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Endoplasmic reticulum stress in health and disease

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Unfolded proteins and other conditions affecting endoplasmic reticulum (ER) homeostasis cause ER stress. The cell reacts to ER stress by activation of the unfolded protein response (UPR), which induces profound changes in cellular metabolism including general translation attenuation, transcriptional upregulation of molecular chaperone genes, and activation of ER-associated degradation. However, prolonged or acute ER stress results in cell death. Recent progress suggests that ER stress and UPR play key roles in the immune response, diabetes, tumor growth under hypoxic conditions, and in some neurodegenerative diseases. Further research on ER stress and UPR will greatly enhance the understanding of these physiological and pathological processes, and provide novel avenues to potential therapies.

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Introduction

Multiple physiological or pathological conditions that affect protein folding and/or calcium homeostasis — including glucose starvation, underglycosylation of glycoproteins, calcium flux across the endoplasmic reticulum (ER) membrane, elevated protein synthesis and secretion, and failure of protein folding, transport or degradation — can cause ER stress, the term given to an imbalance between the cellular demand for ER function and ER capacity [1]. Cells respond to ER stress by activation of the unfolded protein response (UPR) pathway, which emanates from the ER, but requires both the nucleus and the Golgi apparatus for signal transduction. The UPR enables the cell to reduce unfolded protein load in ER by attenuating translation, promoting protein folding, secretion and degradation. However, if ER homeostasis cannot be restored, prolonged UPR may induce apoptosis, which involves mitochondria-dependent or -independent mechanisms. The canonical mammalian UPR pathway has three branches, with

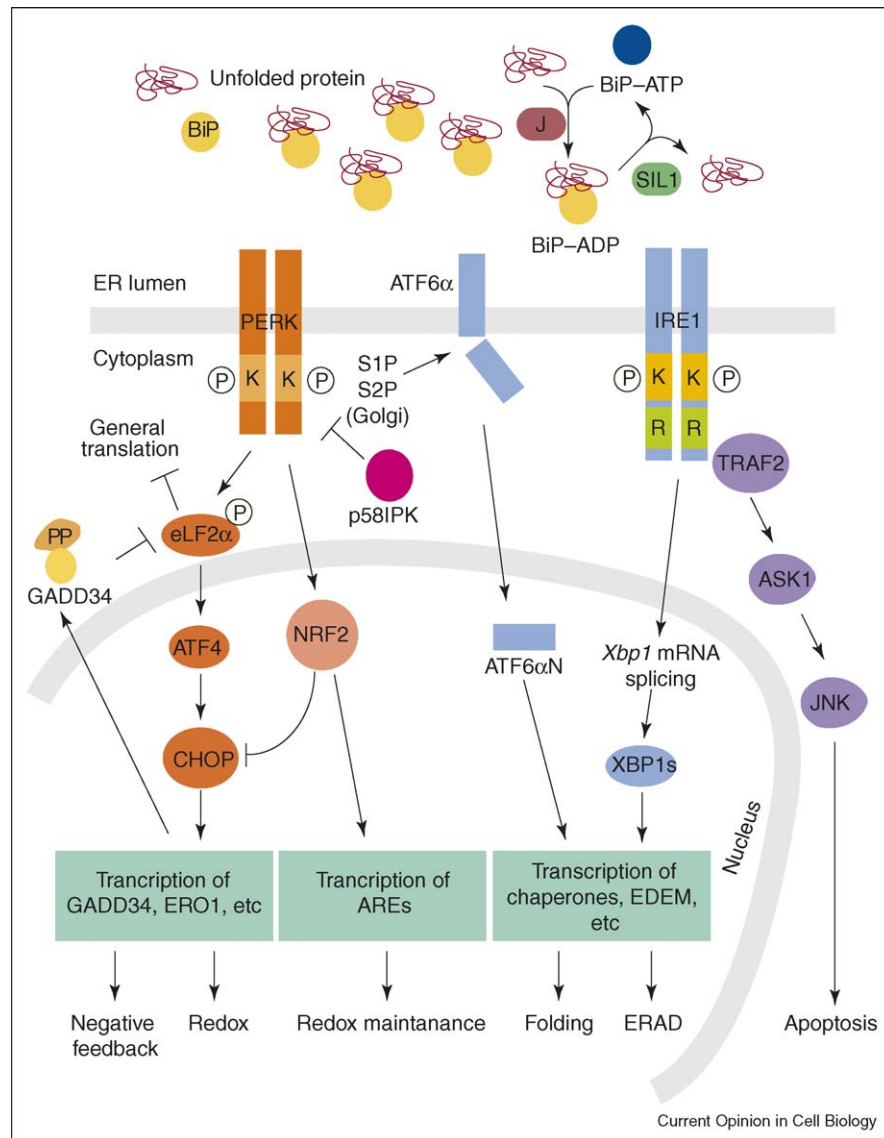
transmembrane proteins ATF6, IRE1 and PERK serving as proximal sensors (Figure 1). Under unstressed conditions, the luminal domains of these sensors are occupied by BiP/GRP78, an ER HSP70 family protein, which represses these signaling pathways. Upon ER stress, sequestration of BiP by unfolded proteins activates these sensors, inducing phosphorylation and oligomerization of IRE1 and PERK, and relocalization of the CREB/ATF family bZIP transcription factor ATF6 to the Golgi where it is cleaved by Site 1 and Site 2 proteases (S1P and S2P) [1].

