Critical Review

Role of the Unfolded Protein Response in Organ Physiology: Lessons from Mouse Models

Víctor Hugo Cornejo[1,2][†]
Philippe Pihán[1,2][†]
René Luis Vidal[3]
Claudio Hetz[1,2,3,4]*

[1]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile
[2]Center for Molecular Studies of the Cell, Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile
[3]Neurounion Biomedical Foundation, Santiago, Chile
[4]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA

Abstract

The endoplasmic reticulum (ER) is a key subcellular compartment involved in the folding and maturation of around one-third of the total proteome. Accumulation of misfolded proteins in the ER lumen engages a signal transduction pathway known as unfolded protein response (UPR) that feedback to recover ER homeostasis or to trigger apoptosis of irreversible damaged cells. The UPR is initiated by three main stress sensors including protein kinase RNA-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring protein 1α (IRE1α), which reprogram the genome through the control of downstream transcription factors. In this article, the authors have reviewed most relevant studies uncovering the physiological function of the UPR in different organs and tissues based on the phenotypes observed after genetic manipulation of the pathway *in vivo*. Biomedical applications of targeting the UPR on a disease context are also discussed. © 2013 IUBMB Life, 65(12):962–975, 2013.

Introduction

The endoplasmic reticulum (ER) is a fundamental subcellular compartment involved in protein synthesis, folding, and maturation. The ER also plays a central role in metabolic processes, including gluconeogenesis and lipid biosynthesis (1). The maintenance of ER protein homeostasis (referred to as proteostasis) is a highly complex process because it involves the dynamic coordination of a large network of chaperones, foldases, and cofactors, in addition to specific environmental and energetic requirements. Perturbation of the protein folding process at the ER causes the accumulation of misfolded or unfolded proteins on its lumen, a phenomenon known as "ER stress." In response to ER stress, cells engage an adaptive signal transduction pathway termed the unfolded protein response (UPR; ref. 2). In general terms, the UPR reprograms gene expression to adjust proteostasis or to induce apoptosis of irreversible damaged cells. UPR signaling events are key to buffer fluctuation in the efficiency of the protein folding process. Thus, the UPR mediates several molecular responses toward increasing ER chaperon expression, enhance quality control mechanisms, and the degradation of abnormally folded proteins, in addition to attenuate protein translation and regulate mRNA stability (2).

TABLE 1

Consequences of the genetic manipulation of UPR components in vivo

Member	Animal model	Phenotype	References
IRE1α	Full knockout	Embryonic lethality after 12.5–13 days of gestation. Impaired development of blood vessels of the placenta caused by reduced VEGF-A secretion.	(26,27)
	Conditional embryo-specific knockout	Mild hypoinsulinemia, hyperglycemia, and a low-weight trend. Abnormal histological structure of the acinar pancreas and salivary serous tissues. Normal liver development.	(28)
	IRE1α/RAG2−/− chimeric mouse	Defects in early and late stages of B-cell lymphopoiesis. Compromised B-cell immunoglobulin gene rearrangement and production of B-cell receptor.	(26)
IRE1β	Full knockout	Increased susceptibility to colitis and higher mortality when challenged with dextran sodium sulfate.	(29–32)
		Hyperlipidemia under high-fat diet due to increased circulating chylomicron.	
		Abnormal ER mucin accumulation leading to reduced mucin secretion in bronchial epithelia and goblet cells.	
XBP1	Full knockout	Hypoplastic fetal liver, reduced hematopoiesis, and embryonic death from anemia. Impaired hepatocyte development.	(33)
	XBP1/RAG2−/− chimeric mouse	Defective B-cell differentiation. Reduced immunoglobulin secretion and unresponsiveness to in vivo antigen and virus challenges. Decreased plasma cells in the spleen and bone marrow.	(34–36)
		Decreased number and reduced survival of conventional and plasmacytoid dendritic cells.	
	Adult liver-specific knockout	No gross liver abnormalities. Reduced plasma levels of cholesterol and triglycerides due to decreased hepatic lipid synthesis and secretion.	(37)
	Conditional β-cell-specific knockout	Mild hyperglycemia and glucose intolerance due to impaired proinsulin processing and reduced insulin secretion. Activation of IRE1α RNase domain leading to proinsulin processing.	(38)
	Conditional α-cell-specific knockout	Altered insulin signaling and glucagon secretion. Glucose intolerance and mild insulin resistance. Overactivation of IRE1α.	(39)
	Conditional intestinal epithelial cell-specific knockout	Spontaneous enteritis and increased susceptibility to induced colitis. Absence of Paneth cells and fewer Goblet cells in the intestinal epithelium due to increased apoptosis.	(40)
PERK	Full knockout	Hyperglycemia and impaired insulin secretion. Increased loss of β-cells leading to diabetes mellitus. Exocrine pancreas insufficiency.	(41–43)
		Growth retardation and skeletal dysplasia characterized by deficient bone mineralization, osteoporosis, and abnormal bone development.	
		Reduced secretion of hepatic IGF-I.	

