulin secretion (37-39). The source of lipids that have functional impact on β -cell function may be paracrine/autocrine (40). In response to glucose stimulation, mouse and human islets generate a variety of lipid molecules, including fatty acyl-CoAs, glycerolipids like 1-monoacylglycerol (41), and eicosanoids like 20-HETE (40), each of which contribute to regulated insulin exocytosis (42, 43).

The many paths leading to amplified secretion allow β cells to closely balance insulin production and glucose homeostasis. However, this robust secretory activity could have deleterious effects on long-term β -cell function, particularly in face of prolonged insulin resistance. For this reason, direct or indirect reduction of β -cell insulin secretory activity could be exploited for therapeutic benefit. The continued stress of insulin secretion can activate the unfolded protein response of the ER (UPR^{ER}), which is a regulated process necessary for β -cell function and survival. The UPR^{ER} and

metabolic amplification also have overlapping regulatory pathways, such as the glutathione antioxidant system, which is important for SUMOylation and deSUMOylation in both the UPR^{ER} (44, 45) and in the amplifying pathway (32, 46-49). SUMOylation can also contribute to RNA processing and senescence in the β cell (44).

Advances in the Understanding of ER Stress and the UPR^{ER} in the β Cell

To maintain sufficient stores of insulin, transcription of preproinsulin mRNA is coupled to nutrient signals and newly synthesized polypeptide enters the ER lumen of the β -cell through cotranslational and posttranslational mechanisms (50). The activities of ER chaperones and catalysts enable proinsulin molecules to attain a mature tertiary conformation that is permitted to enter anterograde transport arriving at the trans-Golgi network for sorting, copackaging into immature secretory granules, and final proteolytic processing (1, 5, 50-52). The ER chaperone binding immunoglobulin (BiP; *Hspa5*) associates with preproinsulin during the early cotranslational insertion of the nascent polypeptide into the ER (53). Subsequently, protein folding is catalyzed by spontaneous formation of correct intramolecular disulfide bond pairings through spontaneous reactions of proximal cysteine residues (interchain cysteines A20 to B19 and A7 to B7; intrachain cysteines A6 to A11) and rearrangements of incorrect disulfide pairings via the actions of protein disulfide isomerases and oxidoreductases (50). The order of bond formation during folding, identity of aberrant bond pairs, and nature of misfolded aggregated proinsulin are critical parameters governing normal β -cell function and the dysfunction that occurs in diabetes (54). The importance of correct folding is underscored by the identification of numerous proinsulin mutations within or near critical cysteine residues of the protein that cause neonatal diabetes because of ER retention of proinsulin, induction of ER stress, and β -cell death (55-59). Additionally, mutations in translation factors, such as the signal sequence receptor *Ssr1* (60) and the tRNA methyltransferase *Cdkal1* (61), can lead to defective insulin processing and folding and contribute to diabetes development (5, 6).

Increased influx of nascent polypeptides into the ER lumen can exceed the ER capacity and will activate an adaptive stress response through at least 3 well-known signal transducers: ATF6, IRE1 α , and PERK. These signaling pathways are part of the UPR^{ER} and control a very broad program of translational and transcriptional control that includes chaperone upregulation, misfolded protein degradation, and both

survival and death ([Fig. 2A](#)). The 3 main UPR^{ER} pathways (ATF6, IRE1 α , and PERK) are all regulated through direct interactions with a chaperone in the ER lumen, BiP. Upon increased protein flux through the ER-Golgi, BiP is titrated away from ATF6, IRE1 α , and PERK to aid in folding and prevent aggregation of secreted proteins ([62](#), [63](#)). This BiP dissociation allows for activation of each of these pathways. Calcium concentrations in the cytosol and ER are tightly regulated ([64](#)), with the ER [Ca²⁺] at ~200 to 500 μ M and cytosolic [Ca²⁺] at ~100 nM; Ca²⁺ is a key cofactor for many ER chaperones like BiP and protein disulfide isomerases ([65](#)). Accordingly, dysregulation of ER Ca²⁺ either by nutrient overload, cytokines, or pharmacological manipulation also causes activation of these UPR^{ER} pathways ([64](#), [66](#), [67](#)). SERCA2 is an ER membrane-localized ATPase that pumps Ca²⁺ from the cytosol into the ER. Defects in SERCA2 lead to β -cell failure and diabetes ([68](#)). Other non-ER pathways can also activate the UPR and may act in β -cell stress adaptation, as discussed next.

Activation of the UPR^{ER} is necessary and β cells are normally equipped to handle this repeated rise and fall in secretory demand ([69](#)). In this regard, the UPR^{ER} helps β cells execute the complicated process of nutrient- and hormone-regulated insulin expression, translation, processing, trafficking, and exocytosis, even under dietary stress ([70](#)). However, in disease, β cells are taxed by the combination of multiple factors, among them genetic mutations or polymorphisms ([71-73](#)), peripheral insulin resistance ([74](#)), nutrient-induced metabolic stress ([34](#), [75](#)), and inflammatory mediators ([76](#), [77](#)). These events can lead to a maladaptive or terminal UPR^{ER}, which eventually results in β -cell dysfunction and apoptosis ([78](#), [79](#)). The need for further evidence of β -cell UPR^{ER} in diabetes has been noted ([80](#)). Such evidence is accumulating through single-cell transcriptomic studies in rodent, primate, and human islets as well as in rodent models of type 2 diabetes progression ([81](#), [82](#)) and histological studies of islets from patients affected by type 2 ([83](#)), and type 1 diabetes ([84](#)). A recent preprint highlighted the limits of the adaptability of β cells to repeated ER stress, showing that although β cells may survive this process, they eventually lose their plasticity, are less able to mount a full ER stress response, and have reduced insulin gene expression ([85](#)). In a simplified view of this process, [Fig. 2B](#) illustrates how β cells exposed to ER stressors induce the UPR^{ER} in response and, depending on the intensity and duration of the insult, undergo a cell fate decision toward either apoptosis or survival. Other contributors to resolution of ER stress include ER-associated degradation (ERAD) ([86](#)) and autophagy ([87](#)).

PERK, ATF4, and CHOP

PERK is activated on dissociation of BiP from its ER-luminal domain. This activation allows the oligomerization and transautophosphorylation of PERK, which promotes its kinase activity and consequent phosphorylation of eIF2 α ([88](#), [89](#)).

Phosphorylation of eIF2 α causes a global suppression of protein translation and preferential production of ER stress response genes including the basic leucine zipper (bZIP) transcription factor ATF4. This process occurs because of a decoy upstream open reading frame in the ATF4 mRNA that under normal conditions leads to a nonfunctional product. When translation is suppressed because of eIF2 α phosphorylation, the ribosome skips this upstream open reading frame and translates a downstream open reading frame that encodes functional ATF4 ([90](#), [91](#)). During adaptation to ER stress, ATF4 regulates the transcription of genes involved in amino acid metabolism and antioxidant response ([92](#)). The induction of these genes by ATF4 contributes to the increase in the folding capacity of the ER, adapting it to the ongoing stress or functional demand. Other functions of this pathway are to enhance the metabolism of the increased amounts of amino acids resulting from the increased protein degradation from ERAD and to adapt the ER to oxidative stress caused by misfolded and unfolded proteins ([93](#), [94](#)). A key gene induced by ATF4 is *Ddit3*, which encodes the pro-apoptotic transcription factor C/EBP homologous protein (CHOP). Early in the UPR^{ER}, CHOP induction may serve as a negative feedback signal to attenuate eIF2 α phosphorylation via the induction of the PPP1R15 members GADD34 and CrEP ([95](#)). During prolonged or excessive UPR^{ER}, CHOP coordinates with ATF4 to induce key elements of β -cell demise ([96](#), [97](#)). Recent findings about CHOP regulation in the β cell have implications for β -cell calcium handling, diabetes, and what is likely insulin-driven liver storage of nutrients leading to steatosis ([98](#)).

