Physiological ER Stress: The Model of Insulin-Secreting Pancreatic β-Cells

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Contents

Abstract

Endoplasmic reticulum (ER) stress and the consecutive activation of the Unfolded Protein Response (UPR) contribute to the pathogenesis of several diseases including diabetes, neurodegenerative diseases and inflammation. However, the UPR also plays a crucial adaptive role in the acquisition and maintenance of the phenotype of cells that secrete large amounts of proteins. After a brief overview of this physiological role of the UPR in immunoglobulin-secreting plasmocytes and pancreatic acinar cells, this chapter will mainly focus on insulin-secreting pancreatic β-cells that play a critical role in glucose homeostasis. Upon their

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stimulation with glucose and other nutrients, these cells display a rise in mitochondrial metabolism, ATP production and Ca^{2+} pumping in the ER, in parallel to the stimulation of protein (preferentially proinsulin) biosynthesis. These metabolic and functional features give rise to a peculiar pattern of acute regulation of the UPR by nutrients. At low non-stimulatory glucose concentrations, when intracellular ATP, $[Ca^{2+}]_{ER}$ and protein synthesis are low, the IRE1-XBP1 branch of the UPR is at its lowest level of activation while the PERK-eIF2α-ATF4 branch of the UPR is maximally activated, with strong upregulation of Integrated Stress Response (ISR) genes. Upon glucose stimulation, the rise in ATP and $[Ca^{2+}]_{ER}$ leads to PERK-eIF2α dephosphorylation, inhibition of the ISR and derepression of protein synthesis. Consequent activation of the IRE1-XBP1 branch of the UPR upregulates the expression of chaperones, foldases, ER to Golgi transport and ER-associated degradation machinery that help the β-cell coping with the large increase in proinsulin biosynthesis. This opposite glucose regulation of the PERK and IRE1 arms of the UPR is rapid and dynamic, suggesting its importance in the physiological adaptation of the β-cell to changes in nutrient supply.

Keywords

Endoplasmic Reticulum Stress (ER stress) · Unfolded Protein Response (UPR) · Pancreatic beta cell · Proinsulin biosynthesis · Gluocose homeostasis

Abbreviations

Aatf	Apoptosis Antagonizing Transcription Factor
Ach	Acetylcholine
Akt	Protein Kinase B
ATF3/4/6	Activating Transcription Factor 3/4/6
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
ATP	Adenosine Triphosphate
BiP	Immunoglobulin heavy chain-Binding Protein
Ca^{2+}	Calcium
CHX	cycloheximide
Ddit3	DNA damage inducible transcript 3
Dnajc3	DNAJ (Hsp40) homologue C3
EDEM	ER Degradation Enhancer Mannosidase α-like
eIF2α	eukaryotic Initiation Factor-2α
EIF2AK3	eIF2α kinase 3
ER	Endoplasmic Reticulum
ERAD	ER Associated Degradation
Erp44	Endoplasmic reticulum protein 44
FFA	Free Fatty Acid
GADD34	Growth Arrest and DNA Damage protein 34
GLP1	Glucagon-like peptide 1

GLUT2	Glucose transporter 2
GSIS	glucose-stimulated insulin secretion
Fkbp11	FK506 binding protein 11
Herp	Hyperhomocysteinemia-induced ER stress responsive protein
Hmox1	heme oxygenase 1
(h)IAPP	(human) islet amyloid polypeptide
Hspa13	heat shock protein 13
HSP90	heat shock protein 90 beta, member 1
IRE1	Inositol Requiring Enzyme 1
ISR	Integrated Stress Response
KATP	K+-ATP-dependent channel
LPS	Lipopolysaccharide
Mt1a	metallothionein 1a
mTOR	mammalian target of rapamycin
Nrf2	Nuclear factor-E2-related factor 2
p38-MAPK	p38- Mitogen Activated Protein Kinase
PDI	Protein Disulphide Isomerase
Pdia4	protein disulfide isomerase family A, member 4
PERK	double stranded RNA-activated protein kinase (PKR)—like ER Kinase
PP1	protein phosphatase 1
RIP	Regulated intramembrane proteolysis
ROS	Reactive Oxygen Species
S1/2P	Site 1/2 Protease
SERCA	Sarco/Endoplasmic Reticulum Ca^{2+} ATPase
TRAF2	Tumor necrosis factor receptor (TNFR) Associated Factor 2
Trb3	Tribbles homologue 3
T2D	Type 2 Diabetes
Ub	Ubiquitin
UPR	Unfolded Protein Response
VDCC	Voltage-Dependent Ca^{2+} Channel
WRS	Wolcott-Rallison Syndrome
XBP1	X-box Binding Protein 1

1 Introduction

The endoplasmic reticulum (ER) is not only the site of synthesis, posttranslational modification and proper folding of proteins destined to the secretory pathway and various intracellular compartments, but also an important intracellular Ca^{2+} store involved in signal transduction, and the site of sterol and lipid synthesis [1]. As detailed in Chaps. 1 and 3, perturbation of one or more of these processes can trigger an ER stress. Although alterations in lipid synthesis may affect the ER, much attention has so far been focused on ER stress induced by disruption of protein folding [2].

Correct folding of proteins is crucial for their proper biological function. This is why the ER is equipped with a battery of ER-resident molecular chaperones and foldases [1], and contains a high Ca^{2+} concentration that ensures proper activity of the latter [3–5]. However, any increase in ER client protein load (*e.g.* increased secretory protein synthesis) or decrease in chaperone function (*e.g.* due to ER Ca^{2+} emptying [3, 6] or ATP depletion [7]) leads to an imbalance between folding demand and folding capacity with accumulation of unfolded proteins in the ER. As a consequence, a cellular adaptive response, the Unfolded Protein Response (UPR), is activated that contributes to restoring ER homeostasis by three means: (i) rapid and transient reduction in protein translation to attenuate ER load [8], (ii) upregulation of the expression of ER-chaperones [9, 10] and increase in ER size [11, 12] to improve the ER folding capacity of the cell, and (iii) activation of the ER-associated degradation (ERAD) machinery [13, 14] and of autophagy [15] to enhance clearance of misfolded proteins from the ER (Fig. 1).

The molecular pathways involved in the fine tuning of the UPR in mammalian cells have been detailed in Chap. 3 of this book. For a correct understanding of this chapter, however, it is useful to describe them as consisting of two main branches. The first branch involves the unconventional splicing of X-box binding protein 1 (*Xbp1*) pre-mRNA by active Inositol requiring enzyme 1 (IRE1) endoribonuclease (also known as ERN1) and subsequent increase in active XBP1s [16], as well as the proteolytic activation of activating transcription factor 6 (ATF6) [17] (Fig. 1). Both transcription factors stimulate the expression of *Xbp1*, chaperones, and components of the ERAD machinery, thereby leading to improved folding capacity and degradation of unfolded proteins [9, 18–20] (Fig. 1).