TABLE 1 *(Continued)*

Member	Animal model	Phenotype	References
	Adult knockout	Acute diabetes phenotype. Hyperglycemia, ER proinsulin accumulation, and increased β-cell apoptosis.	(44)
	Conditional forebrain-specific knockout	Deficits in information processing and enhanced vertical activity. Enhanced perseverative behaviors and reduced behavioral flexibility.	(45)
eIF2α	Homozygous non-phosphorylable knock-in (S51A)	Lethal within 24 hours of birth. Severe hypoglycemia due to impaired liver gluconeogenesis.	(46)
		Disrupted β-cell function and decreased insulin mRNA and protein at birth.	
	Heterozygous non-phosphorylable knock-in (S51A)	Increased weigh gain, glucose intolerance, and impaired insulin secretion under a high-fat diet. β-cell failure.	(47)
	Conditional β-cell-specific knockout	Decrease insulin secretion due to misfolding and mislocalization of proinsulin. Mice develop type II diabetes.	(48)
ATF4	Full knockout	Defective eye lens development leading to microphthalmia in adults.	(20,49–52)
		Severe anemia due to impaired fetal-liver hematopoiesis. Reduced number of hematopoietic progenitors.	
		Resistance to death by oxidative stress in neurons. Smaller infarct area and enhanced recovery after ischemic stroke.	
		Dwarfisms and several skeletal abnormalities, including delayed ossification, osteopenia, and impaired osteoblast and chondrocyte proliferation and differentiation.	
ATF6α	Full knockout	Reduced resistance to acute pharmacologically induced ER stress: liver failure.	(24,53–57)
		Hypoglycemia, insulin resistance, and liver steatosis in response to pharmacologically induced ER stress.	
		β-cell failure, increased glucose intolerance, and impaired insulin secretion in mice fed a high fat diet.	
		Compromised muscle recovery after acute exercise and increased intolerance to exercise.	
ATF6β	Full knockout	No obvious phenotype.[a]	(24)
OASIS	Full knockout	Growth retardation and severe osteopenia. Reduced mineralization and healing after fracture.	(58,59)
		Reduced number or astrocytes in the cortex due to delayed astrocyte differentiation.	
		Compromised differentiation of goblet cells in the large intestine.	
BB2F2H7	Full knockout	Severe condrodysplasia. Mice die shortly after birth by suffocation due to incomplete chest cavity formation.	(59)
LUMAN	Not reported		

TABLE 1 *(Continued)*

Member	Animal model	Phenotype	References
CREBH	Liver-specific knockdown	Reduction in blood glucose levels due to decrease in hepatic gluconeogenesis.	(60)
	Full-mouse knockdown	Reduced C-reactive protein and serum amyloid p-component in the liver at E14.5. No gross developmental or morphological defects.	(61)

[a] ATF6α and ATF6β double knockout is embryonic lethal.

The UPR field is growing exponentially specially in the biomedical area based on the demonstrated role of ER stress in diverse human pathologies, including cancer, inflammatory conditions, neurodegeneration, and diabetes (3). However, most mechanistic studies have been carried out in cell culture systems exposed to artificial pharmacological agents that cause ER stress, and the significance of physiological ER stress has only started to be elucidated in the last years. Accumulating studies in animal models have revealed fascinating and specialized functions of the different signaling branches of the UPR in distinct organs. In this review, we focus in discussing and comparing most relevant studies characterizing the phenotypes of targeting the UPR *in vivo* and the consequences in organ and/or tissue-specific functions.

