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A Switch From Life To Death in ER stressed β cells

Christine M. Oslowski[1] and **Fumihiko Urano**[1,2]

[1]Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

[2]Program in Molecular Medicine University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

Abstract

β cell death is an important pathogenic component of both type 1 and type 2 diabetes. Recent findings indicate that cell signaling pathways emanating from the endoplasmic reticulum (ER) play an important role in the regulation of β cell death during the progression of diabetes. Homeostasis within the ER must be maintained to produce properly folded secretory proteins, such as insulin, in response to the body's need for them. However, the sensitive protein-folding environment in the ER can be perturbed by genetic and environmental factors leading to ER stress. To counteract ER stress, β cells activate cell signaling pathways termed the unfolded protein response (UPR). The UPR functions as a binary switch between life and death regulating both survival and death effectors. The outcome of this switch depends on the nature of the ER stress condition, the regulation of UPR activation, and the expression and activation of survival and death components. This review will discuss the mechanisms and the components in this switch and highlight the roles of this UPR balancing act between life and death in β cells.

I. Introduction

A complete understanding of β cell dysfunction and death prior to and during the onset of diabetes at the system level will have a direct impact on the future of medicine and the treatment of diabetes. In this review, we will focus on the role of the endoplasmic reticulum (ER), a cellular organelle important for the biosynthesis and folding of secretory proteins such as insulin, in β cell failure during the progression of diabetes. Homeostasis within the ER must be maintained to produce properly folded secretory proteins, such as insulin, in response to the body's need for them. However, the sensitive protein-folding environment in the ER can be perturbed by genetic and environmental factors leading to ER stress. To counteract ER stress, β cells activate cell signaling pathways termed the unfolded protein response (UPR). The UPR functions as a binary switch between life and death regulating both survival and death effectors. The outcome of this switch depends on the nature of the ER stress condition, the regulation of UPR activation, and the expression and activation of survival and death components. This review will discuss the mechanisms and the components in this switch and highlight the roles of this UPR balancing act between life and death in β cells.

Address correspondence to: Fumihiko Urano, MD, PhD, University of Massachusetts Medical School, Program in Gene Function and Expression, 364 Plantation Street, Room 522, Worcester, MA 01605, USA. Phone: (508)856-6012; Fax: (508)856-4650; Fumihiko.Urano@umassmed.edu or urano@erstress.com.

II. The Endoplasmic Reticulum

The endoplasmic reticulum is an organelle that performs several important functions including lipid biosynthesis, intracellular calcium homeostasis, and cell signaling. The ER is especially responsible for the processing and folding of proteins destined for secretion, intracellular organelles, or the plasma membrane (Figure 1).

In order to ensure proper folding of proteins into their functional conformations, the ER houses an elaborate machinery of protein folding and processing enzymes including molecular chaperones, glycosylating enzymes, and oxidoreductases with a supporting chemical environment. Through ATP-hydrolytic cycles of binding and release, ER molecular chaperones such as BiP aid in hydrophobic core burial promoting proper protein folding. Glycosylation enzymes modify newly synthesized proteins by covalently attaching glycans. Supported by the ER oxidizing environment, oxidoreductases promote proper oxidative protein folding by forming disulfide bonds and rearrangements between cysteine groups. Several of these enzymes as well as additional components also provide a quality control monitoring system to ensure only properly folding proteins are retained or transported out of the ER. Proteins that misfold are recognized and retrotranslocated out of the ER into the cytoplasm where they are degraded by the ubiquitin-proteasome pathway, a process known as endoplasmic reticulum associated degradation (ERAD).

III. ER stress and the Unfolded Protein Response

ER stress

The ER protein folding and quality control machinery establishes a balance between the ER protein load and the ER folding capacity to process this load therefore maintaining ER homeostasis. ER homeostasis can be perturbed by any environmental or genetic stimuli that disrupt efficient protein folding. Disruption of ER homeostasis causes accumulation of unfolded and misfolded proteins in the ER. This condition is referred as ER stress [1, 2] (Figure 2).

ER stress in β cells

β cells are specialized for the production and regulated secretion of insulin to control blood glucose levels. In the presence of hyperglycemia, β cells secrete insulin from a readily available pool. At the same time, an increase in insulin release activates proinsulin biosynthesis [3]. It has been estimated that each β cell produces approximately one million molecules of insulin every minute [4]. In order to handle this immense ER protein load, β cells have developed a highly specialized ER.