Initially during ER stress, general translation is stalled via the PERK pathway [1]. Activation of PERK results in phosphorylation and inactivation of the translation initiation factor eIF2 α . However, mRNAs containing upstream non-coding open reading frames (uORFs) are still translated to allow for basic cellular survival needs and the recovery from ER stress [1]. Upregulation of UPR effector genes is achieved by transcription factors downstream of the ER stress sensors. The cleaved cytoplasmic domain of ATF6 can function directly as a transcription factor for regulation of genes encoding other transcription factors and molecular chaperones. Activation of IRE1 results in unconventional splicing of mRNA of the *Xbp1* transcription factor, with the resulting open reading frame encoding full length XBP1. Both XBP1 and ATF6 in turn activate transcription of genes encoding molecular chaperones and proteins involved in ER-associated degradation (ERAD).

UPR also incurs profound indirect effects on other cellular processes (Figure 2). For instance, DNA microarray analyses suggest that ER stress upregulates the transcription of many secretory pathway components, including proteins functioning in peptide translocation, glycosylation and folding in ER, proteins involved in anterograde and retrograde transport between ER and Golgi, and proteins implicated in secretion from trans Golgi [2]. Without a functional UPR, protein translocation across the ER membrane is partly impaired [3]. Moreover, proteins involved in lipid metabolism and heme biosynthesis are also upregulated by UPR [2]. UPR also inhibits cell cycle at G1 phase through inhibition of cyclin D1 translation via the PERK/eIF2 α branch [4,5]. In addition, at least in yeast, UPR represses the differentiation process of pseudohyphal growth or invasive growth upon nitrogen starvation [6].

ER stress occurs under both physiological and pathological conditions [7]. Physiological fluctuations of nascent peptides or unfolded proteins in ER may cause temporary

