Stress hypERactivation in the beta-cell

Sonya G. Fonseca
Novartis Institutes for Biomedical Research

Fumihiko Urano
University of Massachusetts Medical School

Mark Burcin
Novartis Institutes for Biomedical Research

1-26-2010
Stress hypERactivation in the beta-cell

Authors
Sonya G. Fonseca, Fumihiko Urano, Mark Burcin, and Jesper Gromada

Rights and Permissions
Citation: Islets. 2010 Jan-Feb;2(1):1-9. Link to article on publisher's site

This article is available at eScholarship@UMMS: http://escholarship.umassmed.edu/pgfe_pp/64
**Stress hypERactivation in the β-cell**

Sonya G. Fonseca,1,* Fumihiko Urano,2,3 Mark Burcin1 and Jesper Gromada1

1Cardiovascular and Metabolism Division; Novartis Institutes for Biomedical Research; Cambridge, MA USA; 2Program in Gene Function and Expression; and 3Program in Molecular Medicine; University of Massachusetts Medical School; Worcester, MA USA

**Key words:** ER stress, UPR, diabetes, β-cell, Wolfram syndrome, WFS1

In pancreatic β-cells, the endoplasmic reticulum (ER) is the crucial site for insulin biosynthesis, as this is where the protein-folding machinery for secretory proteins is localized. Perturbations to ER function of the β-cell, such as a high demand for insulin secretion, can lead to an imbalance in protein homeostasis and lead to ER stress. This stress can be mitigated by an adaptive, cellular response, the Unfolded Protein Response (UPR). UPR activation is vital to the survival of β-cells, as these cells represent one of the most susceptible tissues for ER stress, due to their highly secretory function. However, in some cases, this response is not sufficient to relieve stress, leading to apoptosis and contributing to the pathogenesis of diabetes. Recent evidence shows that ER stress plays a significant role in both type 1 and type 2 diabetes. In this review, we outline the mechanisms of ER stress-mediated β-cell death and focus on the role of ER stress in various forms of diabetes, particularly a genetic form of diabetes called Wolfram Syndrome.

**Introduction**

Pancreatic β-cells have the specialized function of regulating glucose homeostasis by synthesizing and secreting the hormone insulin. Newly synthesized insulin is not immediately functional—it must be folded into its proper three-dimensional structure. The endoplasmic reticulum (ER) has an essential role in the processing and assembly of insulin, with its complex of chaperones, such as immunoglobulin heavy chain-binding protein (BiP) and foldases, such as protein disulfide isomerase (PDI).

The sensitive folding environment of the ER can be disrupted by physiological processes, such as post-prandial insulin biosynthesis which places a heavy load on the ER, as well as a pathological exposure to viruses, toxins, cytokines and mutant protein expression. ER stress occurs when the ER exceeds its folding capacity with such disruptions, leading to an overall dysregulation of protein homeostasis, reflected as reduced maintenance of the quality and quantity of protein and an accumulation of unfolded/misfolded proteins that cannot be processed through the secretory pathway. This stress elicits an ER-to-nucleus signaling cascade, the Unfolded Protein Response (UPR), which mitigates stress in three distinct manners: (1) enhancement of folding activity through increased chaperone/foldase expression, (2) reduction of ER workload through general translational attenuation and (3) clearance of protein aggregation and residual unfolded proteins through ER-associated protein degradation (ERAD). When the UPR does not adequately reduce stress and return the cell to a state of protein homeostasis, the cell undergoes apoptosis.

β-cell apoptosis has recently been shown not only to be a component of type 1 diabetes, but also type 2 diabetes. In type 1 diabetes, β-cells are selectively destroyed by a combination of autoimmune and inflammatory processes leading to an absolute insulin deficiency, whereas in type 2 diabetes, resistance to insulin in peripheral tissues accompanied by a reduction in β-cell mass leads to relative insulin deficiency. Recent data suggests that ER stress contributes to β-cell death, autoimmunity and insulin resistance in the pathogenesis of diabetes. This review outlines the delicate balance of β-cell protein homeostasis and the mechanisms of ER stress-mediated β-cell dysfunction and death.