Human preproinsulin, a precursor for insulin, is synthesized in the cytoplasm containing a signal peptide sequence at its N-terminal, and then is cotranslationally translocated into the lumen of the ER. The signal peptide of preproinsulin is cleaved in the ER to produce proinsulin. In the lumen of the ER, proinsulin undergoes protein folding forming three disulfide bonds assisted by ER-resident oxidoreductases. This oxidative protein folding is essential for proinsulin maturation. Indeed, mutations in the cysteine residues needed for disulfide bonds formation cause proinsulin misfolding and retention in the ER. When properly folded, proinsulin is delivered to the Golgi apparatus and packaged into secretory granules. The conversion of proinsulin to insulin takes place in the secretory granules and mature insulin is released by exocytosis [5]. The frequent fluctuation of blood glucose levels in humans requires regulated proinsulin folding in the ER with exquisite sensitivity in β cells.

Unfolded Protein Response and three types of downstream effectors

Under ER stress conditions, cells activate a signaling network termed the unfolded protein response (UPR). The UPR is initiated by three ER transmembrane sensors: Inositol Requiring 1 (IRE1), PKR-like ER kinase (PERK), and Activating Transcription Factor 6 (ATF6) (Figure 3).

IRE1 is a type I ER transmembrane kinase. Its N-terminal luminal domain acts as a sensor for ER stress signaling [6]. In response to ER stress, IRE1 dimerizes and autophosphorylates to become active. Activated IRE1 splices an intron out of X-box binding protein 1 (XBP1) mRNA [7-9]. Spliced XBP1 mRNA encodes a basic leucine zipper transcription factor that upregulates IRE1 target genes such as chaperones and ERAD components.

Similar to IRE1, PERK is also a type I ER transmebrane kinase sensing ER stress via its N-terminal luminal. When activated by ER stress, PERK oligomerizes, autophosphorylates and then directly phosphorylates Ser51 on the α subunit of eukaryotic initiation factor 2 (eIF2α) [10]. Phosphorylated eIF2α inhibits global protein translation by reducing the formation of ribosomal initiation complexes and recognition of AUG initiation codons. Meanwhile, the translation of the b-ZIP transcription factor, activating transcription factor 4 (ATF4), is preferentially activated and induces transcription of several PERK target genes including chaperones, ERAD components, amino acid homeostasis regulators, and redox regulation [11].

Finally ATF6 is a type II ER transmembrane transcription factor [12]. Upon sensing stress in its C-terminal luminal domain, ATF6 transits to the Golgi where it is cleaved by site 1 and site 2 proteases, generating an activated b-ZIP transcription factor [13]. This processed form of ATF6 translocates to the nucleus to upregulate ERAD components and chaperones [14, 15].

Taken together these three master regulators sense and interpret protein folding conditions in the ER and translate this information across the ER membrane to regulate downstream effectors [1, 2]. These effectors have the following three distinct functions (Figure 3):

(1) Homeostatic effectors—One set of effectors regulated by the UPR elicits three adaptive responses that function to attenuate ER stress and restore ER homeostasis. These responses include the attenuation of protein translation to reduce ER workload and prevent further accumulation of unfolded proteins, upregulation of molecular chaperones and protein processing enzymes to enhance the ER folding capacity, and the increase in ER-associated degradation (ERAD) components to promote clearance of unfolded proteins. The effectors involved in these functions include eIF2α for translational attenuation, BiP (GRP78), GRP 94, PDI and ERO1 for protein folding, and Derlin-1, EDEM, and HRD1 for protein degradation [16-23].

(2) Feedback Effectors—The UPR also regulates a set of effectors that function in negative feedback loops to provide tight control of the UPR and therefore preventing harmful hyperactivation. One example involves an abundant ER chaperone, BiP. BiP binds to the ER luminal domains of the UPR transducers preventing their activation. Upon ER stress, BiP is released to assist protein folding, allowing activation of the UPR transducers. Consequently, the UPR also induces BiP expression to aid in protein folding and to negatively regulate the UPR master regulators [24-26].

Another example involves phosphatases that serve to shut off different components of the UPR. For instance ATF4 upregulates the expression of GADD34 which interacts with protein phosphatase 1 (PP1) causing dephosphorylation of eIF2α to restore protein

translation and shut off the PERK arm once ER homeostasis has been re-established [27]. Recently a similar mechanism has been proposed for IRE1 dephosphorylation mediated by protein phosphatase 2 (PP2A) recruited by the scaffolding protein RACK1 [28].