Figure 1

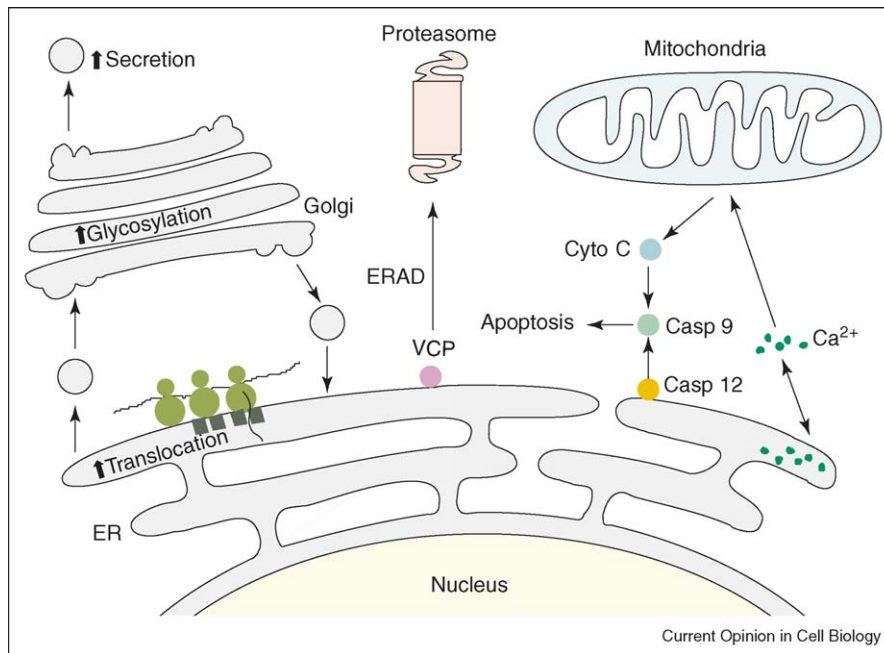


The unfolded protein response pathway in mammalian cells. Upon ER stress, sequestration of BiP by unfolded proteins activates PERK, ATF6 and IRE1. Activated PERK mediates phosphorylation of eIF2 α , resulting in repression of general translation, but permitting ATF4 translation, eventually leading to transcriptional upregulation of multiple genes including CHOP, GADD34 and ERO1. PERK also mediates phosphorylation of NRF2, resulting in its translocation into the nucleus to activate transcription of genes with antioxidant response elements (AREs) in their promoters. The PERK pathway is negatively regulated by p58^{IPK} and the CHOP-induced protein phosphatase (PP) subunit, GADD34. Activation of ATF6 is mediated by translocation into Golgi and cleavage by Site 1 and Site 2 proteases (S1P and S2P). Intramembrane proteolysis releases the N-terminal cytoplasmic domain of ATF6, which translocates into the nucleus as an active transcription factor. Activation of IRE1 results in unconventional splicing and subsequent translation of the mRNA for the transcription factor XBP1. IRE1 also activates the pro-apoptotic ASK1/JNK mitogen activated kinase pathway through TRAF2. BiP cycles between ADP- and ATP-binding forms. Nucleotide exchange factors like SIL1 promote ADP release and thus reload of ATP, while DnaJ family proteins (J) promote BiP ATPase activity, resulting in ATP hydrolysis. K, kinase domain; R, endoribonuclease domain; P, phosphorylation.

attenuation of protein translation and/or upregulation of the protein folding machinery. In contrast, long-term ER stress caused by the accumulation of mutant proteins or acute ER stress induced by chemical agents leads to full mobilization of UPR and often cell death. ER stress has been implicated in diabetes [8], tyrosinemia [9], cardiovascular diseases [10], viral infection [11], cancer [12], the

immune response [13], aging [14], cerebral ischemia [15], neurodegenerative diseases [16,17], α 1-antitrypsin deficiency [18], inclusion body myositis [19] and controversially in mental disorders [20,21] (Table 1). However, it is not clear whether the ER stress observed in some of these conditions is a primary cause of diseases or only a secondary pathological phenomenon.

Figure 2



The effects of unfolded protein response activation extend beyond the ER. ER stress induces profound upregulation of the secretory pathway components, including proteins involved in peptide translocation across the ER membrane, protein transport between the ER and the Golgi, glycosylation of proteins in the Golgi and protein transport out of *trans*-Golgi, indicating that these cellular functions might be enhanced during ER stress as a protective mechanism to reduce the load of unfolded proteins in ER. The ER-associated degradation (ERAD) pathway is also activated during ER stress via transcriptional upregulation to reduce the amount of unfolded proteins in the ER. The removal of unfolded proteins from the ER requires the AAA family protein, CDC48/VCP. Long-term or acute ER stress causes apoptosis, which may be mediated by the ER-localized caspase 12. However, mitochondria-initiated apoptosis may also be involved, due to the calcium exchange between ER and the cytosol, eventually leading to cytochrome c release and the onset of apoptosis. The mitochondria-dependent and -independent apoptotic pathways converge at caspase 9.

This review focuses on recent findings implicating UPR pathway components in the normal physiology of the immune response and the role of ER stress in several common diseases, including diabetes, cancer and neurodegenerative disorders. A few key publications in each field are selected for detailed discussion.

ER stress and the unfolded protein response in the immune response

In addition to having a role in the relief of ER stress, UPR is also used for synthesis of large amount of proteins in ER by some secretory cells. For example, antibody-secreting plasma cells utilize UPR components for cellular differentiation and upregulation of protein synthesis [22^{••}]. *In vivo* cell transplantation experiments demonstrated that while *Ire1*^{-/-} hematopoietic cells can differentiate normally into pro-B cells, they fail to differentiate further into pre-B cells, indicating that *Ire1* is required for differentiation of B cell progenitors but is not important for early hematopoiesis. Moreover, overexpression of the spliced form of *Xbp1* (*Xbp1s*) did not restore pro-B cell differentiation, strongly suggesting that other IRE1-regulated proteins are required for differentiation of these cells [22^{••}].