Deletion of PERK causes diabetes because of β -cell dysfunction in mouse models, and inactivating mutations in PERK cause a genetic form of human diabetes called Wolcott-Rallison syndrome, indicating the essential nature of the UPR^{ER} in β cells ([99](#)). In these diseases, mutations in PERK abolish its kinase activity, which prevents a physiological response to increased flux of protein production through the ER. On the other hand, partial pharmacological inhibition of PERK has beneficial effects in a mouse model of diabetes ([100](#)) and mice missing 1 copy of the *Eif2ak2* gene encoding PERK have improved β -cell function, suggesting there is a “goldilocks” zone for PERK in particular and ER stress sensors in general ([101](#)). Moreover, cross-talk between PERK and IRE1 influences whether or not cells adapt to UPR in ovarian and colorectal

cancer cell lines ([102](#)), although whether this particular mechanism also occurs in the β cell is unknown.

IRE1 α and XBP1s

IRE1 and its relationship to metabolic regulation has been reviewed in detail ([103](#)). IRE1 α is an ER membrane-spanning protein with kinase and nuclease domains facing the cytosol. Like PERK and ATF6, the ER luminal N-terminus of IRE1 α is bound by BiP under normal conditions. When BiP is titrated away during ER stress, IRE1 α oligomerizes and is transautophosphorylated, which activates its nuclease activity. IRE1 α nuclease activity combines with associated ligation machinery to act on its most well-known target, *Xbp1* mRNA. In this process, unconventional splicing of *Xbp1* mRNA removes a short sequence, resulting in translation of the functional bZIP transcription factor XBP1s ([104-106](#)). XBP1s translocates to the nucleus and binds to ER stress response elements (ERSEs) and UPR elements to induce transcription of genes encoding ER chaperones and enzymes involved in protein folding (eg, *Hspa5*), maturation, and degradation, as well as transcription factors such as *Ddit3*, *Atf4*, and *Xbp1* ([107-109](#)). XBP1s also regulates genes involved in ER expansion/biogenesis and the transport and trafficking of vesicles ([109-111](#)). IRE1 α and XBP1s are required to maintain β -cell function and prevent diabetes because IRE1 α deletion in β cells leads to defects in glucose-stimulated induction of genes required for proinsulin processing ([112](#)). IRE1 α also cleaves other mRNAs in a process termed “regulated IRE1-dependent decay” (RIDD), which decreases the load of mRNAs marked for processing in the ER, including insulin mRNA ([113-115](#)). When excessive, RIDD may be a contributing factor to β -cell death ([116](#)). IRE1 α may also contribute to the regulation of insulin synthesis in response to exposure to high glucose, independent from its XBP1 splicing activity ([117](#)).

IRE1 α likely integrates many different signaling pathways through participation in a multiprotein regulatory complex called the UPRosome ([103](#), [118](#)), and IRE1 α deletion in the β cells protects against type 1 diabetes in a mouse model ([119](#)). This effect is proposed to be due to β -cell dedifferentiation and reduced expression of autoantigens, which is in line with findings suggesting that cytokine-induced ER stress signals mostly via the IRE1 α -JNK pathway ([76](#)). This suggests that diminishing IRE1 α activity before the onset of type 1 diabetes could be a strategy to prevent or delay disease.

Relatively less is known about the processes by which adaptive IRE1 α signaling is turned off during sustained ER stress. As referenced previously, sustained PERK activation attenuates IRE1 α activity via the phosphatase RNA polymerase II-associated protein 2 (RPAP2) ([102](#)). RPAP2 dephosphorylates IRE1 α leading to reduced RIDD, terminating IRE1 α 's stress-mitigating activity. IRE1 α is also shut off by the translocon-associated protein Sec63, which facilitates BiP binding to IRE1 α in HEK293 cells ([120](#)). RPAP2 and Sec63 appear to be expressed in human pancreatic islets (TIGER Data Portal; <http://tiger.bsc.es>). Additionally, 2 cytokine-induced proteins, namely N-MYC interactor ([121](#)) and ubiquitin D ([122](#)), prevent IRE1 α -induced JNK activation in β cells. Whether these mechanisms are exploitable therapeutically in β cells is an open question. A recent high-throughput screening campaign successfully identified activators of the IRE1 α -XBP1 pathway using a reporter of *Xbp1* mRNA splicing ([123](#)). The relative specificity of the primary hit IXA4 was confirmed through incorporation of a counter-screen against activators of ATF6 and use of IRE1 α inhibitor 4 μ 8c.

Recent findings from a preprint suggest that in vivo treatment of diet-induced obese mice with IXA4 had beneficial effects in both the liver and pancreatic β cells ([124](#)). The potential for targeting IRE1 α with such a compound to treat diabetes and/or obesity appears promising. However, care must be taken as the β cell-specific loss of IRE1 α ([112](#)) or *Xbp1* ([125](#)), as well as sustained expression of XBP1s ([126](#)) leads to β -cell dysfunction.

ATF6 α and CREB3

ATF6 α is an ER resident protein that transits forward to the Golgi upon ER stress where it is proteolytically cleaved by the same proteases that process SREBPs ([127](#), [128](#)). Cleaved ATF6 is a bZIP transcription factor enters the nucleus and controls a subset of UPR transcription ([129](#), [130](#)).

ATF6 α is acutely required for UPR^{ER} gene induction in response to thapsigargin or tunicamycin in mouse islets ([131](#)). XBP1s-targeted gene expression requires ATF6 α , but ATF6 α -regulated gene expression seems to be independent of XBP1s. ATF6 and XBP1s also contribute to β -cell proliferation in rodents ([132](#)). A recent study discovered a small molecule inhibitor of the SIK kinases that induced a transient UPR^{ER} in β cells involving induction of ATF6 and XBP1s and leading to β -cell proliferation in vivo ([133](#)). Besides ATF6, the CREB3 family of ER-associated proteins

are processed to bZIP transcription factors and regulate expression of secretory pathway genes (134). CREB3 family members aid in ER stress sensing and are expressed in pancreatic β cells; they include CREB3 (aka Luman or LZIP), CREB3L1 (aka OASIS), CREB3L2, CREB3L3, and CREB3L4 (135). During UPR^{ER}, these ER transmembrane proteins are processed in a manner similar to ATF6. Expression of CREB3 was enriched in human β cells with elevated levels of UPR (136) and the metabolic stressor palmitate induced expression of CREB3 and CREB3L3 in human islets (137). CREB3 was also recently implicated as part of a Golgi stress signature identified in human islets treated with brefeldin A (138). A protective action of CREB3 was identified because inhibition of CREB3 by a specific siRNA potentiated palmitate-induced apoptosis in rodent and human β cells (137). In β cells, CREB3L1 could be activated by ER stressors, and induced extracellular matrix genes and genes involved in pancreas development, but did not induce typical ER stress response genes in β cells. The other CREB3 family members are also of uncertain value to β -cell UPR^{ER} (139).