Furthermore we demonstrated that Wolfram syndrome 1 (WFS1) gene is a novel negative regulator of the UPR. WFS1 recruits ATF6α to the E3 ubiquitin ligase HRD1 and proteasome enhancing its ubiquitination and proteasome degradation therefore leading to a suppression of ATF6α signaling. Consistent with these findings, ectopic expression of WFS1 suppresses expression of ATF6α target genes and represses ATF6α-mediated activation of the ER stress response element (ERSE) promoter. WFS1 is a causative gene for Wolfram syndrome, a genetic form of diabetes and neurodegeneration. β cells from WFS1-deficient mice and lymphocytes from patients with Wolfram syndrome exhibit dysregulated UPR through hyperactivation of ATF6α. Therefore higher expression of WFS1 in β cells allows tight regulation of ATF6α activation in these cells that are particularly dependent on the UPR for proper function and survival.

(3) Cell fate effectors—Increasing evidence indicates that the UPR directly regulates both apoptotic and survival effectors. These effectors include C/EBP homologous protein (CHOP), apoptosis signal-regulating kinase 1 (ASK1), Caspase-12, and apoptosis antagonizing transcription factor (AATF) [29-33].

CHOP was identified as an ER stress-induced transcription factor and has been shown to be an important component in ER stress-mediated cell death [29, 30]. Deletion of CHOP promotes β cell survival and improves β cell function in multiple mouse models of diabetes including the Akita diabetes mouse model. CHOP is primarily regulated by ATF4 under the PERK pathways of the UPR. CHOP seems to induce cell death primarily by transciptionally regulating survival and death effectors. For an example, it has been demonstrated that CHOP overexpression decreases expression levels of Bcl-2 [34]. Bcl-2 has been shown to inhibit Bax translocation from cytosol to mitochondria [35]. Because Bax is involved in ER stress-mediated cell death [36, 37], CHOP may execute apoptosis by suppressing anti-apoptotic genes, Bcl-2 and enhancing pro-apoptotic component, Bax.

Caspase-12 was identified as an ER stress-activated caspase [32]. Caspase-12 is localized to the ER and activated by ER stress. Deletion of Caspase-12 in mice protects neurons from amyloid β-mediated apoptosis. The function of Caspase-12 in ER stress-mediated β cell death has not been studied extensively.

ASK1 was identified as an activator for MKK4 and MKK6, which in turn activated JNK and p38 respectively [38]. It has been found that IRE1 is involved in ASK1-mediated JNK activation under ER stress conditions. Unresolvable ER stress leads to the recruitment of TNF-receptor-associated factor 2 (TRAF2) to IRE1 and the activation of ASK1. Activated ASK1 activates c-Jun N-terminal protein kinase (JNK) and leads to neuronal cell apoptosis [31, 39-41]. The role of ASK1 in ER stress-mediated β cell death remains incomplete.

We have recently discovered a novel anti-apoptotic effecter of the UPR, AATF [33]. AATF is an ER stress-induced transcription factor transcriptionally regulated under the PERK-eIF2α pathway. AATF mediates anti-apoptotic effects through transcriptional regulation of AKT1. Ectopic expression of AATF and AKT1 protects β cell lines and mouse primary islets from ER stress-mediated cell death [33].

IV. The UPR binary switch between life and death of β cells

As described earlier, the unfolded protein response regulates both death and survival effectors. How the UPR determines whether a cell will survive or die upon ER stress

conditions is currently incomplete. We propose that the UPR acts as a binary switch between life and death. The outcome of this switch depends on several levels in the ER stress paradigm. These levels include the type of ER stress the cells are exposed to, UPR activation regulation, and the expression and activation of downstream survival and death effectors (Figure 3).

Types of ER stress

Ideally ER stress is mitigated by the UPR machinery promoting cell survival. However in some situations ER stress is unresolvable leading to cell death. Therefore we propose that there are two types of ER stress conditions: tolerable and unresolvable (Figure 4).

