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A Busy Cell – Endoplasmic Reticulum Stress in the Pancreatic β-cell

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The pancreatic β-cell senses nutrients, neurotransmitters and hormones in the circulating blood. The unique function of the cell is to integrate all these ambient signals into an appropriate insulin secretory rate in order to maintain glucose homeostasis. A prerequisite for adequate insulin secretion is proper biosynthesis of the hormone. The rate of biosynthesis needs to be regulated in order to compensate for rapid fluctuations in the secretory rate. The synthesis of insulin includes transcription of its gene to mRNA, translation of mRNA into preproinsulin, and processing of preproinsulin via proinsulin into mature insulin. It also involves the induction of additional components of the secretory pathway to support processing, transport and exocytosis of insulin granules. The endoplasmic reticulum (ER) is the cell organelle playing a paramount role in these processes. A functional ER is crucial to all eukaryotic cells, but especially important in a professional hormone-secreting cell like the β-cell.

In eukaryotic cells, the ER provides a contained environment for the synthesis and modification of membrane proteins and proteins destined for secretion. These co- and post-translational modifications are important for the subsequent folding and assembly of proteins (Helenius 1994). To assist the folding process, the ER contains high levels of protein chaperones including the glucose-regulated proteins calnexin, calreticulin, protein disulphide isomerase and Erp72 (Gething et al. 1992; Ruddon et al. 1997). Disruptions in the ER protein processing system by pathogens, mutations or specific lesions in the protein folding machinery have been implicated in the progression of several human diseases, including neurodegenerative disorders (Lindholm et al. 2006), cystic fibrosis (Vij et al. 2006) and diabetes (Özcan et al. 2004).

This essay will describe the phenomenon of ER stress in pancreatic β-cells with special focus on its involvement in the regulation of β-cell survival and death. The involvement of some ER stress components in the regulation of insulin biosynthesis and secretion will be discussed, along with a short description of the ER stress response (also known as the unfolded protein response). For more in-depth reviews on general ER stress, there are several excellent papers (Boyce et al. 2006) (Wu et al. 2006).

Agents or conditions that compromise protein folding capacity, or evoke an overload of newly synthesized polypeptides, cause an accumulation of unfolded or misfolded proteins in the ER. This event activates three distinct pathways, each controlled by a specific protein: PERK, IRE1 and ATF6 (Boyce and Yuan 2006), respectively. Activation of PERK promotes the phosphorylation and inhibition of the translation initiation factor, eIF2α (Prostko et al. 1993). The resulting attenuation of general protein synthesis prevents further overloading of the ER folding machinery. IRE1 is a protein that, upon its activation, renders endonuclease activity (Calfon et al. 2002). One target for activated IRE1 is the mRNA encoding the X-box-binding protein 1 (XBP-1). This mRNA is alternatively spliced by IRE1 such that it can now be translated into a functional transcription factor. ATF6 is by itself a transcription factor but is
retained in the ER membrane under non-stressed conditions. However, upon ER-stress ATF6 is released from the ER membrane and transported to the Golgi apparatus where it is cleaved into a functional transcription factor (Haze et al. 1999). Activation of IRE1 and ATF6 promotes the expression of ER chaperones, thereby increasing the capacity of the ER to properly fold the accumulated polypeptides. These ER chaperones also function as part of the quality control system that targets misfolded proteins for degradation (Plemper et al. 1997). Thus, the ER stress response normally serves to alleviate ER stress by inhibiting general protein translation, increasing folding capacity and promoting degradation of misfolded proteins (Figure 1).