**The ER Stress Signaling Triad**

The UPR has three master transducers which serve to mitigate stress: inositol requiring 1 (IRE1), PKR-like kinase (PERK), and activating transcription factor 6 (ATF6). The ER chaperone BiP binds to each of these to maintain them in an inactive state until stress: inositol requiring 1 (IRE1), PKR-like kinase (PERK), and activating transcription factor 6 (ATF6). The ER chaperone BiP binds to each of these to maintain them in an inactive state until stress is released from BiP, allowing them to become active. Activation of its kinase domain leads to activation of IRE1α, its only identified target. This in turn inhibits general protein synthesis, while preferentially increasing...
Insulin is secreted by the β-cell in response to acute hyperglycemia which occurs post-prandially. This secretory response stimulates the biosynthesis and translation of the precursor proinsulin in the ER, in which there is approximately a 10-fold increase in synthesis from a low-glucose (<3 mM) to higher glucose concentrations. Preproinsulin is synthesized in the cytoplasm and co-translationally translocated into the ER. Preproinsulin’s signal peptide is cleaved in the ER to generate proinsulin, which then undergoes precise folding in the lumen of the ER, where three disulphide bonds are formed. Once proinsulin is properly folded, it is transported to the Golgi and packaged into secretory granules, where it is processed to yield mature insulin.

β-cells, due to their heavy engagement in insulin secretion, have been found to be very sensitive to disruptions in ER homeostasis. This is reflected in the fact that these cells have a highly developed ER and a high expression of ER stress transducer proteins such as IRE1α, PERK, total XBP1, GRP94 and BiP. Thus, while β-cells have a robust ER signaling system, they are sensitive to disruptions in a system that is already at capacity. These cells are exposed to frequent energy fluctuations (i.e., intermittent changes in blood glucose levels), and thus require precise and proper folding of proinsulin to respond to such changes. Thus, any imbalance between the load of insulin translation placed on the ER and the ER folding capacity will lead to ER stress and a disruption in protein homeostasis in these cells.

The IRE1-XBP1 signaling pathway is important for ER expansion, and the IRE1α isoform is highly expressed in the pancreas. β-cells have been shown to have a baseline phosphorylation of IRE1α, illustrating the high activity of the UPR in these cells. Suppression of IRE1 activity inhibits proinsulin biosynthesis, while activation enhances insulin biosynthesis in an XBP1-independent manner. While the downstream targets of this unique IRE1 pathway have yet to be identified, it is known that the ER-resident oxidoreductase, endoplasmic oxidoreductin 1 (ERO1α) is upregulated. ERO1α is an activator of PDI, which is critical in catalyzing the formation of disulphide bonds during protein folding in the ER. Thus, IRE1 activation may enhance insulin biosynthesis through the upregulation of ERO1α which in turn is responsible for the vital formation of the three disulphide bonds in proinsulin through PDI activation.

PERK is also highly expressed in islets. PERK activation (i.e., phosphorylation) negatively regulates insulin biosynthesis. In PERK null mouse islets, high glucose treatment-induced insulin biosynthesis was enhanced compared to control littermate islets. In β-cell-specific PERK null mice, a connection has been made between the expression of PERK and β-cell development and proliferation. PERK mutations are also the cause of a genetic form of diabetes called Wolcott-Rallison syndrome. Thus, the IRE1 pathway (positive regulator) and PERK pathway (negative regulator) work together to precisely regulate insulin output, again highlighting the importance of the UPR in the β-cell.

Recently, it has been demonstrated that ATF6 may also have a function in regulating insulin. Under ER stress, ATF6 is activated, leading to a decrease in insulin gene expression. This suggests that ATF6 may have dual functions: positive regulation of chaperones and negative regulation of insulin promoter activity. The involvement of the UPR triad (i.e., IRE1, PERK and ATF6) in β-cell proteostasis and insulin biosynthesis illustrates the importance of ER homeostasis in β-cell function.

### Protein Homeostasis in the β-Cell

Insulin is secreted by the β-cell in response to acute hyperglycemia which occurs post-prandially. This secretory response stimulates
the extensive crosstalk in the UPR network which precisely regulates protein secretion.