Tolerable ER stress

Cells are often exposed to physiological or mild conditions that induce tolerable ER stress. Under these ER stress conditions, the UPR can restore ER homeostasis promoting cell survival. For instance, β cells are commonly exposed to transient high glucose leading to an increase in proinsulin mRNA translation and ER protein workload therefore activating the UPR. Several studies indicate that PERK-eIF2α signaling plays a major role in regulating proinsulin mRNA translation under dynamic glucose conditions [42, 43]. Tight control of eIF2α phosphorylation is critical to ensure proper adaptation to increases in ER protein load and to promote β cell survival [42, 44-46]. In islets from Perk knockout mice, insulin biosynthesis stimulated by high glucose is markedly enhanced as compared to that in control mice [42]. As a consequence, Perk knockout mice develop diabetes because of ER stress-mediated β cell death. IRE1α is also activated under transient high glucose conditions. Acute IRE1α activation is required for proinsulin biosynthesis and perhaps enhancing ER proinsulin folding capacity [47]. Therefore under transient high glucose conditions, the UPR promotes cell survival by increasing the ER folding capacity to handle the increase need for insulin production reestablishing ER homeostasis.

Unresolvable ER stress

When the UPR fails to attenuate ER stress and restore ER homeostasis, UPR activation induces apoptosis. This unresolvable ER stress can be caused by genetic mutations as well as environmental factors. For example β cells are exposed to chronic high glucose conditions during diabetes development. Chronic high glucose strongly activates IRE1α and leads to insulin mRNA degradation. Insulin mRNA degradation is mediated by the RNase-like domain of IRE1α.

Another example is Wolcott-Rallison syndrome, a rare autosomal recessive form of juvenile diabetes. In this syndrome, mutations have been reported in the *EIF2AK3* gene encoding PERK [48]. Under ER stress, activated PERK phosphorylates eIF-2α on the Ser51 residue to temporarily shut off global translation to reduce the ER workload. When a high workload is placed on the ER of β cells, for example when insulin demand increases following meal intake, phosphorylation of eIF2α is essential in controlling ER stress levels and thereby promotes cell survival [11]. PERK knockout mouse, a mouse model of Wolcott-Rallison syndrome, develop β cell death and diabetes due to high ER stress caused by excessive activation of proinsuiln biosynthesis in β cells. Therefore, a loss-of-function of PERK and a consequent disruption in translational attenuation during ER stress via decreased eIF2α phosphorylation, could lead to unresolvable ER stress and β cell death. Consistent with this model, mutant mice carrying a heterozygous mutation in the phosphorylation site of eIF2α ($Eif2s1^{+/tm1Rjk}$) become obese and, due to β cell dysfunction, diabetic when fed a high-fat diet [45].

Unresolvable ER stress caused by genetic mutations is also observed in permanent neonatal diabetes. Neonatal diabetes is a rare disorder defined as insulin-requiring hyperglycemia within the first month of life and is typically associated with slowed intrauterine growth. Permanent neonatal diabetes can be caused by several types of mutations. It has recently been shown that mutations in the human insulin gene primarily occurring in critical regions of proinsulin folding can cause this disorder [49]. These mutations presumably lead to improper folding of proinsulin, causing unresolvable ER stress and ultimately leads to β cell apoptosis. A mouse model of this disease, the Akita mouse, has a dominant cysteine^{96}-to-tyrosine missense mutation in the Ins2 gene [50, 51]. This mutation leads to disruption of disulfide bond formation between the A and B chain of proinsulin, causing insulin to misfold and accumulate in the ER of the β cell [50]. This accumulation of misfolded insulin leads to unresolvable ER stress, β cell apoptosis, and consequently diabetes [52].

Unresolvable ER stress can be also caused by environmental factors. For instance, several studies demonstrate that chronic exposure to long chain free fatty acids (FFAs) or cytokines induce β cell apoptosis [53-57]. Treatment of β cell lines with the free fatty acid, palmitate, or cytokines, interleukin-1β and interferon-γ, induce ER calcium leakage disrupting ER calcium homeostasis causing unresolvable ER stress. This unresolvable ER stress activates the UPR inducing β cell apoptosis. The underlying mechanisms are currently under study.

UPR activation regulation

Once ER homeostasis has been reestablished and ER stress is reduced, it is essential that the UPR stress sensors, IRE1, PERK, and ATF6, are turned off. This regulated UPR activation promotes cell survival; however, dysregulation leads to UPR hyperactivation and cell death.