Unfolded Protein Response-related Pathways in β Cells

eIF2 α Kinases

Phosphorylation of eIF2 α at Ser51 is a major determinant of β -cell survival (53, 140, 141) and is linked to the ER stress kinase PERK (142, 143). Three additional eIF2 α kinases—PKR, GCN2, and heme-regulated inhibitor (HRI)—may also influence β cells under specialized conditions (144). Although the mouse knockout of PERK causes overt diabetes because of β -cell dysfunction and degeneration (142, 143), this does not occur with deletion of the other eIF2 α kinases, suggesting they may not be required for glucose homeostasis in the absence of additional genetic or environmental perturbations.

PKR is classically known to be activated in response to double-stranded RNA, such as occurs during certain virus infections (145). PKR can be activated by other stressors as well. Studies of PKR knockout mice did not report metabolic defects (146, 147), but β cells were not put under stress in these models, which may have failed to disclose relevant phenotypes. PKR inhibition with small molecules suppresses ER stress in β cells (148) and has antidiabetic properties in obese mouse models (149). Interestingly, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recently demonstrated to activate PKR (150). Although controversy exists regarding

the potential for SARS-CoV-2 to infect pancreatic islet β cells ([151](#)), there have been several reports of infection and subsequent dysfunction/killing of β cells ([152-154](#)). In one of these studies, PKR was shown to be activated by SARS-CoV-2 in β cells causing β -to- α cell transdifferentiation ([153](#)). This process was blocked by *trans*-ISRIB, a drug that prevents phospho-eIF2 α from binding to eIF2B, thereby alleviating suppression of global translation ([155](#)).

GCN2 is an amino acid sensor activated by decreased charged tRNA levels. GCN2 knockout mice maintained on a standard diet did not exhibit any obvious glucose homeostasis phenotypes ([156](#)). However, a recent study of GCN2-deficient mice showed that when stressed with a high-fat diet, these mice lose pancreatic β -cell mass ([157](#)). Furthermore, humans carrying a type 2 diabetes risk allele of GCN2 have impaired insulin secretion ([157](#)). These findings draw renewed attention to GCN2 and its previously recognized intersection with PERK in β -cell function and ER stress. For example, amino acid starvation still causes eIF2 α phosphorylation in *Gcn2* null mouse embryonic stem cells, although delayed, and conversely phosphorylation of eIF2 α can also occur in response to ER stress in *Perk* null cells ([156](#)). Therefore, GCN2 may work in β cells either as an amino acid sensor or as an auxiliary kinase recruited under specific metabolic conditions where PERK is the primary signal transducer.

HRI, as its name suggests, senses heme levels in erythroid cells, but has both heme-dependent and heme-independent actions across multiple tissues ([158](#)). Even though HRI is apparently well-expressed in human islets, how it aids the β cell or UPR^{ER} is not known. However, a small molecule HRI activator, EPB-53, improved metabolic health in high-fat diet fed mice ([159](#)). This effect required FGF21 as no effects of EPB-53 were seen in FGF21 null mice.

Mitochondrial UPR

Stresses that cause increased amounts of unfolded proteins within the mitochondrial matrix or disrupt oxidative phosphorylation can lead to the mitochondrial UPR (UPR^{mt}) ([160](#)). UPR^{mt} has been observed in the hypothalamus and adipocytes ([161-164](#)). Little is known about the UPR^{mt} in normo- and pathophysiology in the islet. However, transcriptomic data indicate that palmitate induces expression of LonP1, a gene involved in the UPR^{mt}, in human β cells ([137](#)). As a result of the UPR^{mt}, specific mitochondrial chaperones and proteases are expressed, distinct from the factors

involved in the UPR^{ER}, which are presumed to aid in alleviating mitochondrial stress. Phosphorylation of eIF2 α is required for the induction of CHOP, ATF4, and ATF5 during UPR^{mt} (165), although it is not clear which eIF2 α kinase(s) is/are responsible under different conditions leading to mitochondrial stress and the UPR^{mt} (160). Preliminary findings suggest a role for the UPR^{mt}-related protein ubiquitin-like protein 5 in that β cell-specific ubiquitin-like protein 5 null mice exhibit a diabetic phenotype with β -cell impairment and altered islet UPR^{mt} gene expression (166).

Contributions of Lipids to β -cell UPR^{ER}

The UPR^{ER} can both influence and be influenced by lipid metabolism and signaling. Treatment of β cells with the saturated fatty acid palmitate induces the UPR^{ER} including expression of BiP, ATF6, and XBP1s and causes apoptosis likely through CHOP, JNK, and other signals (167, 168). Treatment with the unsaturated fatty acid oleate had opposing effects and, when combined with palmitate, leads to partial protection against ER stress (167). Multiple mechanisms downstream of palmitate lead to β -cell dysfunction and UPR^{ER}, including depletion of β -cell ER calcium stores (169-171), disrupted ER membrane lipid composition (172), altered protein palmitoylation (173), degradation of proinsulin processing enzymes (174), and accumulation of islet amyloid polypeptide (137, 175). In β cells, palmitate can also act through mTORC1 to increase protein translation and ER load, leading to UPR^{ER} (176). Subsequent activation of the XBP1s arm of the UPR^{ER} can also regulate gluconeogenic and lipogenic genes either directly or in conjunction with FoxO1 or SREBP1c in hepatocytes (177); some of these signaling pathways are likely also functioning in β cells. Lipids also accumulate in droplets inside cells and adult human and mouse islet β cells express the lipid droplet-associated perilipins PLIN2 and PLIN3 (178). The expression of PLIN2/3 changes little with age, but lipid droplets accumulate in human islet β cells as individuals age and are increased further in human type 2 diabetes (178). PLIN2 is upregulated in response to fatty acid or pharmacological UPR^{ER} stressors, and knocking out *Plin2* partially rescued β -cell failure in a mouse model of ER stress-induced diabetes, potentially via increased autophagic flux (179). Together, these studies highlight the importance of lipids and lipid storage in β -cell homeostasis and the UPR^{ER}.

Emerging ER Homeostasis Contributors

Discovery of genes potentially implicated in ER stress in different tissues have

emerged in recent years. Here, we highlight a few of these genes with exciting potential; several of these mechanisms are incorporated into an integrated depiction of ER stress signaling ([Fig. 2](#)).

QRICH1.

The transcription factor glutamine rich 1 (QRICH1) controls the apoptotic fate decision during a prolonged unfolded protein response in intestinal cells ([180](#)). This discovery was made applying single-cell RNA sequencing and a whole-genome CRISPR knockout screen for ER stress regulators in primary mouse intestinal organoids. QRICH1 has an upstream inhibitory short open reading frame, similar to ATF4, which allows preferential translation during the UPR. QRICH1 is a member of the caspase activation and recruitment domain family of proteins, implying a role in cell survival. QRICH1 deletion is protective under pharmacological ER stress conditions, placing it as a negative regulator of cell survival. Additionally, QRICH1 can activate its own transcription and bind some of the same gene promoters as ATF4 ([180](#)). This activity up-regulates a program of increased translation and protein secretion. QRICH1 is widely expressed and likely has a conserved function across different cell types. Interestingly, QRICH1 was also a hit in a genome-wide CRISPR knockout screen in β cells ([181](#)), where its deletion led to a decrease in insulin content. These results suggest QRICH1 may regulate β -cell insulin production and ER stress. Interestingly, human loss-of-function mutations in QRICH1 have been identified, leading to Ververi-Brady syndrome ([182-184](#)), characterized primarily by developmental delay. It is presently unclear whether there is any diabetic phenotype in these individuals.

MANF.