**HypERactivation of the UPR**

There are two protein quality control outputs of the UPR: homeostatic and apoptotic. The goal of the UPR is to restore protein homeostasis, however, this may involve apoptosis to ensure that highly stressed cells do not produce damaged (i.e., misfolded) secretory proteins. Indeed, ER stress can be classified into two groups: physiological and pathological.\(^3\) Physiological stress occurs when there is a high demand for protein load. This is acute stress that is readily mitigated by the UPR. One example of this is acute post-prandial ER stress in the \(\beta\)-cell—there is a high demand for insulin biosynthesis following food intake which increases the folding load of the ER. This stress is actually favorable to the \(\beta\)-cell.\(^27\)

When ER stress is severe or prolonged such that it cannot be resolved by the UPR, cell death occurs by apoptosis.\(^36\) This prolonged stress can be attributed to an insufficient UPR response, or hyperactivation of a component of the UPR (i.e., the UPR is not properly regulated). There are at least three pathways involved in ER stress-dependent apoptosis which culminate in the activation of the effector cysteine protease caspase-3: (1) transcriptional activation of CHOP which represses Bcl-2,\(^37\) (2) activation of the Jun N-terminal kinase (JNK) by IRE1-dependent recruitment of TNF-receptor-associated factor 2 (TRAF2)\(^38\) and (3) activation of caspase-12 (caspase-4 in humans).\(^39,40\) (Fig. 2).

Physiological ER stress in the \(\beta\)-cell is beneficial and leads to the highly-regulated activation of insulin biosynthesis.\(^7\) This is attributable to acute hyperglycemia. This physiological stress can also be referred to as Stimulus-Coupling Adaptation to ER Folding (SCAEF). In this state, the downstream apoptotic factors of the UPR are not activated. However, exposure of these cells to chronic, prolonged hyperglycemia induces pathological ER stress which impairs ER function. This leads to \(\beta\)-cell dysfunction and eventually cell death because this pathological stress leads to hyperactivation of the UPR. An example of this can be seen when \(\beta\)-cells are treated with an inhibitor of eIF2\(\alpha\) dephosphorylation, salubrinal.\(^41\) Persistent activation of the PERK-eIF2\(\alpha\) pathway is deleterious to these cells, most likely due to the inhibition of general translation. Hyperactivation of IRE1, as measured by its chronic phosphorylation, also leads to cell death via the JNK pathway\(^42\) and the degradation of mRNAs encoding key stress signaling regulators such as chaperones.\(^43\) Thus, hyperactivation of any of the UPR triad pathways is detrimental, even the ATF6 pathway which controls the expression of the pro-apoptotic CHOP transcription factor.\(^14,44\) In addition to treatment of \(\beta\)-cells with chronic high glucose, there are other causes of pathological ER stress in the cells: exposure to long-chain free fatty acids (e.g., palmitate),\(^45,46\) hyperinsulinemia which occurs in the pre-diabetic stage,\(^47\) glucose deprivation,\(^48\) islet amyloid polypeptide (IAPP) expression\(^49\) and exposure to inflammatory cytokines such as IL-1\(\beta\) and IFN\(\gamma\).\(^50\)

One may consider that through the evolution of the \(\beta\)-cell, there should have been the development of a robust defense mechanism against ER stress-mediated apoptotic outcomes. However, because of the constant demands of insulin biosynthesis, this has rendered the \(\beta\)-cell dependent on a highly efficient UPR. Recent findings show that \(\beta\)-cells are highly susceptible to ER stress-mediated apoptosis and this can be a cause of diabetes.\(^3,51,52\)

**Wolfram Syndrome: A Model of ER Stress-Mediated \(\beta\)-Cell Death**

Current evidence suggests that ER stress is one of the molecular mechanisms of \(\beta\)-cell dysfunction contributing to the pathogenesis of diabetes.\(^41,45,53\) The relationship of ER stress and diabetes can clearly be seen in a rare, autosomal recessive form of juvenile diabetes, Wolfram syndrome.