In the area of diabetes research, ER stress has attracted growing interest since it has been shown that markers of ER stress are up-regulated in both human islets from type 2 diabetes patients (Huang et al. 2007; Laybutt et al. 2007) and in islets from animal models of obesity and diabetes (Özcan, Cao et al. 2004). ER stress in human islets might at least partly be explained by high expression rates of human islet amyloid polypeptide (IAPP) as has been demonstrated by over expression of human IAPP in transgenic rats (Huang, Lin et al. 2007). In accordance with a prosurvival function of the ER stress response, several studies have indicated a necessity of especially PERK signaling for preserved β-cell function. Deletion of PERK results in progressive destruction of β-cells in both humans and mice (Delepine et al. 2000; Harding et al. 2001). In humans, mutations in the PERK gene gives rise to Wolcott-Rallison syndrome, a rare form of monogenic diabetes that manifests as an infantile-onset, insulin-requiring diabetes (Delepine, Nicolino et al. 2000). In perk−/− mice, there is a progressive loss of β-cells and diabetes evolves within the first few weeks after birth (Harding, Zeng et al. 2001). Even more severe β-cell dysfunction is seen in mice with a homozygous serine 51 alanine point mutation in their eIF2α gene (Scheuner et al. 2001). Such mice are resistant to eIF2α phosphorylation and inhibition of general protein synthesis. Mice heterozygous for the same mutation have a normal phenotype under a normal diet but develop obesity and diabetes on a high fat diet (Scheuner et al. 2005). The profound glucose intolerance in these mice results from reduced insulin secretion accompanied by abnormal distension of the ER lumen, defective trafficking of proinsulin and a reduced number of insulin granules. Hence, the inability to activate PERK signaling and phosphorylation of eIF2α in pancreatic β-cells results in reduced function and death of the cell. Thus, specific inhibition of eIF2α dephosphorylation could be a way of improving β-cell function. Indeed, when screening for compounds that promote cell survival in PC12 cells under conditions of ER stress, salubrinal was identified as a specific inhibitor of eIF2α dephosphorylation (Boyce et al. 2005). Despite these effects in PC12 cells, in pancreatic β-cells salubrinal was found to be cytotoxic by itself, and potentiated fatty acid induced ER stress and apoptosis (Cnop et al. 2007). This shows that pancreatic β-cells are very susceptible to any perturbation of ER homeostasis and that the ER stress response is two-faced, promoting both cell survival and death. The molecular basis for this dual action of the ER stress response will be an important research task in the future. An important lesson can be learnt from studies in mouse embryonic fibroblasts (Rutkowski et al. 2006), where an adaptive response to ER stress was found to be dependent on the longer half-life of mRNA encoding molecular chaperones such as BiP/GRP78, GRP94 and calreticulin than those mRNAs that are controlled by the PERK signaling pathway. PERK is considered to be the principle driver of expression of the pro-apoptotic protein CHOP (also known as GADD153 or DDIT3) (Okada et al. 2002). In a recent report, it was suggested that cytokines and palmitate utilize both AP-1 and ATF4 transcription factor to regulate CHOP expression (Pirot et al. 2007). However, neither AP-1 nor ATF4 used endoplasmic reticulum response elements (ERSE), which is in contrast to the SERCA pump inhibitor cyclopiazonic acid (CPA), which utilized ATF4 and ERSE to regulate
CHOP expression. That CHOP is a mediator of cytotoxicity is evident in the Akita mouse. The Akita mouse serves as an example where a mutated protein can induce ER stress and β-cell apoptosis. One allele of the Ins2 gene in the Akita mouse harbors a single-point mutation, resulting in a cysteine-tyrosine substitution at position 96 (Wang et al. 1999). This mutation prevents the formation of a disulfide bond so the mutated protein cannot fold properly and gets stuck in the ER, eliciting an ER stress response. Deletion the CHOP gene in Akita mice delays apoptosis induced by ER stress (Oyadomari et al. 2002). The absence of CHOP was also reported to increase resistance to nitric oxide-induced apoptosis in pancreatic β-cells (Oyadomari et al. 2001). The situation is, however, complicated by the fact that exendin-4, a GLP-1 receptor agonist, augments both thapsigargin and tunicamycin induced CHOP expression, but at the same time improves β-cell function and survival following induction of ER stress (Yusta et al. 2006). ER stress has been proposed to mediate the cytotoxic effects induced by saturated fatty acids, like palmitate (Kharroubi et al. 2004; Karaskov et al. 2006). The mechanism for this is not clear, but there might be an involvement of Ca\(^{2+}\) influxes, since it was found that the Ca\(^{2+}\) channel blocker nifedipine delayed palmitate induced CHOP expression while an opener, Bay K8644, accelerated such process (Choi et al. 2007). Furthermore, diazoxide was recently reported to attenuate fatty acid-induced eIF2\(\alpha\) phosphorylation and CHOP expression and apoptosis in clonal pancreatic β-cells (Ortsäter et al. 2006), indicating that calcium fluxes across the cell membrane are regulating the palmitate induced ER stress response.