The final stage of plasma cell differentiation also requires the IRE1 pathway [22^{••},23]. Plasma cells with dominant-negative mutations in either the kinase or the endoribonuclease domains of IRE1, regions shown to be necessary for *Xbp1* splicing, fail to secrete antibodies efficiently [22^{••}]. Although *Xbp1s* expression is not sufficient for pro-B cell differentiation, ectopic expression of *Xbp1s* fully restores the antibody-producing function of these plasma cells [22^{••},23]. In addition, expression of a dominant-negative eIF2 α did not interfere with plasma cell differentiation or antibody production, suggesting selective involvement of the IRE1 branch of the UPR in B cell differentiation [22^{••}]. In fact, to inhibit protein degradation and cell death while maximizing translation and protein folding, the PERK pathway is repressed in plasma cells via p58^{IPK}, a negative regulator of PERK kinase activity that is indirectly upregulated by XBP1 [24].

ER stress is also involved in the innate immune response [25[•]]. Intramembrane proteolysis of CREB-H, a liver-specific basic leucine zipper transcription factor belonging to the CREB/ATF family, is mediated by UPR in the presence of proinflammatory cytokines or lipopolysaccharide. CREB-H promotes transcription of genes

Table 1**Human diseases linked to ER stress**

Disease	Protein	Function	Major pathology	Ref
Wolcott-Rallison Syndrome	PERK	UPR sensor	Infantile diabetes	[8]
Wolfram Syndrome	WFS1	Involved in ERAD?	Diabetes insipidus, neurodegeneration	[8]
Hereditary tyrosinemia type I	FAH	Tyrosine metabolism	Liver and renal dysfunction; accumulation of a metabolic intermediate causes ER stress	[9]
Familial hypercholesterolemia	LDLR	Low density lipoprotein receptor	Hypercholesterolemia; accumulation of mutant protein causes ER stress	[59]
Z α 1-antitrypsin deficiency	A1AT (α 1-antitrypsin)	Protease inhibitor	Early onset liver disease; accumulation of mutant protein causes ER stress	[18]
Inclusion body myopathy (IBMPFD)	p97/VCP/CDC48	ERAD	Early onset Paget disease and frontal temporal dementia, muscular dystrophy, motor neuron degeneration	[60,61]
Parkinson's disease	Parkin Others	E3 ubiquitin ligase	Tremor, bradykinesia; loss of inclusion-containing dopaminergic neurons in the substantia nigra; Mutation of Parkin causes ER stress	[16]
Familial Alzheimer's disease	PS1 Others	γ -secretase complex	Memory loss, dementia; loss of neurons from frontal cortex, hippocampus, basal forebrains, formation of extracellular plaques and intracellular neurofibrillary tangles; ER stress?	[16, 62,63]
Familial Amyotrophic Lateral Sclerosis	SOD1	Cu/Zn superoxide dismutase	Degeneration of motoneurons in spinal cord, cortex and brain stem; caspase 12 is activated and mutant SOD1 forms aggregates in ER in transgenic mice	[16,64]
Marinesco-Sjögren syndrome	SIL1/BAP/SLS1	BiP adenine nucleotide exchange factor	Cerebellar ataxia, myopathy, cataracts; ER stress and UPR activation are observed in a mouse model	[39*, 40,41]
GM1 gangliosidosis	β -galactosidase	Carbohydrate/lipid metabolism	Severe cerebral neurodegeneration; Accumulation of gangliosides; ER stress is observed in a mouse model	[43*]
Pelizaeus-Merzbacher disease	PLP1	Proteolipid protein 1, myelin component	Spastic quadriplegia, ataxia; dysmyelination; ER accumulation of PLP causes ER stress	[16]
Batten disease/Infantile neuronal ceroid lipofuscinosis	PPT1	Palmitoyl-protein thioesterase-1	Seizures, mental deterioration, blindness; accumulation of toxic fatty-acylated proteins in neurons; may involve ER stress and activation of caspase 4	[65]
Bipolar disease	XBP1?	UPR sensor	Manic/depressive psychosis; <i>Xbp1</i> gene polymorphism?	[20,21]
Transmissible Spongiform Encephalopathy	PrP		Neuronal loss due to accumulation of the misfolded prion protein; enhanced calcium release and ER stress may be involved	[16]
Spinocerebellar ataxia 3/Machado-Joseph disease	SCA3		Ataxia, abnormal ocular movement, spasticity; activation of the IRE1 and PERK branches of UPR	[16]
Huntington's disease	Huntingtin		Neurodegeneration, motor dysfunction, abnormal cognition; mutant huntingtin changes ER calcium homeostasis	[16]
Sporadic inclusion body myositis	APP		Muscle degeneration with vacuolated muscle fibers; inclusions containing either β -amyloid or phosphorylated tau induces ER stress	[19]
Cerebral ischemia			Paresis, memory disturbance; neuron death; ER stress and CHOP activation	[15]
Atherosclerosis			Cholesterol deposition on the artery wall; hyperhomocysteinaemia and accumulation of unesterified cholesterol cause UPR induction	[10, 66,67]
Solid tumors			UPR activation can protect tumor cells from hypoxia-induced apoptosis	[12]
Viral infection			UPR activation upon viral infection	[11]
Inflammation			Utilizes a specific UPR sensor, CREB-H	[25*]
Fluoride tooth			Fluoride causes ER stress in ameloblast, resulting in dental enamel formation	[68]