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER protein that can act intracellularly or be secreted. Truncating mutations in MANF have been linked to rare nonautoimmune diabetes in humans ([72](#)). β -cell dysfunction in these individuals is presumably due to the loss of the protective effect of MANF in context of the UPR^{ER} ([185](#), [186](#)). Recombinant MANF treatment protected human and mouse β cells against cytokine or thapsigargin-induced stress ([185](#)), implying an extracellular activity. In β cells, MANF expression is induced in response to ER stress. This is dependent on thrombospondin-1, an adhesive glycoprotein that protects β cells from lipotoxic and ER stressors. MANF binds to BiP in the ER and is released to

be secreted during ER stress ([187](#), [188](#)). MANF binding to BiP stabilized BiP-client substrate complexes and inhibited BiP nucleotide exchange ([189](#)). Recently, neuroplastin was identified as a receptor for secreted MANF in rat INS-1 β cells, ([190](#)). In that study, MANF was found to bind neuroplastin and suppress downstream nuclear factor- κ B signaling to partially protect against pharmacological ER stressors. Given its properties, MANF and its receptor(s) represent an exciting frontier for potential disease therapies ([191](#)).

YIPF5.

Yip1 domain family member 5 (YIPF5) is an ER transmembrane protein involved in ER-Golgi trafficking ([192](#)) as well as ER-Golgi structural maintenance ([193](#)). Loss-of-function mutations in YIPF5 in humans result in neonatal diabetes, microcephaly, and epilepsy ([71](#)). Diabetes occurs in these individuals because of loss of β cells in response to increased ER stress. YIPF5 is not required for insulin secretion, but it is necessary for resilience against ER stress. ER stressors can induce transcription of YIPF5, suggesting a role in stress adaptation. The related gene YIPF3 was also implicated in β cells because YIPF3 deletion in a genome-wide CRISPR screen resulted in increased insulin content ([181](#)). YIPF3 has limited sequence identity with YIPF5 so it is unclear if they share similar tasks in β cells.

RIPK3.

Receptor interacting serine/threonine kinase 3 (RIPK3) is involved in inflammation and regulated necrosis signaling ([194](#)). RIPK3 is activated in response to β -cell ER stress induced in zebrafish with muscle insulin resistance ([195](#)). In that zebrafish model, RIPK3 activation led to IL-1 β induction and β -cell loss. In further studies, human islets were transplanted into transgenic mice, which permit pharmacological β -cell destruction. RIPK3 was activated specifically in human islet β cells when endogenous β cells were ablated, suggesting a conserved role for RIPK3 across multiple model systems and humans. Interferon-induced RIPK-mediated necrosis also requires PKR ([196](#)), tying the β -cell UPR to cytokine signaling pathways, an idea that is supported in other cell types. In fibroblasts, ER stress induces necroptosis dependent on RIPK1 and RIPK3 ([197](#)), and RIPK3 null mice were protected from ER stress induced by cardiac ischemia-reperfusion ([198](#)). Of note, preexposure of β cells to a mild ER stress, particularly caused by depletion of ER Ca^{2+} , potentiates IL-1 β -induced nuclear factor- κ B activation and the downstream expression of chemokines

and other pro-inflammatory genes ([199](#)). During necrosis in NIH 3T3 cells, RIPK3 interacts with glutamate dehydrogenase and isocitrate dehydrogenase 1, key players in the metabolic amplification of insulin secretion ([194](#)). The contribution of β cell RIPK3 in these responses is yet to be explored.

Technical Advances in β -cell UPR^{ER} Understanding

Our knowledge of the UPR in normal biology and disease states continues to evolve as technical advances drive the discovery and understanding of stimuli, new disease implications emerge, and the UPR is described with higher resolution and further assignment of functions and regulators. Cell lines that represent secretory cell types and disease states, advanced gene expression sequencing, pharmacological tools, UPR reporter assays, genetic manipulation of cells by CRISPR, and production of UPR sensor-null mice are some of the experimental approaches that have defined UPR sensors in normal biology and disease. Next, we highlight some of these recent technical advances.

Temporal Analyses to Better Understand UPR^{ER} Regulation

The dynamics of transcription, splicing, translation, and posttranslational events during the initiation and progression of β -cell UPR^{ER} is not completely understood. Omics experiments measuring outputs in cell types other than β cells could provide some insights. For example, 1 study in HeLa cells used transcriptome, translato^me, and proteome readouts to compare tunicamycin and H₂O₂-induced stress over 0 to 8 hours ([200](#)). Many up- and down-regulated pathways were shared between the ER stress and oxidative stress responses, although the data have yet to be compared with dedicated secretory cell types. The extent to which UPR^{ER} and alternative splicing transcriptional responses to diverse stresses are present and specific to β cells is an area under active investigation ([201](#)). Another example is the recent study identifying the action of QRICH1 in ER stress ([180](#)). The authors succeeded in identifying this factor by performing a time-course tunicamycin treatment in primary mouse intestinal organoids followed by single-cell RNA sequencing. Given the secretory parallels in enteroendocrine cells, the alterations observed in the QRICH1 study may also be relevant to β cells.

ATF6 α and XBP1 were investigated using time-course pharmacological ER stress experiments in isolated mouse islets ([131](#)). The study demonstrated the differing

kinetics of UPR gene induction depending on the stressor: thapsigargin (which depletes ER Ca^{2+}) was quicker than tunicamycin (which prevents protein glycosylation in the ER) to induce splicing of XBP1 and induction of *Hspa5* (BiP) expression. A time-course analysis of cyclopiazonic acid (a drug that depletes ER Ca^{2+} in a reversible way) in rat β cells identified an early degradation of insulin mRNA expression, which was paralleled by induction of the UPR proteins CHOP and BiP. Although the pro-apoptotic protein CHOP returned to a basal level 3 hours after the removal of cyclopiazonic acid, the chaperone BiP remained up-regulated, suggesting a long-term β -cell adaptive response ([115](#)) that may protect these cells against subsequent stresses ([202](#)). Future use of unbiased RNA sequencing analyses will undoubtedly provide additional insights into facets of UPR^{ER} regulation specific to the β cell and hopefully provide a path forward that dissociates the deleterious from the protective effects of the UPR in these cells.

Longer time courses associated with RNA sequencing have been performed. For example, an experimental paradigm was applied in which human islets were treated over the duration of days with palmitate, glucose, or both to model nutrient-induced metabolic stress and poststress recovery ([203](#)). Although human β cells fully recovered when followed for 4 days after a 48-hour exposure to high glucose or palmitate alone, they remained dysfunctional when exposed to both high glucose and palmitate, which was associated with a severe ER stress-induced mRNA signature ([203](#)). Another recent study evaluated the transcriptome during β -cell development and maturation in mice after birth and up to 60 days of age ([204](#)). A unique aspect of this study was the application of an algorithm that clusters gene expression changes based upon their respective temporal patterns ([205](#)). Applying this approach to temporal UPR^{ER} datasets could provide new insights into the UPR in general as well as potentially β cell-specific mechanisms.

Finally, an exciting use of these advanced computational approaches is to compare such changes between datasets from different researchers ([206](#), [207](#)) or target tissues from different diseases with common pathogenic pathways, including ER stress ([208](#)). These analyses open promising research areas that will advance our understanding of diabetes susceptibility, UPR^{ER} biology, and development of novel therapeutic strategies.