The second branch of the UPR involves the phosphorylation of the α-subunit of eukaryotic translation initiation factor 2 (eIF2α) on serine 51 by double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK also known as EIF2AK3). This is followed by rapid and transient reduction in general protein translation, together with a paradoxical increase in the translation of some rare transcripts including activating transcription factor 4 (ATF4) [8, 21]. This leads to enhanced expression of ATF4-target genes including chaperones, several antioxidant response genes like heme oxygenase 1 (*Hmox1*), and proapoptotic effectors such as DNA damage inducible transcript 3 (*Ddit3* also known as *Chop* or *Chop10*), activating transcription factor 3 (*Atf3*), and tribbles homologue 3 (*Trb3*) [8, 22, 23] (Fig. 1).

Noteworthy, eIF2α can be phosphorylated independently from the UPR by kinases other than PERK in response to other kinds of stress, including amino acid starvation [24]. Consequently, an increase in ATF4 protein and the mRNA levels of its target genes including *Ddit3*/*Chop* should not be considered as an indicator of ER stress unless it is paralleled by PERK phosphorylation and/or the activation of other UPR sensors, IRE1 and ATF6. Therefore, we will next refer to this arm of the UPR by the term Integrated Stress Response (ISR) as suggested by Ma and coll. [25].

Although the UPR mainly fulfills an adaptive function, failure of the ER stress response to restore ER homeostasis triggers an apoptotic program that involves the

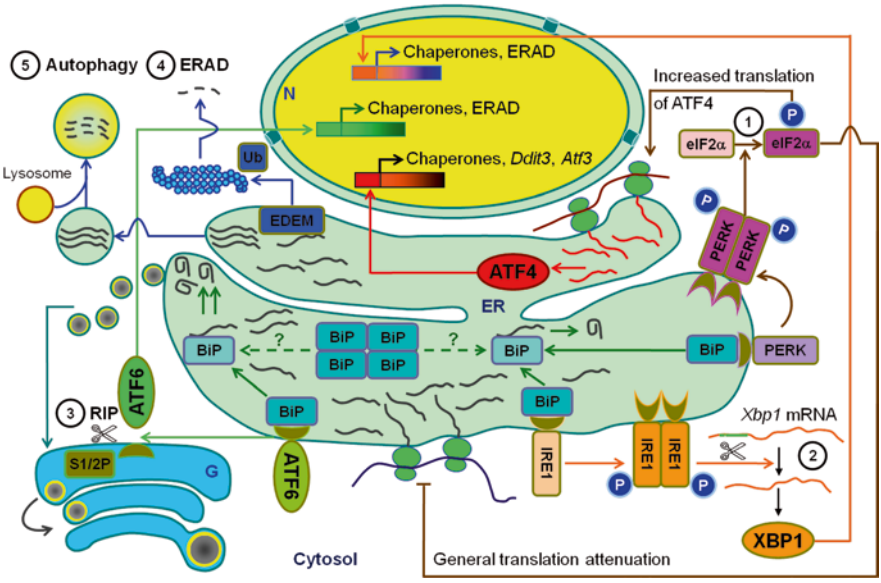

Fig. 1 Schematic representation of the molecular mechanisms of UPR activation in response to the accumulation of unfolded proteins in the ER. The UPR is orchestrated by three master ER stress sensors: PERK, IRE1 and ATF6. In unstressed cells, these sensors are maintained inactive through interaction of their ER luminal domain with the ER-resident chaperone BiP. Then, upon ER stress, accumulation of unfolded proteins triggers their interaction with BiP and promotes dissociation of the latter from PERK, IRE1 and ATF6 [132, 133]. In addition, it has been suggested that BiP has an oligomeric state serving as a storage pool from which BiP is released when unfolded proteins accumulate in the ER [134]. (*1*) PERK-eIF2α-ATF4 branch of the UPR, also referred to as the Integrated Stress Response for events from eIF2α phosphorylation and downstream. (*2*) IRE1-XBP1 branch of the UPR. (*3*) Proteolytic activation of ATF6. Besides the ERAD machinery (*4*), autophagy may also contribute to the clearance of misfolded proteins (*5*). The activation of autophagy by the UPR has been shown to depend on BiP and to require the protein kinase activity of IRE1. Atf3: Activating transcription factor 3; ATF4: Activating transcription factor 4; ATF6: Activating transcription factor 6; BiP: Binding Ig protein; Ddit3: DNA damage inducible transcript 3; eIF2α: eukaryotic translation initiation factor 2α; EDEM: ER degradation enhancer, mannosidase α-like; ERAD: endoplasmic reticulum associated degradation; IRE1: Inositol requiring enzyme 1; PERK: double-stranded RNA activated protein kinase (PKR)-like ER kinase; RIP: Regulated intramembrane proteolysis; S1/2P: Site 1/2 proteases; Ub: Ubiquitin; XBP1: X-box binding protein 1

complex action of several not fully identified effectors. Among them, DDIT3 has been shown to play a major role in the apoptotic response to chronic ER stress in diverse cell types, including pancreatic β-cells [26–28]. The second major player in ER stress-induced apoptosis is part of the IRE1 branch of the UPR. Activated IRE1 has been shown to recruit the adapter protein tumor necrosis factor receptor-associated factor 2 (TRAF2) which is released from the ER-localized procaspase 12 [29]. On the one hand, the complex IRE1-TRAF2 interacts with the apoptosis signal-regulated kinase 1 which induces apoptosis via the activation of c-jun NH2-

terminal kinase and p38-MAPK [30–33]. On the other hand, TRAF2 sequestration by IRE1 promotes procaspase 12 oligomerization and subsequent processing [29, 34, 35]. In addition, it has been proposed that chronic ER stress may alter the specificity of the endoribonuclease (RNase) activity of IRE1, leading to the cleavage of ER-associated mRNAs including that of preproinsulin and thereby, to the alteration of the cell-differentiated phenotype and the promotion of apoptosis [36–40] (for detailed description of the ER stress response and its signal transduction, see chaps. 1 and 2 of this book).

2 Physiological ER Stress Response

During the last decade, the ER stress pathway has emerged as an important contributor to the pathogenesis of several diseases including diabetes [41–45], neurodegenerative diseases [46–49], inflammation [50–52] and cardiovascular diseases [53–55] (see the following chapters for the role of ER stress under pathological conditions). However, the UPR also plays a crucial adaptive role during terminal differentiation of cells that secrete large amounts of proteins, including hepatocytes, thyrocytes, plasma B cells, exocrine pancreatic and salivary acinar cells, and endocrine pancreatic β-cells, as well as in the maintenance of their differentiated phenotype in adulthood [56–65]. In this chapter, we will first present a brief overview of the physiological role of the UPR in immunoglobulin-secreting plasmocytes and pancreatic acinar cells, and will next focus on insulin-secreting β-cells that play a critical role in glucose homeostasis. We will also review data suggesting the existence of two different types of physiological ER stress responses in β-cells, depending on their energetic status, ER Ca^{2+} content and level of protein synthesis.