encoding the inflammatory components of the acute phase response.

ER stress and the unfolded protein response in diabetes

Diabetes is a prevalent metabolic disease caused by pancreatic failure associated with autoimmunity (type I) or insulin resistance in peripheral tissues (type II). Type I diabetes can result from misfolded mutant insulin that causes chronic ER stress in pancreatic β -cells, as in the Akita mouse [26]. Deregulation of translational repression that causes protein overload also leads to type I diabetes, as shown by mice and human carrying mutations in the PERK branch of UPR [27,28]. Over-activation of the PERK branch of UPR signaling can also cause ER stress in pancreatic β -cells and result in diabetes. Derepression of translation attenuation is required for ER stress recovery and is mediated by p58^{IPK}, a PERK kinase inhibitor [29,30]. Recently, p58^{IPK} knockout mice have also been found to exhibit symptoms of type I diabetes (Table 2), including high blood glucose levels and decreased insulin levels concomitant with the apoptosis of pancreatic islet β -cells [31*].

Recent studies suggest that ER stress might also be a key player in obesity-induced type II diabetes. Increased levels of the phosphorylated forms of PERK and eIF2 α , and elevated activity of the proapoptotic kinase JNK, which is activated by IRE1, are found in the liver and the adipose tissue of obese mice. Activation of JNK results in aberrant serine phosphorylation and repression of insulin receptor substrate 1 (IRS1), leading to insulin resistance in peripheral tissues [32**]. Furthermore, *Xbp1*^{+/-} mice on a high-fat diet develop a phenotype resembling type II diabetes [32**] that may be due to ER stress caused by an insufficient UPR. Lastly, mice heterozygous for a

targeted mutation of *eIF2 α* on a high-fat diet become obese and exhibit glucose intolerance and slower insulin release upon glucose infusion, indicative of diabetes [33]. In these mice, more proinsulin remains bound to BiP, which serves as a major ER chaperone in addition to its ER stress sensing function, suggesting saturation of the protein folding machinery.

ER stress and the unfolded protein response in cancer

ER stress and UPR have been observed in tumors [12], but how UPR activation contributes to cancer cell survival is not clear. Recently, it has been reported that UPR is activated by hypoxia, a condition often found in the cores of solid tumors [34]. *In vitro*, hypoxia has been shown to upregulate *Xbp1* splicing and transcription of many UPR-related proteins, including BiP and GRP94 (an ER HSP90 family protein) [35]. *Xbp1*-deficient cells exhibit sensitivity to hypoxia that is inversely correlated with the amount of *Xbp1* expression, and generate smaller tumors when implanted into SCID mice [35].