The UPR Signaling Network

Several cellular perturbations can alter ER proteostasis, triggering ER stress. These alterations can include, for example, a drastic depletion of ER calcium, altered ER-to-Golgi vesicular trafficking, impaired ER-associated degradation (ERAD), altered chaperone function, and local oxidative stress (4). The UPR orchestrates adaptation to protein folding stress by modulating at least three parallel signaling pathways initiated by the activation of the stress sensors inositol-required enzyme 1 (IRE1, α and β), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6, α and β). The IRE1α signaling branch is the most conserved arm of the UPR and the only one present in yeasts. IRE1α is a kinase and endoribonuclease that on activation catalyzes the processing of the mRNA encoding the transcription factor X box-binding protein 1 (XBP1), removing a 26-basepair intron (5–8). This nonconventional splicing event changes the coding reading frame of the mRNA, resulting in the expression-spliced XBP1 (XBP1s). XBP1s is a potent and stable transcription factor that regulates a subset of UPR targets involved in folding, quality control, and lipid synthesis (8,9). IRE1α also activates other stress signaling events through the binding of adapter proteins such as tumor necrosis factor receptor-associated factor 2 (TRAF2), triggering the activation of c-Jun N-terminal kinase

(JNK) and apoptosis signal kinase-1 (ASK1), which have been implicated in processes such as autophagy and apoptosis (2,10,11). Besides, IRE1α can degrade a subset of mRNAs and miRNAs through its RNAse domain in a process termed regulated IRE1α-dependent decay (RIDD), which can contribute to both adaptations to stress by decreasing protein expression or the induction of apoptosis (12–15).

On activation, PERK dimerizes and autotransphosphorylates leading to phosphorylation of the eukaryotic translation initiator factor 2α (eIF2α), a molecular event that arrests protein synthesis and thus contributes to alleviate the overload of misfolded proteins inside the ER (16,17). In addition, phosphorylation of eIF2α allows the specific and selective translation of the mRNA encoding activating transcription factor 4 (ATF4). ATF4 is a key transcription factor involved in the regulation of genes related to redox balance, amino acid metabolism, protein folding, autophagy, and apoptosis (18–20). Many different mediators of ER stress-induced cell death have been described that depend on PERK signaling. One of the most characterized proteins is C/EBP-homologous protein (CHOP/GADD134), which is controlled by ATF4 (21). In addition, expression of GADD34 could contribute to cell death due to a reestablishment of protein synthesis through eIF2α dephosphorylation and the enhancement of ROS production (22,23). Finally, under stress conditions, ATF6 translocates to the Golgi apparatus, where it is proteolytically processed by site-1 and site-2 proteases. This processing event releases an active cytosolic fragment (ATF6f) that operates as a basic leucine zipper (bZIP) transcription factor. ATF6f regulates a subset of UPR target genes involved in ERAD and also modulates XBP1 mRNA levels (24). ATF6f and XBP1s may also synergize in the control of a specific pattern of gene expression by the formation of heterodimers (25). Thus, the UPR represents a global homeostatic feedback network that integrates information about the intensity and duration of the stress stimuli toward recovering of proteostasis or inducing cell death of damaged cells.

The biological relevance of proximal UPR signaling modules *in vivo* have gained much insight in the last 5 years because of the discovery of unexpected novel physiological functions (Table 1). Overall, most studies illustrate a fundamental role of the UPR in maintaining the function of

specialized secretory cell function where the high demand of protein folding and secretion constitutes an endogenous source of ER stress. Interestingly, many recent studies also suggest unexpected functions of the UPR in the control of energy metabolism and lipid and cholesterol synthesis, in addition to behavior/cognition and innate immunity. In the next sections, we review important aspects of physiological activities of the UPR *in vivo* assessed using mouse models to target most relevant components of the pathway.

PERK

PERK is one of the most studied UPR sensor *in vivo*, possibly because the knockout animals for PERK were viable and were generated early in 2000. The phenotypes associated with PERK loss of functions involve strong changes in glucose metabolism and pancreatic β-cell survival. In addition to modulate the levels of insulin in pancreas and blood, the production of most digestive enzymes by exocrine pancreas was also altered (41,42,44). Because of these drastic phenotypes, PERK knockout newborn animals gain weight very slowly and develop early diabetes and premature death. Similarly, acute PERK deletion using a tamoxifen-induced system in the pancreas triggered spontaneous diabetes mellitus (44). So far, multiple studies indicate that PERK operates as a physiological sensor to adjust ER folding function of specific secretory tissues that are essential for early development (Fig. 1; examples in refs. 41,42,44,62, and 63). This modulation of the secretory capacity of a developing tissue through PERK may occur through controlling differentiation, increasing proliferation, and/or maturation of the secretory apparatus of β-cells and other cell types (43,64).