UPR Biosensors

The complexity of the UPR^{ER} signal has demanded development of a multitude of reporter assays for use both *in vitro* and *in vivo*. Early in the characterization of the UPR, cell-based assays relied upon the coupling of ERSE and UPR element transcriptional control elements of the UPR with expression of colorimetric or fluorescent reporters that were induced in response to pharmacological modulators of the UPR (209). Also, the upstream open reading frames that govern ATF4 translation upon eIF2 α phosphorylation enable repression of translation and stress-induced induction of reporter proteins (210, 211). The inhibition of mRNA translation from unspliced XBP1 sequences and the production of a functional mRNA on stress has been incorporated into the design of numerous *in vitro* and *in vivo* reporter assays that drive the production of a fluorescent product on IRE1 α activation (212, 213). Secreted alkaline phosphatase and fluorescent proteins also report upon the integrity of the ER and anterograde transport under conditions of stress (214, 215). The innovative impact of reporters of ER function is highlighted by the identification of a novel mechanism of ER reflux of material to the cytosol using ER-targeted superfolder GFP and ER-targeted mCherry as biosensor molecules (216). ERSE- and UPRE-driven mCherry has also been used as a fluorescent imaging-based readout of UPR^{ER} induction, for example, to demonstrate the cell–cell transmissibility of the UPR^{ER} in hepatocytes (215).

A powerful approach toward increasing understanding of β -cell UPR is the combination of different reporter assay readouts for ER homeostasis and secretory capability. A dual-luciferase reporter assay was developed for simultaneous readings of both ER calcium and UPR status (217). The assay combines a Gaussia luciferase-based ER calcium-monitoring protein (218) and a new UPRE-driven Nano luciferase. This tool could be used for high-throughput screening in β cells or other islet cell types alone or perhaps in combination with additional reporter assays. For example, a spliced XBP1-luciferase fusion protein was developed (219). Swapping one of these luciferases for a distinct substrate-specific luciferase or an alternate readout could allow assay multiplexing and greater extraction of information from high-throughput experimentation. Advances in the monitoring of preproinsulin processing in β cells using a fusion of insulin and superfolder GFP and mCherry (220) could be combined with measures of ER sensory pathways in experimental designs (especially luciferase-based) and in screening for therapeutic agents for treatment of diabetes. Incorporation of specific biosensors downstream of other stress signaling pathways could also provide valuable insights.

Single-cell Variation and β -cell Heterogeneity Contributions to ER Stress Responses

There may be distinct subtypes of β cells ([221-223](#)), but in certain cases these apparent subpopulations may actually be β cells transitioning between different states. Insulin expression and activity also vary between subpopulations of β cells ([224](#)), which may involve bursting of insulin gene transcriptional activity ([225](#)). β -cell heterogeneity and state transitions have been extensively reviewed ([226-230](#)), yet we would like to draw attention to a few key studies that are starting to unveil aspects of ER stress related to β cells transitioning through different states of relatively high and low insulin production and high and low UPR^{ER} ([136](#)). To handle stress, β cells are able to reduce insulin expression and increase UPR and antioxidant gene expression. Reduced insulin expression in that state was correlated with increased markers of β -cell proliferation and the cells also had increased metabolic gene expression.

In another approach, clusters containing β -like cells were derived from induced pluripotent stem cells (iPSCs) ([231](#)) obtained from individuals with neonatal diabetes resulting from mutations in the insulin gene at cysteine codons, which causes misfolding of proinsulin. The authors corrected 1 of these mutations in iPSCs using CRISPR/Cas9 and studied the β -like cells derived from mutant and corrected iPSCs. Applying single-cell RNA sequencing to these iPSC-derived β -like cells indicated subtle gene expression differences among the multiple cell types contained in the clusters after differentiation. β -like cells with mutated insulin had up-regulation of many genes involved in ER stress and ER-associated degradation, and down-regulation of genes involved in mitochondrial respiration, β -cell proliferation, and function. When these clusters were transplanted into mice, the mutant cells exhibited elevated expression of ER stress markers (eg, BiP, MANF) and reduced mTOR signaling, but there was no increase in cell death ([231](#)), possibly because the physiology of the normal recipient mice did not challenge the transplant. These findings suggest that early UPR^{ER} in differentiating β cells, in this case β cells with mutant insulin, causes dysfunction immediately and results in lower β -cell proliferation.

Single-cell RNA sequencing was applied to pancreatic islets from cynomolgus monkeys to investigate changes that occur with aging ([232](#)). β -cell function is known to decline with age ([233-235](#)), but because of the heterogeneous nature of islet

endocrine cells, it is difficult to determine the major contributing factors. Single-cell transcriptomic analysis of young and aged monkey islets revealed hundreds of up- and down-regulated genes associated with aging in both β and α cells. In particular, there was significant enrichment of UPR^{ER} gene expression in aged islet β cells. For example, increased expression of the ER chaperone HSP90B1 with age contributes to inhibition of the β cell's secretory response to glucose. Aged β cells exhibit down-regulation of genes involved in regulated exocytosis, protein trafficking, translation, and GPCR signaling pathways. Finally, islet amyloid polypeptide expression and amyloid formation is increased in aged islets, concomitant with a switch from adaptive to pro-apoptotic ER stress (232). These alterations combined with the enhanced UPR likely contribute to impaired β -cell function in aging.

Cell-cell Connections and the Spreading of the UPR

Islet β cells are highly interconnected through gap junction proteins, the connexins. In the case of β cells, Cx36 is a major player (236, 237). These junctions are dysfunctional in diabetic mice (238). ER stress can spread from cell to cell, a phenomenon previously observed in cancer (239, 240). Recently, work in hepatocytes established that ER stress can indeed be transmitted intercellularly through gap junctions (215), although via a distinct connexin, namely Cx46. The identity of the small molecule messenger(s) that propagates this stress signal is unknown. Given the interconnectedness of islet β cells, the same property that permits synchronous responses to nutrients and hormones may also predispose β cells to the propagation of stress signals. Whether stressed β cells can induce ER stress in adjacent β cells is an open question.

Potential for β -cell Rest Therapies

Often β cell-targeted therapies in type 2 diabetes aim to enhance the amount of insulin that is produced and secreted in response to hyperglycemia. It has been proposed that such an approach eventually leads to so-called " β -cell burnout" and contributes to progressive β -cell failure in type 2 diabetes (241-243). This scenario does not always occur and is likely influenced by genetic and environmental factors, as suggested by the existence of individuals in whom β -cell function is preserved despite decades of insulin hypersecretion to compensate for obesity-induced insulin resistance (244-248). The chronic use of sulfonylureas, which directly cause closure of the K_{ATP} channel to elicit insulin secretion, leads to β -cell dysfunction (242, 249),

and β -cell function declines in type 2 diabetes across multiple distinct treatment strategies ([250](#), [251](#)). In contrast, glucagon-like peptide 1 analogs both increase insulin release and protect human β cells against ER stress, by favoring BiP up-regulation ([252](#), [253](#)).

“ β -cell rest” involves suppressing endogenous β -cell activity pharmacologically ([254](#)), either indirectly by treating with exogenous insulin or by combination therapies that lower blood glucose while increasing insulin sensitivity ([Fig. 1](#)). The strategy based on intensive insulin therapy was first proposed and tested in 1940 in patients with type 1 diabetes ([255](#)). This intensive insulin treatment approach was later applied to individuals with type 2 diabetes by the 1970s, with promising results ([256](#)). Although subsequent trials have been limited in size, a review of the results suggests positive outcomes ([250](#)). More recently, the combination therapy approach was used to rest β cells and restore function in a strain of *db/db* mice with impaired β -cell compensation ([257](#)). These mice develop obesity and severe diabetes, but a combination of thiazolidinedione and SGLT2 inhibitor therapies allowed β -cell recovery. Work in *db/db* mice also demonstrated the benefits of β -cell rest via sequentially inhibiting GIPR signaling during the light cycle and activating GLP-1 receptor signaling during the dark cycle over a 4-week course of treatments ([258](#)).