The first report of this disease was documented in 1938 when Wolfram and Wagener analyzed eight siblings, four of which had a combination of juvenile diabetes and optic atrophy.\(^54\) This disease has been characterized as having four cardinal components: diabetes insipidus, diabetes mellitus, optic atrophy and deafness, of which only diabetes mellitus and optical atrophy are required to make a diagnosis.\(^55-58\) Diabetes mellitus typically becomes symptomatic in the first decade of life, with a mean age of 6, while optic atrophy follows in the second decade (mean age of 12).\(^59,60\) (Fig. 3). Of the other symptoms, 73% of patients develop diabetes insipidus, 62% develop sensorineural deafness, 58% develop renal tract complications, and 62% develop neurological defects.\(^59\) A high percentage of patients also present...
function of WFS1. It must be noted that the heterogeneity of this

tions in patients with Wolfram syndrome thus lead to a loss-of-

pound heterozygotes for two mutations, most of which have one

seem to elicit more severe symptoms than mildly inactivating

one, such as missense mutations, and mutations outside of exon

8 typically present with a more mild phenotype. WFS1 muta-

tions in patients with Wolfram syndrome thus lead to a loss-of-

function of WFS1. It must be noted that the heterogeneity of this
disease has led to the recent discovery of another locus for this
disease, WFS2/Miner1/CISD2, which accounts for only a fraction of Wolfram syndrome cases. 76-78

WFS1 is a 100 kDa glycosylated protein that is localized to the
ER membrane. 79 Its N-terminal is cytoplasmic, while its C-
terminal is located in the ER lumen. The C-terminal of WFS1 is
highly conserved in the mouse, rat and human. N-glycosylation is
its only predicted post-translational modification and it is pro-
jected to have nine transmembrane domains. 80 There is evidence
which suggests that WFS1 may function as a calcium channel;
ectopic expression of WFS1 produces an increase in calcium con-
centration in the cytosol and exhibits cation-selective channel
activities in the ER membrane. 81 This suggests that WFS1 may
function in ER calcium homeostasis. WFS1 may also regulate
ion homeostasis of the canalicular reticulum. 82 Hence, inactiva-
tion or suppression of WFS1 may cause an imbalance in ER cal-
cium homeostasis.

While ubiquitously expressed, WFS1 is highly expressed in
the pancreas. Although Wolfram syndrome patients are gener-
ally not obese, nor do they have insulitis, postmortem studies
reveal a selective loss of β-cells in their pancreatic islets. 82 In line
with this, WFS1 is not only highly expressed in β-cells, but is
localized to these cells in the pancreas, with no detectable expres-
sion in the various other pancreatic cell types. 83

While it was known that the loss of β-cells in patients is not
auto-immune related, the cause of cell death has been elusive
until recently. WFS1 has been shown to be a component of IRE1
and PERK signaling and is important in the maintenance of ER
homeostasis, particularly in β-cells. It has also been reported
that glucose-induced cytosolic calcium production is lower in the islets
of WFS1 knockout mice as compared to controls. 84 Suppression of
WFS1 leads to high levels of ER stress in β-cells, suggesting that
WFS1 protects β-cells against ER stress and, conversely, chronic
ER stress is caused by a loss-of-function of WFS1 protein. 85-86
Together, these findings suggest that a lack of functional WFS1
causes abnormal calcium homeostasis in the ER, elicits ER stress,
and triggers apoptosis in pancreatic β-cells. This suggests that
Wolfram syndrome can be attributed to chronic, unresolved ER
stress in the β-cell.

The precise function of WFS1 in ER stress signaling has
remained somewhat elusive. However, there is several lines of evi-
dence which suggest that WFS1 may be a multi-functional protein.
For example, it was reported that the C-terminal and transmem-
brane domains of WFS1 interact with the Na+/K+ ATPase β1
subunit. WFS1 mutations thus disrupt this interaction which is
speculated to decrease α1 and β1 sodium pump subunit expres-
sion in β-cells leading to cell dysfunction. 87 The question remains
whether this interaction is a result of ER stress and is it primary
or secondary to the pathology of Wolfram syndrome. In addition,
WFS1 can bind the Ca2+/CaM complex, which suggests that WFS1
may modulate the actions of Ca2+ as an intracellular second mes-
senger. 88 Impairment of binding of this complex may also cause
a loss-of-function of WFS1. There is also preliminary evidence
that WFS1 regulates a key transcription factor of the UPR, ATF6,
through the ubiquitin-proteasome pathway. Higher expression of
WFS1 in β-cells, prevents hyperactivation of ER stress signaling

![Wolfram Syndrome Disease Progression](image-url)
in these cells which are particularly sensitive to disruption of ER homeostasis and dysregulation of the UPR. Thus, WFS1 has a role in protecting β-cells from premature death by acting as an ER stress signaling suppressor.