2.1 The Immunoglobulin-Secreting Plasma B Cells

The IRE1-XBP1 arm of the UPR plays a critical role during the transformation of B lymphocytes in plasmocytes specialized in the secretion of large amounts of immunoglobulins (Igs). Indeed, *Xbp1*-KO B cells were unable to differentiate and secrete Igs [58]. Moreover, *Xbp1* mRNA splicing has been shown to be essential for Ig synthesis during B cell differentiation. Thus, overexpression of spliced XBP1 (XBP1s) rescued plasma cell differentiation and restored Ig production in *Xbp1*-KO B cells. Furthermore, XBP1s has been shown to be involved in interleukin-6 expression and secretion. Noteworthy, this cytokine is necessary for plasma cell survival [66]. In agreement with these observations, B cells overexpressing a dominant-negative mutant of IRE1 protein that prevents classical IRE1 signaling and subsequent *Xbp1* mRNA splicing, did not express B cell receptors and were defective in Ig production upon lipopolysaccharide (LPS) stimulation. Interestingly, infection of these cells by a retrovirus expressing XBP1s protein restored, and even

enhanced, the levels of secreted Ig [61]. Moreover, IRE1 conditional-KO mice expressing IRE1 only in extra-embryonic tissues displayed markedly reduced serum Ig levels upon LPS stimulation [67]. The ATF6 arm of the UPR has also been shown to be activated in differentiating B cells. However, whether and to what extent it is required for Ig synthesis and secretion is unknown [68, 69]. Finally, UPR activation in B lymphocytes has been shown to be accompanied by the upregulation of several XBP1-ATF6-target genes including Ig-binding protein (*BiP*), ER degradation enhancer mannosidase α-like (*Edem*), hyperhomocysteinemia-induced ER stress-responsive protein (*Herp*) and DNAJ (Hsp40) homologue C3 (*Dnajc3* also known as $p58^{IPK}$) [61, 69, 70].

In contrast with XBP1-ATF6-target genes, the ISR does not seem to play a significant role in the process of B cell maturation and Ig synthesis. Thus, the ISR genes *Ddit3* and growth arrest and DNA-damage-inducible protein 34 (*Gadd34*) were not induced during B cell differentiation [61, 69, 70]. Besides, fetal hematopoietic cells carrying a mutation in eIF2α that prevents its phosphorylation were able to give rise to mature B cells. The latter secreted normal amounts of Igs upon stimulation [61]. Likewise, *Perk*-KO B cells did not present developmental or functional defects [69]. Moreover, it has been proposed that PERK-mediated signaling is repressed during plasma cell differentiation, consistent with the need to maintain high rates of Ig synthesis upon LPS stimulation. Thus, B cell differentiation has been shown to stimulate the expression of the cytosolic chaperone DNAJC3 [69, 70], a proposed negative regulator of PERK activity [71]. In addition, pretreatment of a B cell line with LPS has been shown to block further induction of the downstream ATF4-target DDIT3 by classical pharmacological inducers of ER stress [70].

2.2 The Exocrine Pancreatic Acinar Cells

The ER stress signaling network has also been reported to play an important role in the development and function of exocrine pancreatic acinar cells. The latter synthesize and secrete large quantities of digestive enzymes in the form of inactive precursors/zymogens. These cells present the highest rate of protein synthesis among all human tissues and thereby, require an intact operative UPR. Indeed, pancreatic acinar cells lacking the *Xbp1* gene undergo important apoptosis during embryonic development [56]. In addition, mutant mice neonates expressing *Xbp1* only in the liver had a small pancreatic gland with sparse acini, the acinar cells of which contained few zymogen granules and a poorly developed ER. These mutant pancreases also presented reduced expression of several XBP1-target genes, including protein disulfide isomerase (*Pdi*), *Edem* and *Sec61α*, while presenting a marked increase in the mRNA levels of *Ddit3* in parallel with reduced expression of digestive enzymes [60]. These observations were extended by a recent report showing that *Xbp1* ablation in adult acinar cells induced important alterations in cell structure with reduced cytoplasm and zymogen granules, as well as a poorly developed and dilated ER. These cells also presented increased apoptosis in parallel to the stimulation of eIF2α phosphorylation, ATF6 activation, and enhanced expression of the proapop-

totic effector *Ddit3* [72]. Finally, it has been shown that the stimulation of isolated rat acini, with physiological concentrations of secretagogues that do not cause cell damage, stimulated amylase secretion in parallel to the upregulation of *Xbp1* mRNA splicing and BiP protein levels. On the other hand, PERK phosphorylation and increased *Ddit3* expression were only observed with the secretagogue that caused cell damage [63]. Taken together, these observations highlight the fundamental role of XBP1 in the maintenance of acinar cell function and integrity under physiological conditions.

However, unlike B lymphocytes, PERK has also been shown to play an important physiological role in acinar cells. Thus, *Perk*-KO mice displayed postnatal exocrine pancreatic insufficiency as evidenced by steatorrhea. Besides, exocrine cells of these mutant mice presented altered ER morphology and increased apoptosis [57]. These alterations have been suggested to stem from protein over-synthesis and uncontrolled ER stress. However, a subsequent report has proposed that PERK was essential for acinar cell viability, but was not required for normal protein synthesis and secretion. Thus, protein synthesis in excised pancreatic lobules of mice lacking *Perk* specifically in acinar cells and amylase secretion under basal or stimulated conditions were similar to that in control littermates before the onset of massive cell death. However, protein synthesis was markedly reduced in the pancreases of adult mutant mice, likely as a consequence of the death of the majority of acinar cells in these pancreases. Interestingly, these *Perk*-deficient acinar cells did not present significant changes in the expression of *BiP, Ddit3*, or *Xbp1* mRNA splicing [73], and displayed an overall normal morphology, in contrast with the initial study by Harding and coll. [57]. Finally, it has been shown that *Atf4* KO mice also exhibited markedly reduced exocrine pancreas. However, this event was observed in the early postnatal period as a consequence of hypotrophy and not cell death [73].

These examples illustrate the important and complex physiological roles of the different ER stress signaling branches in the preservation of function and survival of cells with a high protein secretory capacity. In the next sections of this chapter, we will depict in detail our current knowledge about the role and the regulation of physiological ER stress response in insulin-secreting pancreatic β-cells. The latter are particularly sensitive to ER stress due to their high rate of proinsulin biosynthesis in response to glucose stimulation [74–76].