Additionally, metabolically active cells have adaptive mechanisms to maintain the optimal amount of reactive oxygen species generation downstream of glucose metabolism, and this points to an avenue that could lead to therapeutic benefits in β cells ([259](#)). One such example is to suppress β -cell function as a therapeutic strategy by reducing the activity of glucokinase ([260](#)), the enzyme that controls glucose entry into the glycolytic pathway ([261](#)). For example, chronically treating islets from diabetic (*db/db*) mice with the glucokinase inhibitor mannoheptulose reversed the left-shifted glucose dose-response ([262](#)). Although, β cell-specific targeting strategies should be considered given that glucokinase in the α cell may have an opposite action ([263](#)). This is in line with previous suggestions that prolonged glucose-stimulated insulin secretion itself is a causative factor in β -cell stress through increased generation of reactive oxygen species ([264](#)). In agreement with this hypothesis, resting β -cell lines and rodent islets under lipotoxic stress using diazoxide protects against ER stress and secretory dysfunction ([265](#)). Recent evidence supports the idea that even short-term hyperglycemic stress can impair β cells ([266](#)). Conversely, a recent study of a subset of Diabetes Remission Clinical Trial participants demonstrated that dietary weight loss provides sustained improvements in β -cell

function ([267](#)).

Similar to intensive insulin/diazoxide therapies, suppression of β -cell function by other means has therapeutic benefits not only in type 2 diabetes, but also in type 1 diabetes, given the results of studies using the calcium channel blocker verapamil ([268](#), [269](#)). It is possible that preventing β cells from hypersecreting insulin may lower the exposure of potential autoantigens, allowing β cells to be less visible to the immune system and thus delaying disease ([270](#)).

Conclusions

The findings reviewed here suggest that further studies are warranted regarding both the mechanisms of the UPR in β cells and mechanisms/strategies to alleviate this stress—potentially through β -cell rest. Targeting these pathways in a β cell-specific manner could be a highly effective approach to improve β -cell health in disease. Toward this end, there are some key questions and topics, among many, that remain to be addressed:

- Do stressed β cells transmit UPR stress signals to adjacent β cells within the islet? If so, can this process be modulated pharmacologically for therapy? Given β cells' interconnection and their juxtaposition to γ and δ cells, whether such signals can propagate between these endocrine cells to affect global islet function is an open question.
- Can our current understanding of stimulus-secretion coupling and UPR biology be used in conjunction with β -cell targeting methods to therapeutically and specifically restore β -cell function without affecting other tissues?
- IRE1 α can be inhibited by Sec63 ([120](#)) and RPAP2 ([102](#)). However, whether these mechanisms are conserved or potentially exploitable in β cells is unknown. Can IRE1 α activity be specifically modulated in β cells through these regulators or through other means? One approach could be to link an IRE1 inhibitor (eg, IXA4) to GLP1 to target β cells, similar to GLP1-linked antisense oligonucleotides ([98](#)) and GLP1-linked estrogen ([271](#)). For an extensive discussion of pharmacological targeting of the UPRER as a therapeutic strategy in disease, please see ([272](#)) and see ([273](#)) for a β cell-focused review.
- Are there additional MANF receptors in addition to neuropilin and is their expression altered and/or activity disrupted in diabetes? Can MANF or its receptor(s) be targeted therapeutically in β cells in vivo or ex vivo?

- Advanced analysis of dynamic transcriptomic data at both the bulk and single-cell level should continue to be actively pursued to gain new understanding of how β cells handle physiological fluctuations in insulin demand and which aspects go awry in disease. Factors that influence the cell fate decision between survival and apoptosis in the β -cell UPR^{ER} (Fig. 2B) are of particular interest.
- A better understanding of the potential cross-talk between UPR pathways in the β cell may lead to therapeutically exploitable options. Can “protective pathways” (eg, BiP) be stimulated while inhibiting the pro-apoptotic ones (eg, CHOP, JNK)? PERK-IRE1 α cross-talk has been established in non- β cells in the context of UPR adaptation (102), although whether this mechanism also occurs in the β cell is unknown.

Pursuing lines of research that bring us closer to addressing any of these points will undoubtedly result in many new questions. Such pursuits represent critical opportunities to discover therapeutic in-roads for diabetes and other diseases of secretory biology.

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Glossary

Abbreviations

BiP	binding immunoglobulin
bZIP	basic leucine zipper
CHOP	C/EBP homologous protein
CoA	coenzyme A
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum-associated degradation
ERSE	endoplasmic reticulum stress response element

FFA	free fatty acid
GDH	glutamate dehydrogenase
GPCR	G protein-coupled receptor
HRI	heme-regulated inhibitor
iPSC	induced pluripotent stem cell
KATP	ATP-sensitive potassium
LDTF	lineage-determining transcription factor
MANF	mesencephalic astrocyte-derived neurotrophic factor
QRICH1	glutamine rich 1
RIDD	regulated IRE1-dependent decay
RIPK3	receptor interacting serine/threonine kinase 3
RPAP2	RNA polymerase II-associated protein 2
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCS-ATP	succinyl-CoA synthetase ATP
SCS-GTP	succinyl-CoA synthetase ATP
SDTF	signal-dependent transcription factor
TCA	tricarboxylic acid

UPRER	endoplasmic reticulum stress-induced unfolded protein response
UPRmt	mitochondrial unfolded protein response
YIPF5	Yip1 domain family member 5

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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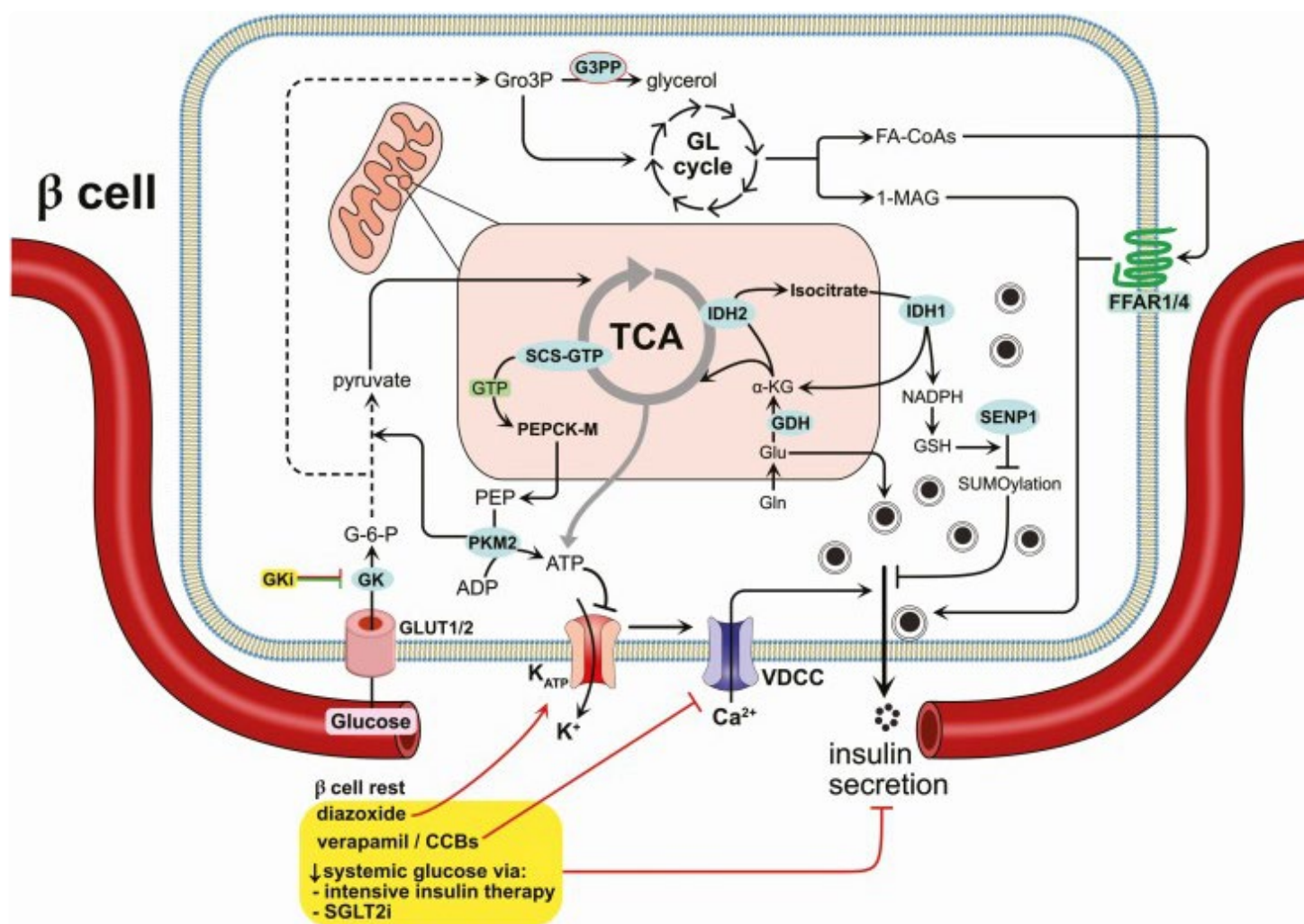
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Figures and Tables