If the cytotoxic role of PERK signaling in β-cells remains controversial, the beneficial effect of chaperone activity is clearer. Chemical chaperones can reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes (Özcan et al. 2006). Orally active chemical chaperones, 4-Phenyl butyric acid and taurine-conjugated ursodeoxycholic acid, alleviated ER stress in cells and whole animals. Treatment of obese and diabetic mice with these compounds resulted in normalization of hyperglycemia, restoration of systemic insulin sensitivity, resolution of fatty liver disease, and enhancement of insulin action in liver, muscle, and adipose tissue. The results demonstrate that chemical chaperones enhance the adaptive capacity of the ER and act as potent antidiabetic compounds, with potential clinical utility in the treatment of type 2 diabetes.

Besides being parts of ER stress response, recent data has shown that IRE1 and eIF2\(\alpha\) are also involved in the regulation of insulin biosynthesis. Treating INS-1E or primary β-cells with CPA results in a 40 % decrease in Ins1 and Ins2 gene expression after a 2 hour exposure (Pirot et al. 2007), an effect that occurs in parallel with enhanced XBP-1 splicing indicating the involvement of IRE1 signaling. However, IRE1\(\alpha\) phosphorylation is also coupled to enhanced insulin biosynthesis and occurs in response to a transient, 1 hour, exposure to high glucose (Lipson et al. 2006). Inactivation of IRE1\(\alpha\) signaling by siRNA treatment, or inhibition of IRE1\(\alpha\) phosphorylation, hinders insulin biosynthesis. IRE1 activation by high glucose does not accompany XBP-1 splicing or BiP dissociation but up-regulates its target genes, such as WFS1. Thus, IRE1 signaling activated by transient exposure to high glucose uses a unique subset of downstream components that differ from the activation of IRE1 seen during ER stress. In contrast, long term exposure to elevated glucose concentrations is associated with a classical IRE1 signaling response (Figure 2). When it comes to glucose regulation of eIF2\(\alpha\) phosphorylation, it has been shown that the degree of phosphorylation is high at 2 mM glucose, low at 10 mM glucose and at 30 mM glucose it is slightly higher than at 10 mM glucose (Elouil et al. 2007). The fact that glucose can reduce the degree of eIF2\(\alpha\) phosphorylation has been known for some time (Gomez et al. 2004). This is not due to
decreased activity of any eIF2α kinase (Vander Mierde et al. 2006) rather, by using a series of protein phosphatase inhibitors, this effect was shown to be the result of enhanced activity of ser/thr protein phosphatase-1 (PP1). Hence, glucose activates a PP1-mediated signaling pathway that enhances the overall translation rate in β-cells. Thus, the steady-state level of eIF2α phosphorylation in β-cells is the net result of a balance between folding-load-induced phosphorylation and PP1-dependent dephosphorylation (Figure 3).

Both a hyperglycemic (Wang et al. 2005) and a hyperlipidemic (Karaskov, Scott et al. 2006; Cnop, Ladriere et al. 2007) environment can induce ER stress in pancreatic islets and β-cells in vitro. What then can be done to help these cells adapt to such metabolically stressful situation? To aid in this process, there is a need to dissect the ER stress response in β-cells in greater detail and gain deeper insight into what molecules that exert regulatory control of the different arms of the ER stress response. In this respect, it will be interesting to dissect which protein phosphatases that can exert feed-back control of PERK and IRE1 signaling.