3 The Model of Pancreatic β-Cells: Metabolic Features and Susceptibility to ER Stress

Pancreatic β-cells are the guardians of glucose homeostasis in mammalians. They secrete insulin after meals to maintain glycemia within a narrow range. Glucose is the main physiological regulator of β-cell function. This effect requires glucose transport across the plasma membrane and its oxidation by glycolysis and the mitochondrial Krebs cycle, thereby leading to an atypical rise in cytosolic ATP/ADP ratio [77] (reviewed in [78]). The latter induces the closure of ATP-sensitive K^+

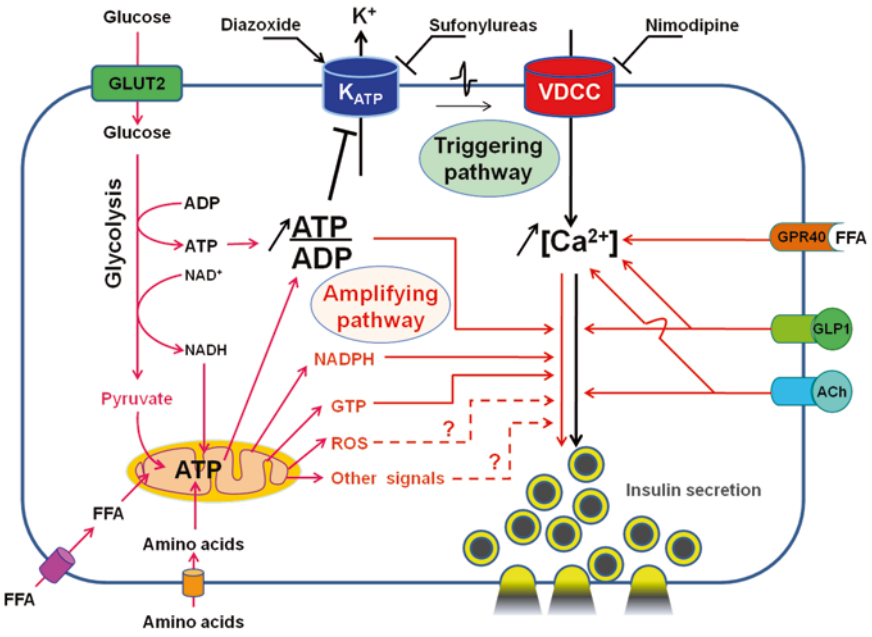

Fig. 2 Schematic representation of the triggering and amplifying pathways of the stimulation of insulin secretion by glucose and other nutrients in pancreatic β-cells, and the mechanisms by which pharmacological agents, hormones and neurotransmitters modulate insulin secretion. ACh: Acetylcholine; FFA: Free fatty acid; GLP1: Glucagon-like peptide 1; GLUT2: Glucose transporter 2; K_{ATP}: K^+-ATP-dependent channel; ROS: Reactive oxygen species; VDCC: Voltage-dependent Ca^{2+} channel

channels, plasma membrane depolarization and opening of voltage-dependent Ca^{2+} channels. The ensuing Ca^{2+} influx induces a rise in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) that is the triggering signal for insulin secretion (triggering pathway of glucose-stimulated insulin secretion (GSIS), Fig. 2) [79]. In addition, metabolic coupling factors generated by glucose metabolism and intracellular messengers affected by hormones and neurotransmitters such as glucagon-like peptide 1 and acetylcholine can amplify the efficacy of Ca^{2+} on insulin granule exocytosis (metabolic and hormonal amplifying pathways of insulin secretion, Fig. 2) [79].

Besides increasing the ATP/ADP ratio and stimulating insulin secretion [77, 80, 81], glucose acutely stimulates β-cell protein synthesis in a concentration-dependent manner, with a preferential effect on proinsulin biosynthesis. As for GSIS, the latter effect requires glucose metabolism. It is, however, independent from changes in $[Ca^{2+}]_i$ [82–85]. Glucose modulates proinsulin biosynthesis in the short-term through the stimulation of preproinsulin mRNA translation. This process involves specific sequences in the 5'- and 3'-untranslated regions of the preproinsulin mRNA that favor translation of the latter over other mRNAs and confer its stability [86, 87]. In addition, a selective recruitment of preproinsulin mRNA from

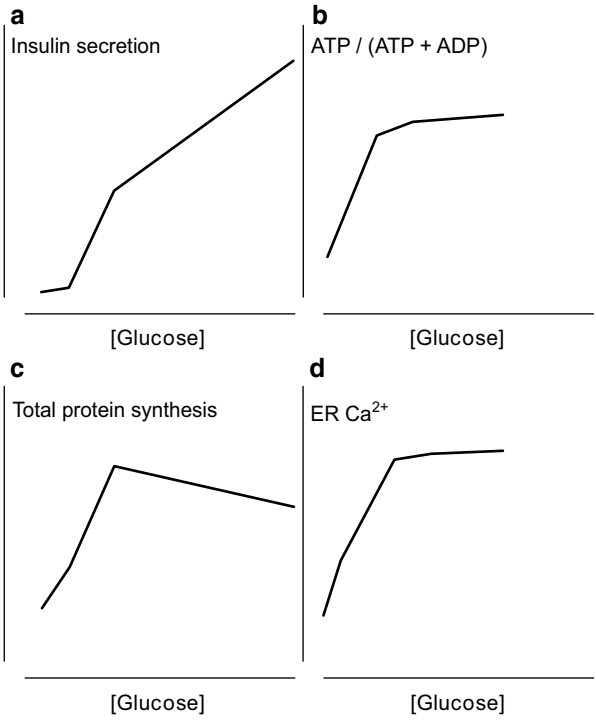

Fig. 3 Schematic representation of the effects of increasing glucose concentrations on the principal functional and metabolic parameters in pancreatic β-cells. **a** and **c**, GSIS and total protein synthesis in isolated rat islets cultured for 18 h in G2–30. Data adapted from [94]. **b**, glucose-induced changes in the ATP/ADP ratio in isolated rat islets incubated for 30 min in 0.5–20 mmol/l glucose. **d**, Glucose-induced changes in $[Ca^{2+}]_{ER}$ in mouse β-cells. Data adapted from [100]

a cytosolic pool to the ER occurs upon glucose stimulation [88, 89]. Glucose also increases the rate of preproinsulin mRNA translation by modulating the activity of translation initiation factors [90–92]. On the other hand, the glucose-dependent activation of the mammalian target of rapamycin (mTOR) pathway may also contribute to proinsulin biosynthesis [93]. Then, in the long-term, glucose also regulates proinsulin biosynthesis by stimulating preproinsulin gene transcription [84, 87].