One example is observed in Wolfram syndrome. Wolfram syndrome is a rare autosomal recessive disorder characterized by childhood onset of diabetes mellitus, followed by optic atrophy, deafness and death from neurodegeneration in the third or fourth decades [58-60]. Postmortem studies reveal a non-autoimmune-linked selective loss of β cells [61]. Mutations in the WFS1 gene are responsible for this syndrome [62-64]. The WFS1 protein is localized to the ER and highly expressed in β cells [65, 66] [67]. As mentioned previously, WFS1 plays an important role in negative regulation of ATF6α signaling and therefore provides tight regulation of the UPR. β cells are particularly dependent on utilizing the UPR to reestablish ER homeostasis under transient ER stress conditions such as high glucose. Thus the lack of WFS1 causes hyperactivation of ATF6 leading to β cell death [67-70]. Apoptotic components regulated by ATF6 are currently under study.

Another example is exemplified in the regulation of PERK. Loss of function of p58^{IPK}, a negative regulator for PERK, also causes β cell death and diabetes [71, 72]. p58^{IPK} is induced several hours after the UPR has been initiated and negatively regulates PERK. By the time p58^{IPK} is produced, if cells haven't yet returned to homeostasis, the UPR may switch into apoptotic mode. In the absence of p58^{IPK}, a continued translational block through PERK causes dysregualted UPR and unresolvable ER stress, leading to β cell death.

The UPR regulates both survival and death factors

IRE1, PERK and ATF6a regulate the transcription and activation of both survival and death effectors. The identification of these components remains incomplete. More importantly is to understand when and how the UPR determines to favor one set of effectors over the other tipping the balance between life and death.

(1)Regulation of Survival and Death Factors by IRE1—Upon sensing ER stress, activated IRE1 cleaves an intron from the mRNA encoding X-box binding protein 1 (XBP1)

[7-9]. The spliced variant of XBP1 mRNA encodes a transcriptional activator for several UPR genes including chaperones, protein folding catalysts, and ERAD components [73, 74]. Apart from homeostatic functions, IRE1 also regulates apoptotic effectors. In the presence of unresolvable ER stress, IRE1 activates JNK through ASK1 and elicits apoptosis [31, 39]. This pathway has been shown to block the function of the anti-apoptotic Bcl-2 by phosphorylating it, thus causing apoptosis in β cells [75, 76]. IRE1 is also involved in the decay of mRNAs encoding ER homeostatic proteins, including PDI, and BiP [77-80].

(2) Regulation of Survival and Death Factors by PERK—PERK has been shown to protect β cells from ER stress-mediated cell death through the attenuation of protein translation [42, 81]. In addition, we have recently found that PERK upregulates a novel anti-apoptotic effector, apoptosis antagonizing transcription factor (AATF) and mediates survival in part through the transcriptional regulation of AKT1 [33]. In contrast, PERK also regulates expression of CHOP; which is an important component of ER stress-mediated β-cell death [29, 30, 52, 82, 83].

(3) Regulation of Survival and Death Factors by ATF6α—ATF6α is a major regulator of BiP and has been shown to protect cells from ER stress-mediated apoptosis [84-86]. Contrary to the previous findings, we recently found that hyperactivation of ATF6α causes β cell death [70]. This hyperactivation of ATF6α was observed in the islets of WFS1 knockout mice and lymphocytes from Wolfram syndrome patients [70]. The death factors regulated by ATF6α should be identified.

(4) Regulation of Life and Death by controlling stability of pro- and anti-apoptotic effectors—Survival and death effectors are also regulated at the post-transcriptional level. It has been shown that survival is favored during mild and tolerable ER stress as a consequence of the intrinsic instabilities of mRNAs and proteins that promote apoptosis compared to those that facilitate protein folding and adaptation. As a consequence, the expression of apoptotic proteins is short-lived as cells adapt to stress. This observation indicates that post-transcriptional mechanisms regulating the ratio between survival and apoptotic effectors are important for controlling life and death of ER stressed β cells.

Future work on understanding the components and mechanisms of the UPR binary switch paradigm

Even though recently we have made some progress on the UPR binary switch model, several unanswered questions remain. It is important to mention that the UPR is context specific. The type of ER stress, species, and cell should be considered when building the UPR binary switch model. Also studying “heterogeneity” among cells in the same context in which some cells adapt to ER stress while others cannot and ultimately die may shed some light in this area. Therefore investigating the differences between and within cell populations under different types of ER stress will aid in identifying missing components and mechanisms of our binary switch model between life and death.