A key function of PERK is the regulation of mRNA translation through eIF2α phosphorylation. Under physiological conditions, PERK controls the capacity of the organelle to fold proteins by limiting the translocation of newly synthesized polypeptides into the lumen of ER lumen (1,2). PERK knockout animals display a number of severe defects in addition to pancreatic dysfunction, including growth retardation, multiple skeletal dysplasias, severe spinal curvature, and reduced locomotor activity (41,42). Most these phenotypes are associated with a primary dysfunction of specialized secretory cells.

As mentioned previously, PERK knockout mice develop hyperglycemia and reduced serum insulin levels (41,44). However, the exocrine and endocrine pancreas develop normally in this animal model, suggesting that altered differentiation of pancreatic cells is not the principal cause for the phenotypes observed. In addition, experimental data indicate that the loss of insulin-positive cells in PERK-deficient animals is not due to the depletion of insulin stores but rather due to the progressive loss of cells capable of synthesizing the hormone (41). Cells of the exocrine and endocrine pancreas of PERK mutant mice had characteristic abnormal ER luminal distension and accumulation of protein inclusions inside this compartment (41).

The most striking feature of the affected tissues in PERK knockout animals is the occurrence of extensive apoptosis of
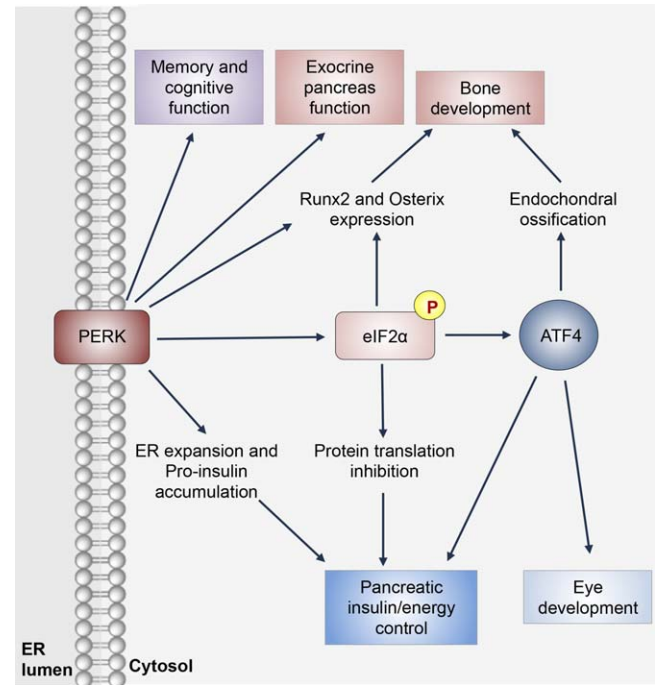

Fig 1 The function of PERK signaling in diverse organs. The figure summarizes the phenotypes described in animals where PERK-mediated events were targeted with genetic manipulation. The PERK pathway regulates metabolie processes related with pancreatic function, development of the skeleton, and memory and cognitive functions. In addition, this signaling branch may modulates eye development through ATF4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

endocrine and exocrine pancreatic cells (41,42,44), which is preceded by an abnormal accumulation of immature insulin and increased β-cell proliferation (43,44). In addition, global markers of ER stress are upregulated in PERK-deficient animals, suggesting a general disturbance of ER proteostasis (41,43,44). These studies uncovered the function of PERK in islet function, insulin production, and its role in maintaining quiescence in the fasted state by controlling protein synthesis and maturation (42).

PERK signaling also plays an important role in the differentiation of osteoblasts probably because these cells secrete abundant extracellular matrix components. PERK knockout mice exhibit severe osteopenia that is manifested as decreased cortical bone thickness and mineral apposition rate and this is correlated with reduced trabecular bone volume and thickness (42,64,65). Analysis of gene expression patterns during the development of extracellular matrix indicated that PERK regulates bone development through controlling differentiation and proliferation programs in osteoblasts and the secretion of type I collagen. In addition, key genes related to matrix deposition, such as osteocalcin, are regulated by PERK (64,65).