It has been shown in purified rat β-cells that glucose stimulation from 1 to 10 mmol/l (G1 to G10) increased total protein and proinsulin biosynthesis by 13- and 25-fold, respectively. However, stimulation with higher glucose concentrations did not further increase these rates. Interestingly, under basal condition, synthesized proinsulin represented 13% of total protein synthesis while glucose stimulation increased this percentage to ~50% [74]. Similarly, in purified human β-cells, glucose stimulation from G0 to G10 has been shown to increase total protein synthesis by 2-fold, and proinsulin biosynthesis by 6-fold. Proinsulin represented 7% of newly formed proteins under basal condition, and 25% at G10. On the other hand, the protein synthetic activity of insulin-negative islet cells was high irrespective of the glucose concentration [76]. Besides, in isolated rat islets cultured in the presence of increasing glucose concentrations, glucose stimulated total protein synthesis between G2 and G10 in parallel to the augmentation of the ATP/ADP ratio. This activation occurred at lower glucose concentration than the stimulation of insulin secretion which is mainly observed between G5 and G30 [80, 94] (Fig. 3a–c). Of note,

G5 is just below the threshold concentration for the stimulation of insulin secretion and G10 is the optimal glucose concentration for the maintenance of rat β-cell function and survival during prolonged culture *in vitro*. Thus, culture under either lower or higher glucose concentration (*vs.* G10) is deleterious and triggers alterations in function and gene expression, and leads to β-cell apoptosis if prolonged (reviewed in [95] and [96]).

In parallel to the above described metabolic events, the acceleration of β-cell metabolism upon stimulation with increasing glucose concentrations enhances ER Ca^{2+} uptake by sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA2B) between G0 and G10 in mouse β-cells (Fig. 3d) [97–100]. This effect likely results from the double dependency of SERCA2B activity on ATP and Ca^{2+} concentrations [101]. Interestingly, the ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) has been shown to play an important role in proper function of several ER-resident molecular chaperones and foldases [3–5] and in the processing of proinsulin [102]. Thus, it is possible that Ca^{2+} pumping in the ER plays a role in the modulation of chaperone activity and in the adaptation of the ER folding machinery to the important increase in protein synthetic load triggered by glucose stimulation in β-cells.

Besides proinsulin, β-cells also synthesize islet amyloid polypeptide (IAPP), a 37-amino acid polypeptide of unclear physiological function, which is coexpressed and cosecreted with insulin [103]. Human IAPP (hIAPP) has been shown to form toxic oligomers in the secretory pathway and to alter β-cell function and survival [104–106]. Its forced expression in rodent β-cells has been shown to induce an ER stress, in parallel with the accumulation of polyubiquitinated proteins [107, 108]. Moreover, increased expression of hIAPP in rat β-cells has been shown to alter the autophagy pathway, which also plays an important role in the clearance of misfolded proteins [109]. Therefore, the operative ERAD system and autophagy may be critical for the clearance of hIAPP oligomers upon physiological stimulation of human β-cells.

Altogether, these observations suggest that a constant and fine-tuned physiological UPR is crucial to cope with the high rates of proinsulin biosynthesis, and, at least in humans, with the degradation of hIAPP oligomers, in order to maintain the functional β-cell mass.

4 Role of the ER Stress Signaling Pathway in the Maintenance of the Functional β-Cell Mass

Pancreatic β-cells have been shown to express high levels of several UPR genes, and both exaggerated activation and genetic disruption of various components of this response trigger β-cell apoptosis and induce insulin-dependent diabetes mellitus in man and rodents. For instance, the Wolcott-Rallison Syndrome (WRS), an early-infancy non-autoimmune insulin-dependent form of diabetes, has been shown to stem from non-sense or frame-shift mutations of *Perk* [41, 42]. In addition, polymorphisms of the *Perk* gene have been associated with type 1 diabetes

in an Indian population [110]. Interestingly, *Perk*-deficient mice showed a phenotype similar to human WRS: β-cells of these mice displayed enhanced glucose-stimulated proinsulin biosynthesis and degenerated progressively after birth [57, 111], in agreement with the observed reduction of the β-cell mass in WRS patients [112]. In addition, *Perk* deletion specifically in β-cells has been shown to reduce ER to Golgi trafficking of proinsulin and to impair the ERAD machinery [113]. Similarly, the prevention of eIF2α phosphorylation through a knock-in substitution of serine 51 by alanine, induced the alteration of glucose homeostasis and diabetes in mice, in association with increased proinsulin biosynthesis, accumulation of proinsulin in the ER, distention of the latter, oxidative stress, and β-cell apoptosis [64, 114, 115]. It has been initially proposed that these alterations result from the inability of these mutated/KO β-cells to appropriately attenuate protein translation in response to glucose stimulation. Alternatively, it has been suggested that PERK is required for insulin trafficking and quality control independently from ER stress [113, 116]. Anyway, these observations demonstrate the important role of the PERK branch of the UPR in β-cell physiology. However, excessive activation of this branch, such as in type 2 diabetes (T2D), is deleterious and has been shown to markedly alter the β-cell phenotype, particularly through increased expression of DDIT3 [28] (see Chap. 9 of this book for the role of ER stress in diabetes).

It has also been shown that *Ire1* conditional-KO mice were mildly hyperglycemic, glucose intolerant and hypoinsulinemic. Interestingly, these mice displayed normal pancreatic insulin content and normal islet morphology, suggesting a defect in insulin secretion [67]. Concerning XBP1, although a primary report has shown that the development of the endocrine pancreas was unaltered in mutant mice expressing *Xbp1* only in the liver [60], a recent report has demonstrated that mice lacking *Xbp1* specifically in β-cells present altered glucose homeostasis as a consequence of the alteration of both β-cell function and survival [117]. In addition, mice lacking *Dnajc3* or *Sec61*α display a gradual onset of diabetes associated with important β-cell loss [118, 119]. On the other hand, it has been demonstrated that prolonged exposure of mouse islets to supraphysiological glucose levels leads to hyperactivation of IRE1 [38]. The latter effect has been proposed to be involved in the glucotoxic degradation of preproinsulin mRNA and subsequent reduction in proinsulin biosynthesis [37, 38, 120]. Furthermore, *Xbp1s* overexpression in dispersed rat islets has been shown to alter GSIS and islet cell viability [121].

Finally, polymorphisms in ATF6 have been associated with T2D in Pima Indians and Dutch Caucasians [44, 122]. However, although mice lacking ATF6α and β isoforms displayed a lethal embryonic phenotype, animals KO for one or another isoform develop normally [10]. Thus, an experimental proof of a role of ATF6 in β-cell function under physiological conditions is still missing.

There is thus ample evidence that the ER stress signaling pathway plays a critical role in the preservation of the β-cell phenotype. In contrast, only a few studies have tried to characterize the effect of acute physiological changes in nutrient availability on the ER stress response in pancreatic β-cells.