V. Conclusion

Increasing evidence indicates that ER stress is directly related to β cell dysfunction and death during the progression of genetic forms of diabetes such as Wolfram syndrome, Wolcott-Rallison syndrome, and permanent neonatal diabetes [87, 88]. Recent evidence also suggest that ER stress-mediated β cell death is also involved in the pathogenesis of type 1 and type 2 diabetes [89]. ER stress activates the signaling network termed the unfolded protein response (UPR). The UPR acts like a binary switch performing a balancing act between life and death upon ER stress. The complete understanding of the mechanisms and

components involved in this switch may reveal new information on how ER stress induces β cell death and perhaps novel targets for diabetes prevention or treatment.

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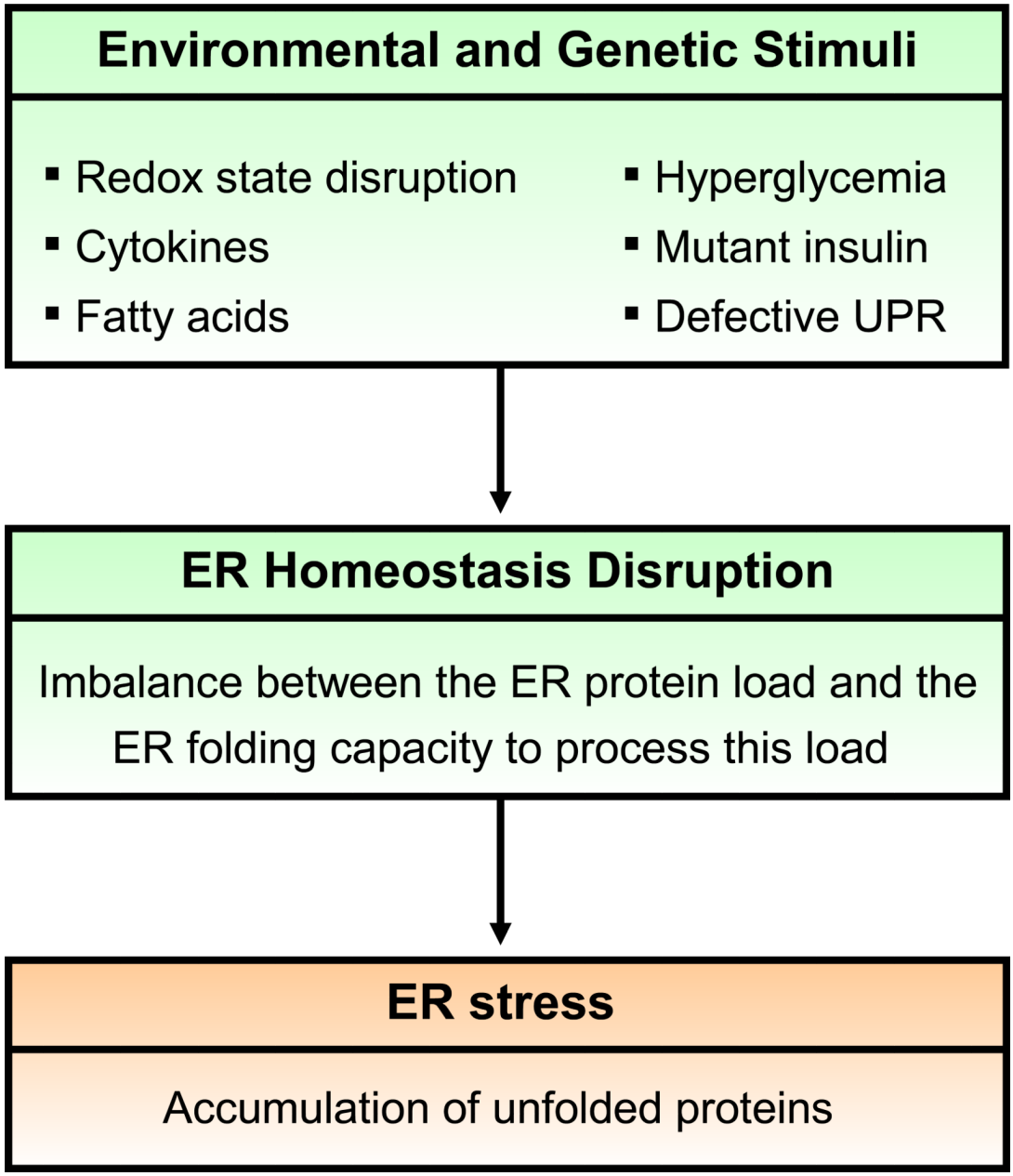

Figure 1. ER stress

In order for proteins to fold within the endoplasmic reticulum properly, there must be a balance between the ER protein load and the folding capacity to handle this load. Several genetic and environmental stimuli can disupt this ER homeostasis therefore causing an accumulation of unfolded and misfolded proteins within the ER lumen. This condition is known as ER stress.