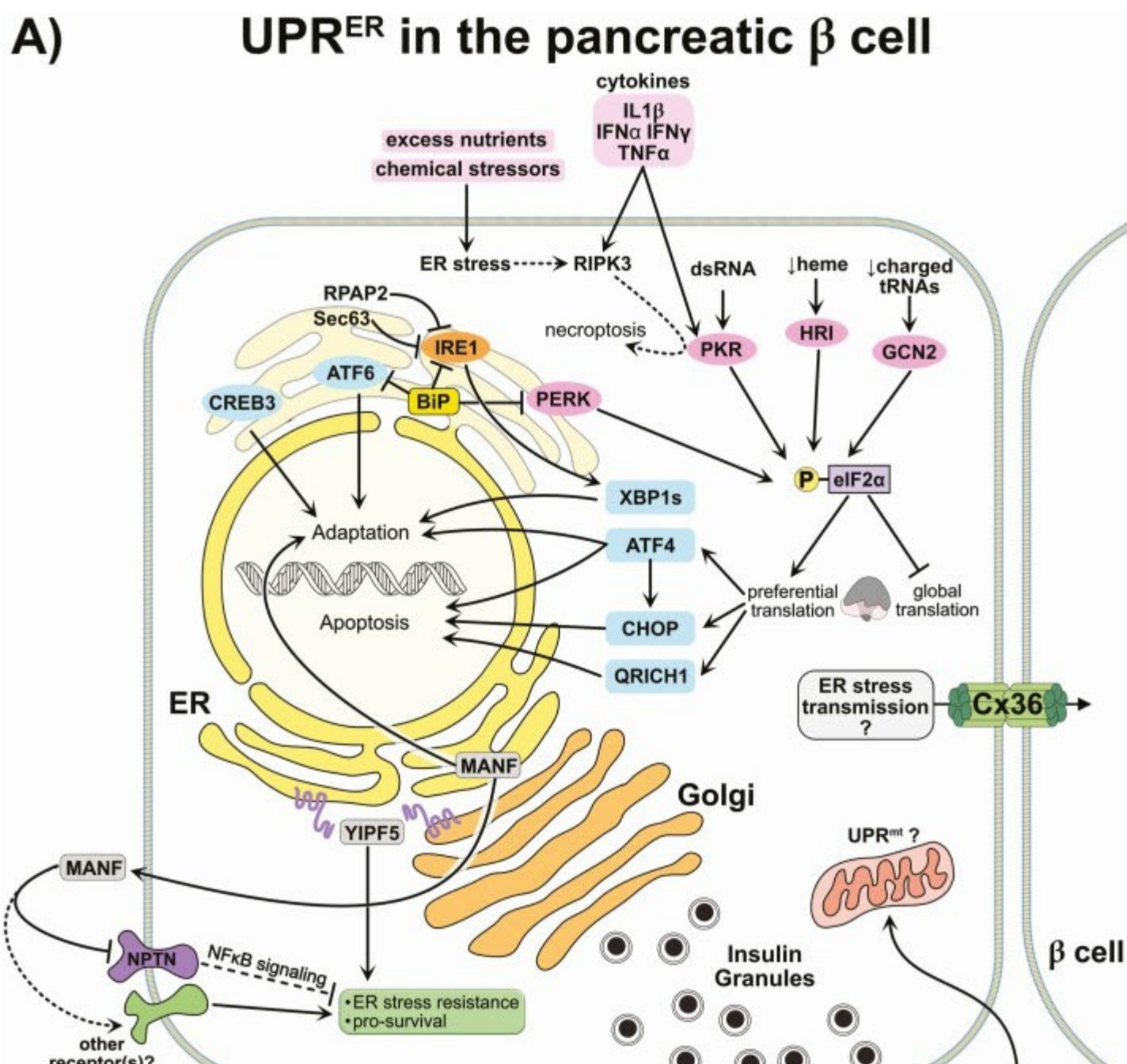
Figure 1.