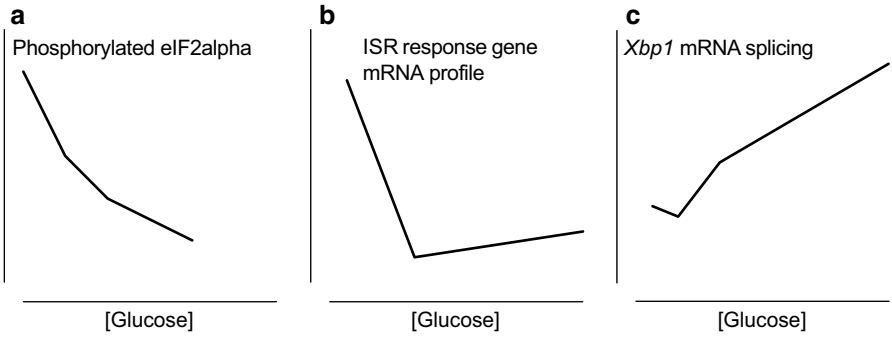

Fig. 4 Schematic representation of the effects of increasing glucose concentrations on UPR markers in β-cells. **a**, the profile of eIF2α phosphorylation in MIN6 cells in response to glucose stimulation between 0 and 20 mmol/l glucose. This schematic representation is based on data published in [92] and [99]. **b**–**c**, profile of ISR gene mRNA levels (*Ddit3 and Atf3*) (**b**) and *Xbp1* mRNA splicing (**c**) in rat islets cultured in 2–30 mmol/l glucose. These schematic representations are based on data published in [62] and [94]

5 Physiological Modulation of the ER Stress Response by Glucose in Pancreatic β-Cells

In β-cells as in other cell types, ER Ca^{2+} emptying with the pharmacological agent thapsigargin, ATP-depletion, or overexpression of mutated proteins that cannot fold correctly, all trigger a strong ER stress with sustained activation of both the IRE1-XBP1 and PERK-ATF4 arms of the UPR, followed by cell apoptosis. In contrast, physiological changes in nutrient availability surprisingly exert distinct effects on the two branches of the UPR in β-cells. Thus, in contrast with *Xbp1* mRNA splicing, XBP1s protein levels, and XBP1-target gene expression that are low at low glucose and increase with glucose stimulation, the ISR is regulated in the opposite way, *i.e.* maximally activated at low glucose concentrations and rapidly decreased upon glucose stimulation [62, 91, 92, 99, 123]. In the following paragraphs, we will first detail the glucose regulation of each of these two branches of the UPR in rodent β-cells before proposing a model that may explain these results and could be experimentally tested.

5.1 Modulation of the ISR

Several studies have shown that the ISR is maximally activated in rodent β-cells cultured in a low glucose concentration (0–2 mmol/l), as evidenced by increased eIF2α phosphorylation, ATF4 protein levels, and ATF4-target gene mRNA and protein levels, and that the ISR is rapidly reduced by glucose in a concentration-dependent manner (Figs. 4 and 5) [62, 94]. In agreement, it has been demonstrated

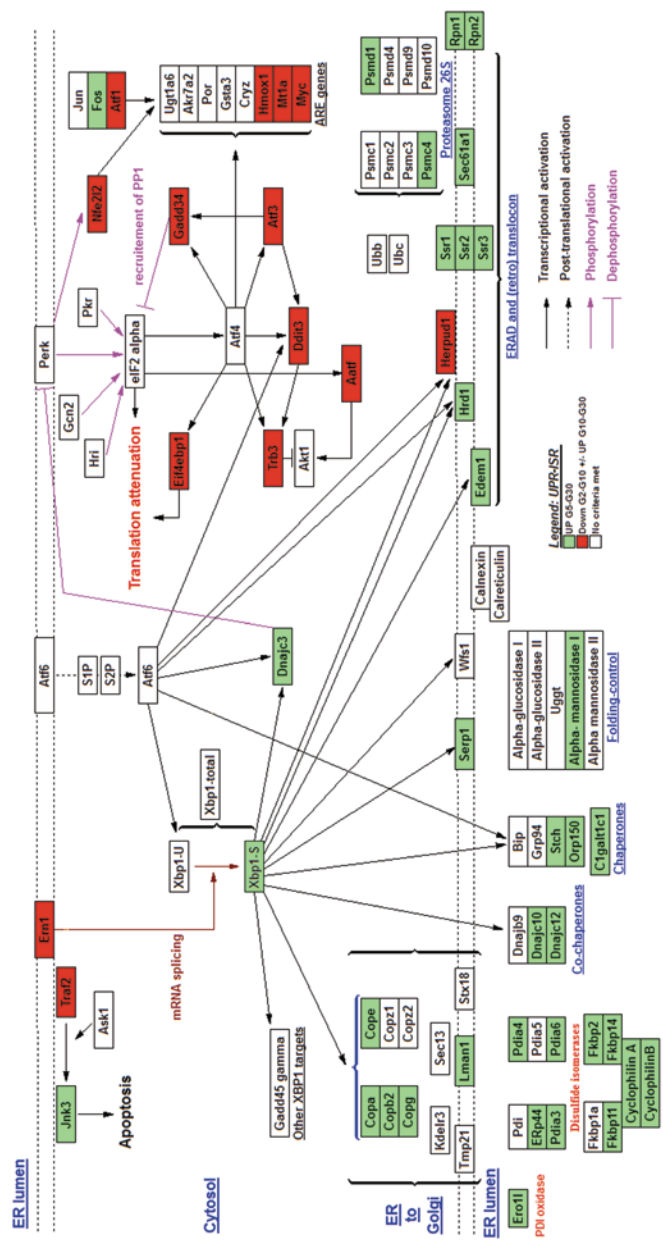

Fig. 5 Simplified representation of the glucose-induced changes in the mRNA levels of UPR genes in isolated rat islets cultured for 18 h in G2–30. This biological map depicting the components of the ER stress response was created using MAPP Builder software [135]. Then, genes significantly affected by glucose stimulation in our microarray data [94] were colored according to their expression profile using GeneMAPP [135]. Genes that are upregulated by glucose stimulation between G5 and G10 and between G5 and G30 are colored in green. On the other hand, genes downregulated between G2 and G10 and moderately upregulated between G10 and G30 are colored in red? Genes displaying other expression profiles, such as those upregulated between G10 and G30, are not colored here for simplification. For more complete details, the reader is referred to [94]

in insulin-secreting MIN6 cells that glucose, within the physiological range, dose-dependently stimulates the dephosphorylation of PERK and eIF2α in parallel with the enhancement of total protein synthesis (Fig. 4) [91, 92, 99]. The dephosphorylation of eIF2α has been shown to involve the activation of a protein phosphatase 1 (PP1)-related pathway [92]. Interestingly, an inverse relationship between eIF2α phosphorylation in pancreatic extracts and blood glucose concentration has also been reported *in vivo* in fasting mice and mice that received an intraperitoneal glucose load [111]. Recently, PERK-eIF2α activation at low glucose levels has been shown to depend on the decrease in the energy status of the β-cell, the inhibition of SERCA pumps, and subsequent Ca^{2+} efflux from the ER [99, 123]. If the glucose reduction in PERK-eIF2α phosphorylation likely contributes to the glucose stimulation of protein synthesis, it is difficult to quantify the relative contribution of this effect in comparison with that of the concomitant reduction of AMP-dependent protein kinase activity and activation of mTOR.