Interestingly, WFS1 may also have a role in insulin biosynthesis and secretion. WFS1 expression is induced during glucose-induced insulin secretion. This data, along with the localization of WFS1 to the β-cell, suggests that WFS1 is an important component of proinsulin folding and processing in the ER of the β-cell. The role of WFS1 as a regulator of the ATF6 branch of the UPR may also be related to its role in regulating insulin biosynthesis and/or secretion, demonstrating the multi-functional aspect of this protein. Indeed, activation of ATF6 by ER stress has been shown to decrease insulin gene expression. ATF6 polymorphisms and haplotypes are also associated with impaired glucose homeostasis and type 2 diabetes. Thus, this raises the possibility that the interaction between WFS1 and ATF6 not only functions as a method to regulate the UPR, but also insulin biosynthesis: by restricting ATF6 activation, WFS1 prevents ATF6-mediated suppression of insulin gene expression.

Studying rare diseases, such as Wolfram syndrome, can help lead to answers to more common diseases, because there is a single genetic defect which can allow researchers to focus on the function of a gene or protein that is often involved in common diseases. Indeed, a link can be made between Wolfram syndrome and common forms of diabetes in which loss of β-cell mass via apoptosis also occurs. For example, it has been shown in a Japanese study that the WFS1 allele variants R456H, H611R and I720V significantly correlate with type 1 diabetes. There is also a link between Wolfram syndrome and type 2 diabetes. In type 2 diabetes, peripheral resistance to insulin action leads to hyperinsulinemia. This overwhelms the β-cell ER and activates ER stress signaling pathways. This chronic stress may lead to β-cell apoptosis in patients that are genetically susceptible to ER stress (i.e., they have an insufficient/defective UPR). In line with this, recent candidate-gene approaches and genome-wide association studies confirmed with WFS1 a type 2 diabetes susceptibility region. Studies have also suggested that the WFS1 risk allele may be associated with impaired β-cell function and the progressive loss of insulin secretion may predispose carriers of the WFS1 variant to develop type 2 diabetes. Recently, the WFS1 risk variant, rs10010131, has been shown to specifically impair GLP-1-induced first phase and second phase insulin secretion. It has been suggested that this impaired response to GLP-1 decreases post-prandial insulin secretion and may influence β-cell growth and differentiation.

The question that one may pose is: why are β-cells so sensitive to WFS1 mutations? Firstly, it has been demonstrated that WFS1 may directly play a role in the primary function of the β-cell, insulin biosynthesis. However, more research needs to be done to confirm this. Another theory is that there exists WFS1-like molecules in other professional secretory cells and that WFS1 confers specificity on the β-cell. While WFS1 is expressed in other cell types, it is highly expressed in the β-cell, therefore, mutations will primarily affect these cells. In line with this, the first symptom of Wolfram syndrome is diabetes. This could be defined as a cell-type specific ER stress response. There are other examples of this, such as OASIS, which regulates the signaling of the UPR specifically in astrocytes. CREBH has also been identified as a hepatocyte-specific UPR transducer. Together, the current research on WFS1 demonstrates that indeed there is a link between ER stress and the pathogenesis of various forms of diabetes: genetic, type 1 and type 2. Thus, WFS1 may be an important target for diabetes prevention and/or therapy. Currently, a patient registry has been initiated by Dr. Alan Permutt’s group at Washington University to better define the natural history of this disease and find further links between ER stress and common forms of diabetes.