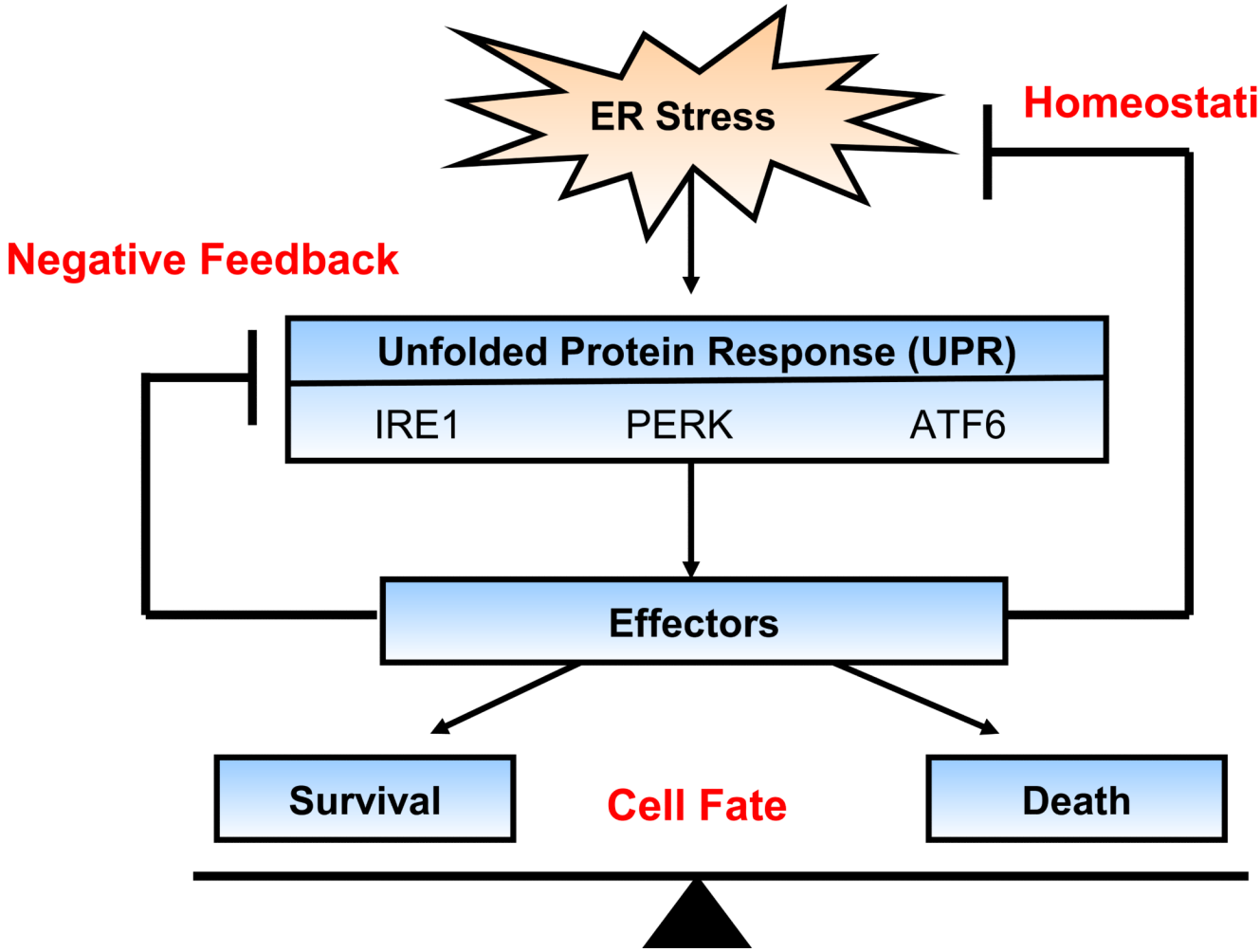

Figure 2. The Unfolded Protein Response

Upon ER stress, a signaling network termed the unfolded protein response (UPR) is activated. The UPR has three master transducers: IRE1, PERK, and ATF6. These transmembrane proteins sense ER stress within the ER and translate this information across the ER membrane to induce expression and activation of downstream effectors. These effectors have three functions: homeostatic, to mitigate ER stress and restore ER homeostasis; feedback regulators, to turn off the UPR transducers when ER homeostasis is restored; and finally cell fate regulators including both survival and death components that play a role in determining whether the cell will live or not upon ER stress.