Low glucose-activated ISR stimulates the expression of several pro-apoptotic ATF4-target genes, including *Ddit3*, *Atf3* and *Trb3* [62, 94, 124] (Fig. 5). Besides, we observed that high glucose stimulation from G10 to G30 slightly increased the expression of these ATF4-target genes, giving rise to an asymmetric V-shaped expression profile (Fig. 4b). Noteworthy, this profile resembles that of rat islet cell apoptosis, raising a possible link between ISR and the apoptosis of β-cells exposed to extreme low or high glucose concentrations [62, 94, 96]. However, the activation of the PERK arm of the UPR at low glucose has also been suggested to play a protective role in this cell type [99]. Such beneficial effect is unlikely to result from the repression of protein synthesis since the latter has been demonstrated to induce β-cell apoptosis [125]. Instead, PERK activation may act through the stimulation of the expression of anti-apoptotic genes. One potential candidate is the apoptosis antagonizing transcription factor (*Aatf*), a novel ISR effector that has been shown to promote β-cell survival under ER stress through transcriptional upregulation of v-Akt murine thymoma viral oncogene homolog (*Akt1*) gene [126]. Remarkably, in a microarray study of glucose-induced changes in the transcriptome of cultured rat islets, we found that *Aatf* was highly expressed in the presence of low glucose (G2–G5) and markedly reduced by glucose stimulation between G2 and G10 [94] (Fig. 5). Alternatively, PERK has been shown to regulate the activity of nuclear factor erythroid derived 2 like 2 (NFE2L2, also known as NRF2). This transcription factor, together with ATF4, drives the expression of several antioxidant response genes, including metallothionein 1a (*Mt1a*) and *Hmox1*, and has been proposed as a cytoprotective effector that opposes ER stress-induced redox imbalance (reviewed in [127]). However, the upregulation of these genes at low glucose may just be a marker of oxidative stress, as it has been demonstrated that culture of purified rat β-cells and MIN6 cells in a low glucose concentration triggers an oxidative stress that can be abrogated by physiological stimulation with various nutrients including glucose [128, 129]. Interestingly, we have shown that the expression of *Nfe2l2*, *Mt1a* and *Hmox1* in rat islets is downregulated by glucose between G2 and G10 [94, 130] (Fig. 5).

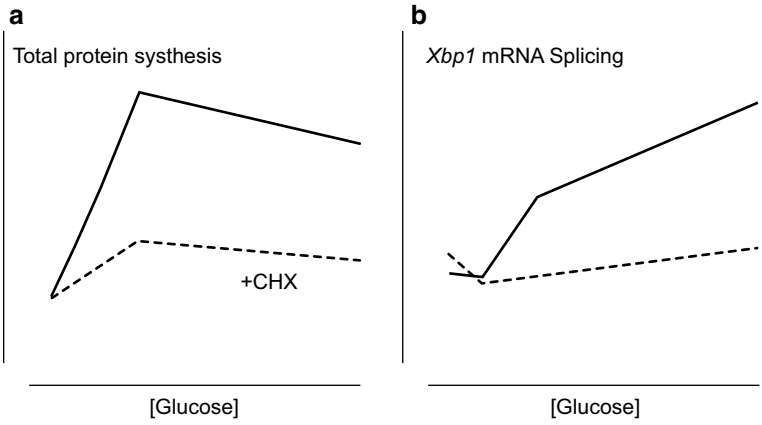

Fig. 6 Schematic representation of the effects of cycloheximide (CHX) on glucose-induced changes in rat islet total protein synthesis **a** and *Xbp1* mRNA splicing **b**. Isolated rat islets were cultured for 18 h in G2–30 without or with CHX 0.1 μmol.l^{-1}. Data are adapted from [62]

5.2 Modulation of the IRE1-XBP1 Branch of the UPR

Several studies indicate that physiological stimulation of pancreatic β-cells with glucose and other nutrients acutely and dynamically modulates the UPR, and that this effect may play a role in β-cell phenotypical plasticity and preservation of insulin secretory response. Thus, we have previously demonstrated that stimulation of isolated rat islets with glucose and other nutrients rapidly induces *Xbp1* mRNA splicing, a very specific and sensitive marker of IRE1 and UPR activation (Fig. 4c). Interestingly, this effect was maximal only 2 h after glucose stimulation and was rapidly reversed upon return to a non-stimulatory glucose concentration. This stimulation of *Xbp1* mRNA splicing by glucose was concentration-dependent and paralleled the stimulation of insulin secretion, therefore presenting a shift to the right compared with the stimulation of total protein synthesis.

The glucose stimulation of *Xbp1* mRNA splicing was independent from Ca^{2+} influx and insulin secretion, as evidenced by the lack of effect of diazoxide, a pharmacological opener of the K_{ATP} channels that prevents glucose-induced Ca^{2+} influx and insulin secretion in β-cells (Fig. 2). In contrast, it was prevented by low concentrations of cycloheximide that inhibited the glucose-induced stimulation of protein synthesis while preserving its basal level in G2 (Fig. 6). These observations therefore suggest that the glucose activation of the UPR in pancreatic β-cells results from the upregulation of protein synthesis and subsequent increase in ER synthetic load [62].

The shift to the right of the glucose-dependence of *Xbp1* mRNA splicing *vs.* total protein synthesis may seem in contradiction with a causal link between both events (Fig. 6). However, it is possible that the stimulation of Ca^{2+} pumping into the ER that occurs between G2 and G5, together with the increase in ATP synthesis (Fig. 3),

enhances ER-resident chaperone function. This, in addition to the stimulation of BiP expression via the PERK-ATF4 arm activated at low glucose [62], could be sufficient to handle the synthetic load at these glucose concentrations.