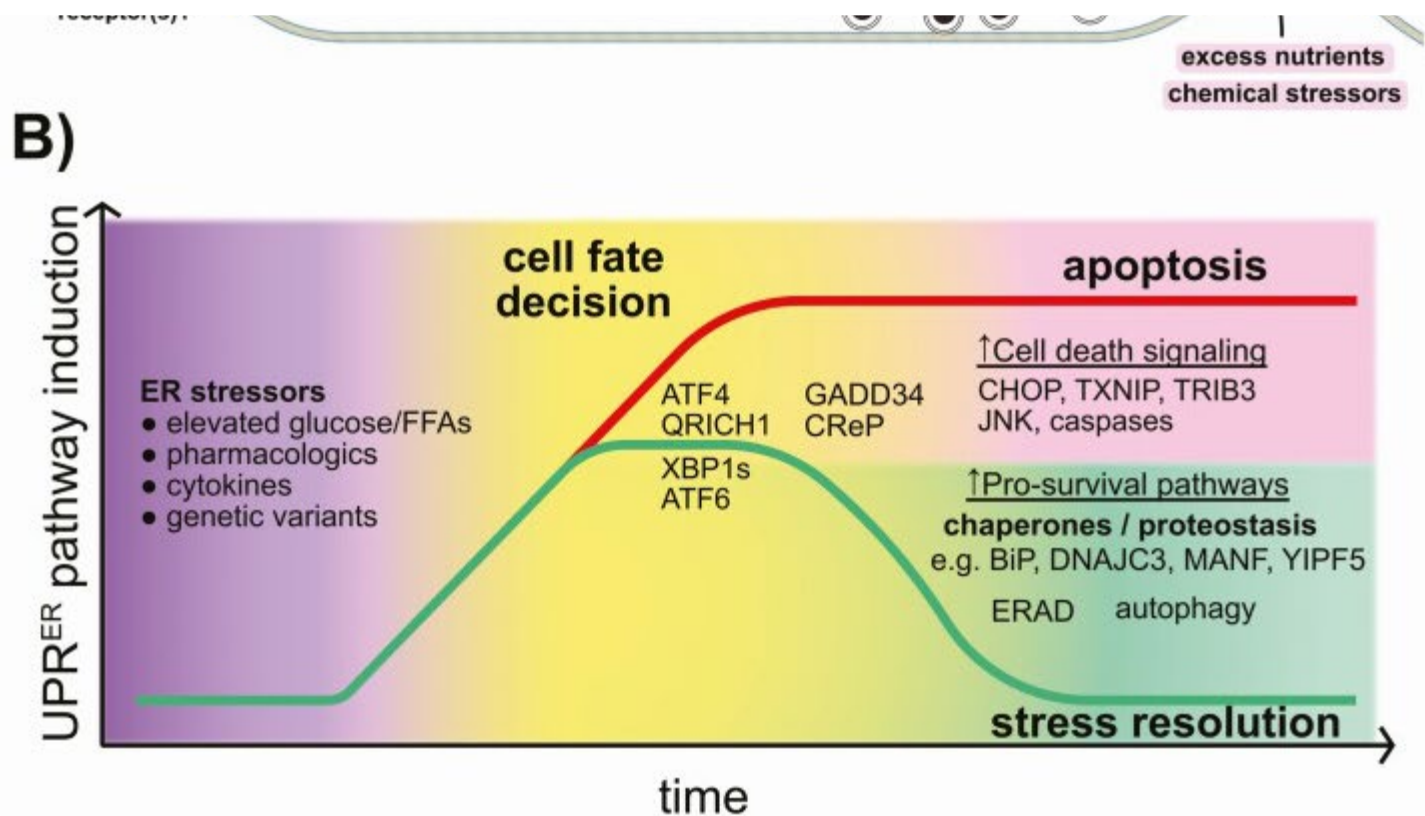


Intersection of the metabolic amplifying pathway of insulin secretion and β cell rest. Metabolism of nutrients through glycolysis and the tricarboxylic acid (TCA) cycle feed pathways generating metabolites including phosphoenolpyruvate (PEP), ATP, isocitrate, as well as intermediates of the pentose phosphate pathway and nucleotide biosynthesis pathways. The enzyme SCS-GTP generates succinate and GTP; this GTP, combined with oxaloacetate from the TCA cycle, is converted to PEP by PEP carboxykinase (PEPCK-M or *Pck2*). Recent work showed that membrane-localized pyruvate kinase (PKM2) converts this PEP to ATP, depleting local ADP concentrations, and promoting the closure of K_{ATP} channels and subsequent membrane depolarization. Another highlighted pathway is that of glutamate dehydrogenase (GDH), which converts glutamine to α -ketoglutarate (α -KG) in the mitochondria. There, α -KG may fuel the generation of other TCA cycle intermediates or be converted into isocitrate to replenish the downstream reducing equivalent glutathione (GSH), which is required for deSUMOylation of exocytotic proteins. The glycerolipid (GL) cycle also feeds into metabolic amplification via supplying 1-monoacylglycerol, which acts directly on exocytotic machinery to promote secretion. Fatty-acyl-CoAs (FA-CoAs) are also released and/or reach the β cell from the circulation to activate the free fatty acid receptors 1/4 (FFAR1/4), GPCRs that promote ER calcium release. Additionally, glycerol-3-phosphate (Gro3P) derived from glycolysis can be degraded by glycerol-3-phosphate phosphatase (G3PP) to regulate the impact of Gro3P on insulin secretion. There are direct and indirect therapeutic strategies that aim to reduce the insulin secretory demand on β cells (ie, β -

cell rest, highlighted in yellow). These include, but are not necessarily limited to: (1) the use of diazoxide to open K_{ATP} channels and prevent membrane depolarization even in the face of increased glucose concentrations; (2) targeting of β -cell calcium channels to directly prevent calcium influx-mediated exocytosis; (3) glucokinase inhibitors (GKis) that could reduce metabolic flux of glucose into glycolysis and the TCA cycle, but also lead to improved secretory regulation; and (4) intensive insulin treatments or sodium-glucose transporter 2 inhibitors (SGLT2i), each of which results in a decrease in circulating glucose and therefore indirectly alleviate the demand on β cells. Supporting references are provided in the text. The arrow from GK_i to GK is red and green, indicating it suppresses β -cell insulin secretory activity but also enhances β -cell glucose dose-response.

Figure 2.





Overview of endoplasmic reticulum (ER) stress and non-ER stress UPR pathways in β cells and the fate decision of adaptive vs terminal ER stress-induced unfolded protein response (UPR^{ER}). (A) The β cell repeatedly responds to nutrient fluxes over its lifetime and uses the UPR^{ER} as a mechanism to handle this constant demand for insulin production. Increased secretory protein production causes the titration of the ER chaperone binding immunoglobulin (BiP) away from ER membrane resident stress sensors, leading to their activation. These include PERK, IRE1 α , and the ATF6/CREB3 family. Each of these sensors activates downstream transcription factors that enable cells to tolerate or mitigate the stress. ATF6/CREB3 are cleaved and release the respective active transcription factors; IRE1 α and cofactors catalyze the cleavage and splicing of XBP1 mRNA, causing expression of the transcription factor XBP1s; and the eIF2 α kinase PERK phosphorylates eIF2 α , inhibiting global translation and causing the preferential translation of additional transcription factors (ATF4, CHOP, QRICH1). These transcription factors induce expression of pro-survival as well as pro-apoptotic genes, depending on the factor and also on the duration/intensity of the stress. Distinct non-ER-related stresses can intersect with UPR signaling through the remaining eIF2 α kinases HRI, GCN2, and PKR (in pink), which each similarly inhibit global translation. ER and Golgi localized factors including YIPF5 and MANF promote ER stress resistance, although the mechanisms are under investigation. Finally, other mechanisms may be at play during the β -cell response to stress. For example, it is unknown what the mitochondrial UPR (UPR^{mt}) does, or whether the highly interconnected islet β cells may transmit stress signals via gap junctions, as has been shown in other cell types. Supporting references provided in the text. (B) In response to physiologic, pathophysiologic, or pharmacologic stressors, β cells activate the UPR^{ER} (as described in [Fig. 2](#)). Genetic variation or predisposal also contributes to baseline stress and the ability of the β cell to respond appropriately. The

duration and intensity of the stress response may dictate a cell fate decision wherein β cells progress either toward stress resolution or programmed cell death. Key factors in stress resolution include the early induction of ER chaperones (eg, DNAJC3/p58^{IPK}, GRP78/BiP), protein disulfide isomerases (PDIs), and ER membrane/luminal pro-survival factors (eg, MANF, YIPF5), induction of ER-associated degradation (ERAD) and autophagy, culminating in the clearance of misfolded proteins and restoration of homeostasis. When the stress persists unmitigated, continuous ATF4 expression leads to C/EBP homologous protein (CHOP) production and downstream induction of pro-apoptotic factors (eg, TXNIP, TRIB3). Novel factors like glutamine rich 1 (QRICH1) may play a role in this cell fate decision and progression toward apoptosis. Intense UPR^{ER} can induce negative feedback of eIF2 α phosphorylation via the expression of eIF2 α phosphatases GADD34 and CReP. Attempting to restart translation before sufficient stress mitigation may then antagonize recovery and promote cell death.