**Type 1 and Type 2 Diabetes and the ER Stress Connection**

Genetic forms of diabetes, such as Wolfram syndrome, are just the tip of the iceberg when it comes to linking ER stress with the metabolic syndrome. There is recent evidence which links type 1 diabetes and ER dysfunction. The production of pro-inflammatory cytokines by phagocytes, such as monocytes and macrophages, plays a key role in the pathogenesis of this disease. This includes the production of TNFα, IL-12, IL-1β and IFNγ. These cytokines have been shown to decrease ER calcium and cause severe ER stress and ER stress-mediated apoptosis in β-cells. In addition, IFNγ and IL-1β in β-cells induce the production of nitric oxide (NO). This in turn leads to β-cell failure and apoptosis, thus NO also plays an important role in the pathogenesis of type 1 diabetes. There is evidence that this NO-induced β-cell apoptosis is mediated by ER stress signaling. NO production leads to a reduction of ER calcium levels through the attenuation of the sarcoplasmic reticulum Ca2+-ATPase2b (SERCA2b), causing severe ER stress and induction of pro-apoptotic genes. The ER stress connection includes the data which shows the ER density and volume are over two-fold higher in human diabetic ER of the β-cell. Recent studies have demonstrated an induction of BiP and spliced XBP1, which was not noted in non-diabetic counterparts. There is also a deeper link between the insulin resistance feature of type 2 diabetes and ER stress. Glucolipotoxicity (i.e., the exposure of the β-cell to chronic stress. Glucoliptoxicity (i.e., the exposure of the β-cell to chronic
There is abundant evidence to support the role of ER stress and stress signaling in the physiological function of the β-cell, as well as the pathogenesis of different forms of diabetes. This has been exquisitely demonstrated through studies of monogenic forms of diabetes such as Wolfram syndrome. Such studies have allowed researchers to better understand components of the UPR pathway and their functions, as well as the importance of ER stress signaling in the pancreatic β-cell. This has been highlighted by the link between the functional absence of a UPR component, such as WFS1, and ER stress-mediated β-cell death.

There are several aspects of ER stress signaling which need to be further investigated in order to appreciate the role of this pathway in the pathogenesis of diabetes. This will also answer the question whether components of the UPR, such as WFS1, would make “druggable” targets for the treatment of various forms of diabetes—this would address the issue of whether β-cells can be protected from hyper- or hypo-activation of ER stress signaling pathways without compromising their primary function as glucose sensors and regulators. The areas of research which need future exploration include: (1) understanding the mechanisms of the UPR in regulating insulin biosynthesis and secretion, (2) understanding the mechanisms of UPR activation in the β-cell by glucolipotox and (3) further defining the key regulators, pathways, and crosstalk of UPR-mediated apoptosis specific to the β-cell. Such research will further strengthen the link between ER stress, β-cell death, and the pathogenesis of diabetes.

Acknowledgements

Laboratory of F. Urano is supported by grants from NIH-NIDDK (R01DK067493), the Diabetes and Endocrinology Research Center at the University of Massachusetts Medical School, the Juvenile Diabetes Research Foundation International, Massachusetts Technology Transfer Center, and the Worcester Foundation for Biomedical Research.

References


Future Perspectives

There is abundant evidence to support the role of ER stress and stress signaling in the physiological function of the β-cell, as well as high glucose and long-chain fatty acids. This has recently been shown to lead to excessive ER stress which may perpetuate the cycle of β-cell exhaustion and the strain already placed on the ER of the β-cell by hyperinsulinaemia. In addition, ER stress is involved in insulin resistance of the liver, muscle and adipose. Figure 5 summarizes the link between ER stress and the different forms of diabetes.

Figure 4. Nitric oxide and ER stress-mediated apoptosis. Nitric oxide production in the β-cell due to exposure of inflammatory cytokines, can cause apoptosis of the β-cell through two pathways: (1) ER stress-mediated and (2) non-ER stress-mediated. In the ER stress mediated pathway, nitric oxide causes calcium depletion from the ER, leading to the accumulation of unfolded and misfolded proteins (i.e., ER stress) and the activation of the pro-apoptotic gene, CHOP.
**Figure 5.** Summary of the link between ER stress and different forms of diabetes. The three forms of diabetes: genetic, type 1 and type 2 can all be linked to ER stress and UPR-mediated apoptosis of the β-cell.

30. Anding AL, Chapman JS, Barnett DW, Curley RW Jr, Clagett-Dame M. The unhydrolyzable fenretinide analogue 4-hydroxybenzylretinone induces the proapoptotic genes GADD153 (CHOP) and Bcl-2-binding component 3 (PUMA) and apoptosis that is caspase- and independent of the retinoic acid receptor. Cancer Res 2007; 67:6270-7.
37. Anding AL, Chapman JS, Barnett DW, Curley RW Jr, Clagett-Dame M. The unhydrolyzable fenretinide analogue 4-hydroxybenzylretinone induces the proapoptotic genes GADD153 (CHOP) and Bcl-2-binding component 3 (PUMA) and apoptosis that is caspase- and independent of the retinoic acid receptor. Cancer Res 2007; 67:6270-7.