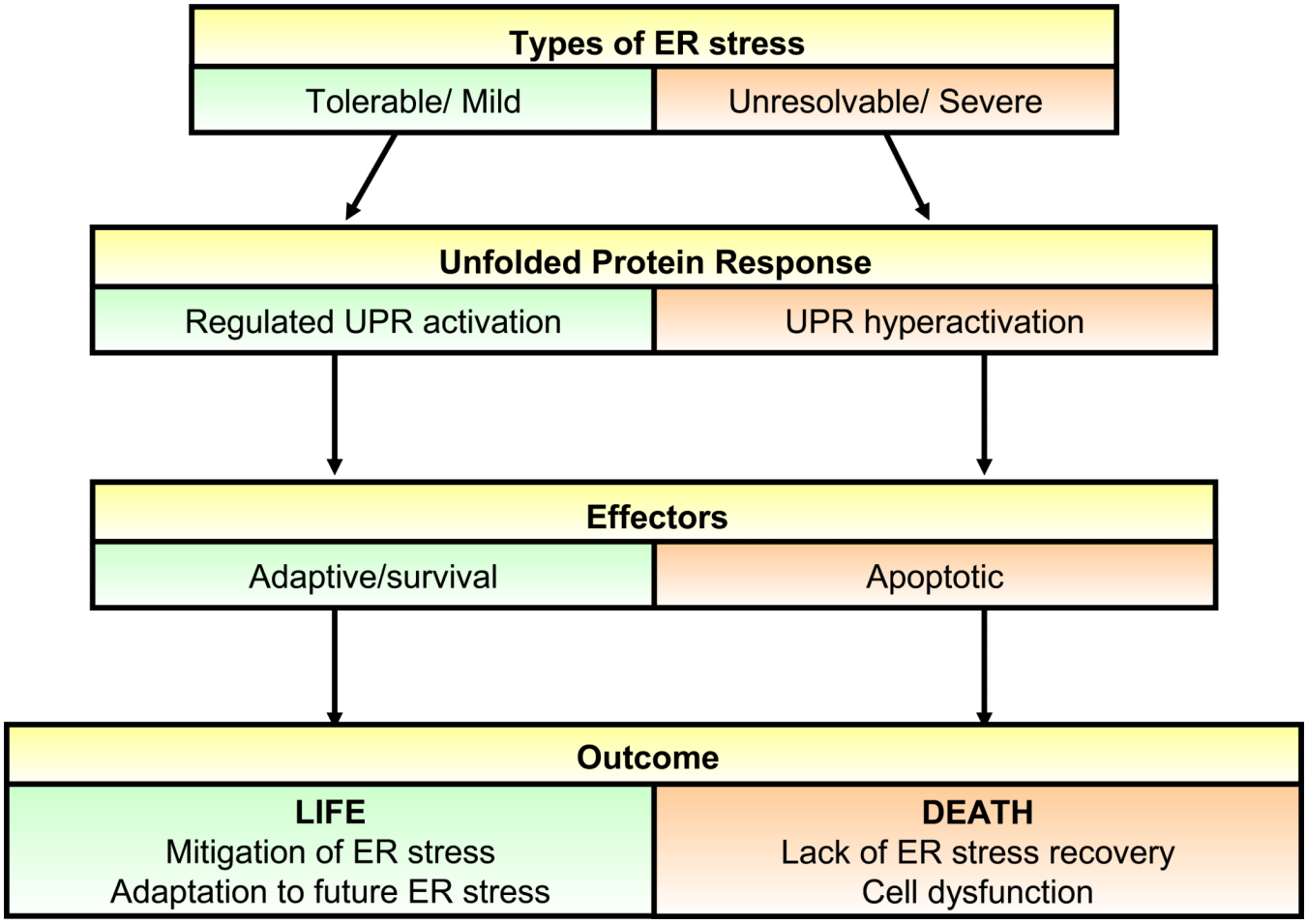

Figure 3. The UPR Binary Switch Model

We propose that the UPR acts as a binary switch between life and death. the outcome of this switch depends on many levels of the ER stress paradigm. These levels include the type of ER stress, UPR activation regulation, and the expression and activation of survival and death effectors. Cells will live or die depending on a switch between one or more of these levels.