On the other hand, although glucose stimulation beyond G10 did not further enhance total protein synthesis, we observed a further increase in *Xbp1* mRNA splicing, XBP1s protein levels, and XBP1-target gene mRNA levels. One possible explanation is that the slight activation of the PERK-ATF4 pathway at high glucose levels represses protein translation (Fig. 4a). Indeed, it has been shown that mouse islets carrying a Ser51Ala mutation of eIF2α PERK phosphorylation site displayed enhanced glucose-stimulation of total protein synthesis and proinsulin biosynthesis in comparison with control islets [64]. Additionally, supraphysiological glucose stimulation is accompanied by the activation of several glucotoxic pathways including oxidative stress and hypoxia [96]. These conditions may worsen protein misfolding by altering the ER redox status, and thereby hampering the formation of the three disulfide bonds in proinsulin. Therefore, increased expression of foldases, including several disulfide isomerases as well as components of the ERAD pathway, may be required to preserve β-cell function under this condition (Fig. 5).

In agreement with these findings, it has also been shown that transient exposure of mouse islets and INS-1 cells to high glucose levels induces IRE1 phosphorylation. Besides, the IRE1 signalling has been shown to be essential for proinsulin biosynthesis. Thus, IRE1 knockdown in INS-1 cells specifically inhibited proinsulin biosynthesis. However, the glucose-dependent activation of IRE1 in this study was not accompanied by *Xbp1* mRNA splicing, which is surprising [131]. Although one can hypothesize that IRE1 may act through other targets, a fundamental physiological role of XBP1 in the maintenance of functional β-cell mass has been recently demonstrated in mice lacking *Xbp1* gene selectively in β-cells. These animals were hyperglycaemic and glucose intolerant as a consequence of reduced β-cell mass, reduced content in insulin secretory granules, and impaired GSIS. Further investigation in MIN6 β-cells revealed that XBP1 also plays a role in proinsulin processing and maturation [117]. However, additional functional studies on isolated islets from these mice are needed to determine the nature of the β-cell defect.

UPR activation in pancreatic β-cells is rapidly modulated by changes in the ambient glucose concentration and has been shown to play a role in proinsulin biosynthesis and GSIS. Therefore, the dynamic nutrient challenge imposed to β-cells by alternations of food intake and starvation may be paralleled by synchronous changes in IRE1 activation and *Xbp1* mRNA splicing. This could contribute to the adaptation of the ER folding capacity to the fluctuations of protein synthesis through the modulation of the expression of an array of genes. Indeed, we found that glucose activates *Xbp1* mRNA splicing and subsequently upregulates XBP1s protein levels in parallel to an increase in the expression of several XBP1-target genes, such as heat shock protein 90 beta, member 1 (*Hsp90b1* also known as *Grp94*), *Edem* and *BiP* [62]. Further analysis of the transcriptome of rat islets cultured under the same conditions allowed us to identify other UPR genes regulated by glucose in β-cells, including the chaperones heat shock protein 13 (*Hspa13*) and *Dnajc3*, foldases such as *Pdi*, endoplasmic reticulum protein 44 (*Erp44*), protein

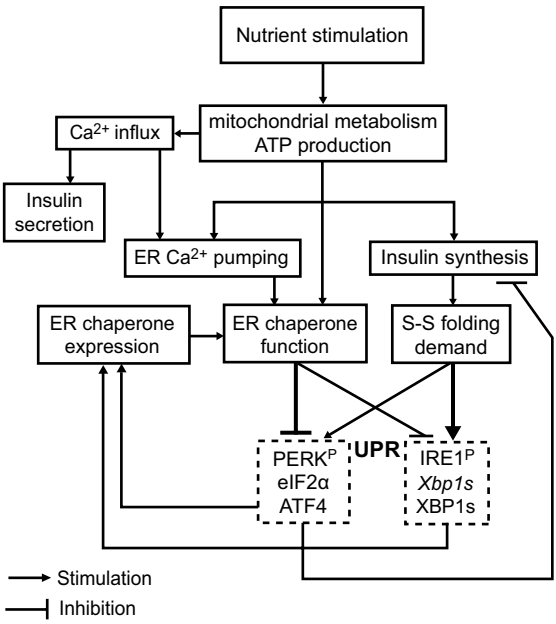

Fig. 7 Proposed model of nutrient regulation of the UPR in pancreatic β-cells. Depending on the ambient glucose concentration, β-cells seem to bring into play two different kinds of UPR: a low glucose-activated UPR when intracellular ATP, $[Ca^{2+}]_{ER}$, and protein synthesis are low, and characterized by the main activation of the PERK-ATF4 arm and a modest activation of the IRE1-XBP1 arm, and a high glucose-activated UPR when intracellular ATP, $[Ca^{2+}]_{ER}$, and protein synthesis are high, and characterized by the main activation of the IRE1-XBP1 arm and a modest activation of the PERK-ATF4 arm. See the conclusion for details

disulfide isomerase family A, member 4 (*Pdia4*) and FK506 binding protein 11 (*Fkbp11*), genes involved in ER to Golgi transport, and components of the ERAD pathway [94] (Fig. 5).

6 Conclusion

The ER stress signaling pathway plays a fundamental physiological role in the preservation of the phenotype of highly secretory cell types. This holds true for insulin-secreting pancreatic β-cells that require an adequate UPR regulation to face the important ER synthetic load enhanced by glucose stimulation. The latter exerts opposite effects on the IRE1-XBP1 and PERK branches of the UPR. These actions are tightly related to the particular metabolic and functional features of β-cells. Thus, in the presence of a non-stimulatory glucose concentration, mitochondrial metabolism, ATP production, and insulin secretion are at basal levels. Under this situation, the low SERCA pump activity and subsequent fall of $[Ca^{2+}]_{ER}$ results in strong PERK activation and ensuing eIF2α phosphorylation. This ensures the repression of global protein synthesis and the activation of a complex transcriptional program including both pro-survival and pro-death effectors. The final outcome seems likely to depend on the duration of exposure to low glucose. Under these conditions, the activity of IRE1-XBP1 arm is maintained at basal level. On the other hand, glucose stimulation acutely induces an acceleration of mitochondrial metabolism, a rise in the ATP/ADP ratio, Ca^{2+} influx and insulin secretion, in parallel to the enhance-

ment of SERCA pump activity and subsequent rise in $[Ca^{2+}]_{ER}$. These events lead to PERK dephosphorylation as well as the activation of PP1, thereby leading to eIF2α dephosphorylation and derepression of protein synthesis. The ensuing important increase in client protein load in the ER triggers *Xbp1* mRNA splicing and upregulates the expression of several chaperones, including *Dnajc3* which may apply an additional repression on the ISR pathway by inhibiting PERK kinase activity, and components of the ERAD system. In addition, Ca^{2+} pumping into the ER may acutely modulate the activity of ER chaperones. These events ultimately lead to the upregulation of the folding capacity of the ER and the degradation of misfolded proteins (Fig. 7). Both IRE1-XBP1 and PERK arms of the UPR are rapidly and reversibly modulated by changes in the ambient glucose concentration. This complex regulation is fundamental for the preservation of the β-cell secretory response and glucose homeostasis of the organism.

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