The PERK/eIF2 α pathway may also be a determinant in tumor survival under hypoxic conditions. Selective translation of ATF4 and CHOP, two PERK-induced transcription factors, occurs under low oxygen conditions [36*]. As seen in *Xbp1*-deficient cells, apoptosis of *Perk*^{-/-} mouse embryonic fibroblasts or cells homozygous for a phosphorylation-deficient form of eIF2 α increases under hypoxic conditions [36*]. In addition, these cells when transformed form smaller tumors in transplantation experiments. Although these studies suggest that the UPR may play an important role in the survival of cancer cells, it remains elusive how hypoxia induces the UPR. As suggested, it is possible that low oxygen levels disrupt the pro-oxidative environment necessary for disulfide bond

Table 2

Targeted UPR pathway proteins in mice

Protein	Phenotype of targeted mice	Reference
ER stress sensors		
PERK	<i>Perk</i> ^{-/-} mice exhibit type I diabetes, bone abnormalities, small body size.	[27]
IRE1 α	<i>Ire1α</i> ^{-/-} mice are embryonic lethal.	[22**]
UPR transducers		
XBP1	<i>Xbp1</i> ^{-/-} mice are embryonic lethal; <i>Xbp</i> ^{+/-} mice show insulin resistance and type II diabetes on high fat diet.	[32**,69]
eIF2 α	Homozygous eIF2 α S51A mice die perinatally with diabetes and pancreatic β cell deficiency.	[28]
ASK1	<i>Ask1</i> ^{-/-} mice have no obvious phenotype.	[70]
ATF4	<i>Atf4</i> ^{-/-} mice exhibit embryonic or perinatal lethality.	[71]
CHOP	<i>Chop</i> ^{-/-} mice are viable and normal.	[72]
NRF2	<i>Nrf2</i> ^{-/-} mice are susceptible to oxidative stress, exhibit low glutathione levels, and have regenerative immune-mediated hemolytic anemia.	[73,74]
Other		
GADD34	<i>Gadd34</i> ^{-/-} mice have no obvious phenotype.	[75]
p58IPK	p58 ^{IPK} ^{-/-} mice exhibit type I diabetes.	[31*]
Caspase 12	<i>Casp12</i> ^{-/-} mice develop normally but are resistant to ER-stress-induced apoptosis.	[47]

formation during protein folding, resulting in misfolded proteins and ER stress [36[•]]. Nor is it clear how ER stress promotes survival of cancer cells. A recent report suggests that ER stress results in phosphorylation of the tumor suppressor protein p53 by GSK3 β , leading to its relocalization to the cytoplasm and suppression of the p53-dependent apoptosis pathway, which may contribute to the growth of some tumors [37[•]].

ER stress and the unfolded protein response in neurodegeneration

Neuronal loss in both familial and sporadic forms of neurodegenerative disorders is often accompanied by formation of irregular protein inclusions or fibrillar aggregates composed of misfolded proteins [38]. Upregulation of ER stress markers has been observed in postmortem brain tissues and cell culture models of many neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and expanded polyglutamine diseases such as Huntington's disease and spinocerebellar ataxias [16]. However, the significance of the upregulated UPR observed in these diseases is not clear. Two recent *in vivo* studies suggest that, at least in some cases, ER stress may be tightly correlated with neurodegeneration.

Cerebellar Purkinje cell degeneration and subsequent ataxia occurs in mice homozygous for a spontaneously occurring truncation of the *SiI1* transcript, which encodes an adenine nucleotide exchange factor of BiP [39[•]]. In humans, mutations in *SIL1* cause Marinesco-Sjögren syndrome, a rare disease associated with cerebellar ataxia, progressive myopathy and cataracts [40,41]. With BiP, as with other HSP70 family members, ATP/ADP exchange is likely to play an important role in unfolded protein binding and release [42]. Detailed studies suggest that Purkinje cells in the *SiI1*-deficient mouse have ubiquitinated nuclear- and ER-associated protein aggregates containing several ER resident proteins. Moreover, prior to the formation of obvious protein aggregates, upregulation of the ER stress markers BiP, CHOP and ORP150 occurs in *SiI1*-deficient Purkinje cells [39[•]] (Zhao *et al.*, unpublished). Together these data suggest that while the cycle between the ATP- and the ADP-binding forms of BiP is critical for chaperone function, the UPR signaling function of BiP is relatively unaffected by alteration of nucleotide-binding states.