The incidence of type 2 diabetes is rapidly growing globally, reaching epidemic proportions in the Western world (Zimmermann-Belsing et al. 2004). More than 100 million people world wide have diabetes and will suffer a substantially reduced quality of life as a consequence. A sedentary life style, along with an excessive caloric intake, contributes to the development of the disease by imposing a increased functional demand on the β-cell. The frequent ingestion of blood sugar elevating snacks makes the pancreatic β-cell – a busy cell. Hopefully, the knowledge evolving from ongoing and future studies of the ER stress pathways can be harnessed to advantage in the quest for drugs conferring protection of the β-cell from functional exhaustion and demise.
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Figure legends

Figure 1.
Schematic representation of the endoplasmic reticulum stress response. The accumulation of misfolded proteins in the endoplasmic reticulum leads to the release of BiP/GRP78 from ATF6, PERK and IRE1, which allows these three proteins to become active. Activation of PERK promotes the phosphorylation and inhibition of the translation initiation factor, eIF2α, leading to a reduced translation rate. However, the translation rate of some mRNAs, like ATF4 mRNA, is actually increased during eIF2α phosphorylation. ATF4 is a transcription factor for GADD34 that activates a negative feedback loop to dephosphorylate eIF2α and the proapoptotic protein CHOP. IRE1 is a protein that, upon its activation, renders endonuclease activity. A target for activated IRE1 is the mRNA that encodes the X-box-binding protein 1 (XBP-1). This mRNA is alternatively spliced by IRE1 and can now be translated into a functional transcription factor. ATF6 is by itself a transcription factor but is retained in the ER membrane under non-stressed conditions. However, upon ER stress ATF6 is released from the ER membrane and transported to the Golgi apparatus where it is cleaved into a functional transcription factor. Activation of IRE1 and ATF6 promote the expression of ER chaperones and thereby increase the capacity of the ER to properly fold the accumulated polypeptides. These transcription factors also enhance the expression of proteins involved in protein degradation. Figure is inspired by Rutkowski and Kaufman (2004) Trends Cell Biol vol. 14:20-28.

Figure 2.
Exposure to high glucose time-dependently activates two phases of IRE1α signaling. On the short time scale an exposure to an elevated glucose concentration results in phosphorylation of IRE1α, evoking an enhanced expression of the target gene WFS1 and enhanced rate of insulin synthesis but does not induce alternative splicing of XBP-1. On the other hand, when the exposure time is extended, XBP-1 mRNA is alternatively spliced and insulin biosynthesis rate is decreased below normal levels. The figure is produced from data presented in reference (Lipson, Fonseca et al. 2006).

Figure 3.
The degree of eIF2α phosphorylation is a balance between ER stress associated phosphorylation and glucose-induced protein phosphatase-1-mediated dephosphorylation. The figure is produced from data presented in reference (Vander Mierde, Scheuner et al. 2006).
Figure 1

Misfolded proteins lead to the activation of ATF6, PERK, and IRE1. ATF6 is cleaved, and its cleaved form activates transcription factors such as ATF4, CHOP, and GADD34. ATF4 promotes the synthesis of chaperones like BiP/GRP78 and PDI, which help in the degradation of misfolded proteins. PERK phosphorylates eIF2α, leading to translational attenuation, which is also mediated by ATF4. IRE1 mediates alternative splicing of XBP-1, leading to the production of functional transcript factors.

Alternative splicing of XBP-1 giving a functional transcription factor

Translational attenuation

Chaperones BiP/GRP78 and PDI

Degradation
Figure 2

*High glucose activates two phases of IRE1α signaling*

![Graph showing two phases of IRE1α signaling: Insulin biosynthesis phase and ER stress phase.](image)
Figure 3

The diagram illustrates the relationship between ER stress, glucose stimulation, and the translation rate. ER stress is balanced against glucose stimulation, with the expression levels of p-eIF2α and eIF2α as factors influencing the translation rate.