Not only is ER stress caused by protein misfolding in neurons, but it is also induced by accumulation of certain lipids. A recent report highlighted ER stress as the cause for neuron death in G_{M1} gangliosidosis caused by a null mutation in β -galactosidase [43[•]]. This mutation results in accumulation of G_{M1} gangliosides, which causes calcium efflux from the ER and ER stress. Consistent with ER-stress-induced death, CHOP, JNK upregulation and caspase 12 activation were observed in the dying neurons.

Interestingly, BiP upregulation occurred much later than upregulation of other UPR-induced proteins, indicating that protein misfolding is a late event in the brains of these mice, consistent with the primary cause of the disease being a defect in lipid storage.

Although ER stress has been linked to neurodegenerative diseases, in many cases it may not be the primary cause of neuron death. However, it may modify the progression and severity of these complicated diseases. Therefore, additional research on the role of ER stress in neurodegeneration may provide insights into the mechanisms of these diseases and potential therapies.

The mechanisms of ER-stress-induced cell death and crosstalk of UPR with other cellular stress response pathways

The mechanisms by which unresolved ER stress causes cell death are still not fully understood. Recent studies suggest that multiple pathways can contribute to ER-stress-induced apoptosis [44]. IRE1 can activate the ASK1/JNK mitogen-activated protein kinase pathway [45] or p53 [44], resulting in commitment of the cell to apoptosis via the classic mitochondria-initiated apoptotic pathway. Recently, proapoptotic BCL-2 family proteins, BAX and BAK, have been found to interact with IRE1 α directly on ER [46]. Interestingly, proper induction of the IRE1 branch of the UPR requires BAX and BAK, but not their proapoptotic function, which resides in a separate protein domain. Further research is needed to determine if the ER-localized BAX and BAK are also involved in apoptosis. ER stress may also cause apoptosis via non-mitochondrial pathways, as shown by the resistance of cells deficient for the ER-localized caspase 12 (*Casp12*) to ER stress-induced apoptosis [47]. Whether this caspase is necessary for death of all cell types during ER stress is unclear, given two recent reports showing that caspase 12 is dispensable under some ER stress conditions [48,49]. Cell death due to long-term ER stress may also involve the induction of oxidative stress. The gene encoding the ER oxidoreductase ERO1 α is a target of the transcription factor CHOP [50^{••}]. ERO1 α participates in protein disulfide bond formation during protein refolding in the ER to help relieve ER stress, but in doing so also transfers electrons to molecular oxygen, forming reactive oxygen species (ROS), which are toxic to the cell and may lead to apoptosis.

The UPR pathway is intricately connected to other stress response pathways through shared components. In response to different cellular stresses including viral infection, heme deficiency in erythroid cells and amino acid starvation, eIF2 α can be phosphorylated by PKR, HRI and GCN2, respectively, in addition to PERK [51]. PERK also mediates phosphorylation of transcription factor NRF2, which translocates into the nucleus to upregulate genes involved in redox maintenance [52].

Moreover, the ASK1/JNK stress-activated kinase pathway can be induced by both ER stress and oxidative stress, leading to apoptosis [53]. In addition, Ca^{2+} released from mitochondria can enter the ER, causing UPR induction [54]. The crosstalk between different cellular stress response pathways may allow versatile adaptation of cells to stress using a limited number of proteins.

Conclusions

ER stress has been implicated in many diseases and a large body of work on regulation of the canonical UPR and on ER-stress-induced cell death has been accumulated. Recent research demonstrates that some novel components of the UPR, or molecules that regulate the canonical UPR, are expressed in a cell-type-specific fashion. These findings suggest that different types of cells may have evolved unique responses for adaptation to ER stress [24,25,55,56]. Most of the studies on ER stress have been conducted in cell cultures with non-physiological ER stress inducers, and hence it may not be correct to extrapolate these results to the *in vivo* situation, where different mechanisms cause ER stress. Recently, a transgenic mouse strain has been established to monitor ER stress *in vivo* [57]. Taking advantage of this mouse and other animal models may yield results more relevant to human diseases. Further studies on the integration of the UPR with other stress responses of the cell, the cellular mechanisms involved in sensing and transduction of various ER insults, and the molecular pathways underlying ER-stress-induced cell death, will also significantly enhance our understanding of ER stress in disease. Lastly, the identification of ER stress inhibitors, such as the recently described inhibitor of eIF2 α dephosphorylation [58], may provide therapeutic interventions in the progression of numerous diseases.

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