Although sustained activation of PERK has been classically associated to the engagement of apoptosis (66), there is an

interesting recent study indicating that the artificial activation of PERK in oligodendrocytes can provide survival signals in an experimental model of multiple sclerosis, reducing demyelination and axonal degeneration (67). These data suggest that PERK could have divergent roles in neural tissues. Besides, in a recent study, the role of PERK in cognitive function has been examined. Disruption of PERK in the adult mouse forebrain showed a decrease in eIF2α phosphorylation and ATF4 expression and was associated with altered behavior in several tests (45). Remarkably, a recent report indicated that ablation of *Perk* improves learning and memory of an experimental model of Alzheimer's disease (68). These reports uncovered an important function of PERK in cognitive functions of the nervous system. In summary, accumulating evidence indicates a fundamental role of PERK in adjusting protein synthesis in diverse tissues, having pleiotropic activities in organ homeostasis.

eIF2α Phosphorylation in Serine 51

eIF2α is part of a major machinery implicated in the initiation of protein translation in all eukaryotic cells. eIF2α phosphorylation at serine 51 inhibits the formation of the translation initiation ternary complex and causes attenuation of global protein synthesis (69). In addition to PERK, three alternative kinases can phosphorylate eIF2α at serine 51, including the general control nonrepressed 2 (GCN2) kinase (activated by amino acid starvation conditions), the double-stranded RNA-dependent (PKR) kinase (activated by viral infections), and the heme-regulated inhibitor (HRI) kinase (responds to iron deficiency; ref. 69).

Knock-in mouse models have been developed to substitute serine 51 for alanine, creating a nonphosphorylable form of eIF2α. Homozygous eIF2α^S51A mutant mice are born at the expected Mendelian ratios and show no obvious developmental differences, but die within 24 h after birth (46). This phenotype is caused in part by the occurrence of severe hypoglycemia owned to impaired postnatal gluconeogenesis and decrease glycogen synthesis and storage in the liver, both essential processes for postnatal survival (46). At birth, eIF2α mutant mice also show signs of disrupted β-cell function, displaying decreased levels of insulin mRNA and protein when compared with wild-type animals (46). Interestingly, this phenotype is less severe in PERK knockout mice, which show a decrease in insulin levels only several weeks after birth (42), suggesting possible compensatory effects mediated by other eIF2α kinases, such as PKR and GCN2.

Despite displaying reduced levels of eIF2α phosphorylation, eIF2α heterozygous mutant mice develop normally into adulthood. Nevertheless, when fed with a high-fat diet, eIF2α mutant heterozygous mice gain more weight than their wild-type counterparts, developing glucose intolerance and impaired insulin secretion (47). The reduced insulin secretion observed in eIF2α^S51A heterozygous animals is caused by β-cell failure, as evidenced by a distended ER and diminished number of insulin granules. Moreover, it was shown that proinsulin

is retained in the ER through its association with the ER chaperone BiP, suggesting an altered folding process (47).

To determine the specific function of eIF2α in the liver and the pancreas, tissue-specific genetic ablation of this factor has been carried out. Overexpression of GADD34 in the liver, a component of the eIF2α phosphatase complex, causes eIF2α dephosphorylation and results in hypoglycemia and reduced hepatic glycogen content (70). In addition, hepatic expression of GADD34 c-terminal domain also resulted in reduced body weight gain, improved glucose tolerance and insulin sensitivity, and decreased liver steatosis in adult mice fed a high-fat diet. This phenomenon was proposed to be due to a direct regulation of genes involved in fatty acid synthesis and lipid metabolism (70). Conditional deletion of eIF2α in pancreatic β-cells of adult mice also results in hyperglycemia and increased glucose intolerance due to reduced islet mass (48). β-cell-specific deletion of eIF2α led to decreased secretion of insulin due to higher proinsulin translation, mislocalization of secretory and plasma membrane proteins, reduced expression of ER stress-response genes, and increased oxidative stress (48). All these events were correlated with β-cells apoptosis and a diabetic phenotype.

Taken together, at this point, the phenotypes uncovered on targeting PERK expression or eIF2α phosphorylation are fairly similar, indicating that this pathway acts in concert to control of energy metabolism, having a fundamental role in sustaining β-cell survival and function, and therefore glucose homeostasis (Fig. 1).

ATF4

ATF4 is a member of the cAMP-responsive element-binding protein (CREB) family of bZIP-containing proteins and has broad roles in developmental pathways, cell differentiation, and organ morphogenesis (49,50,71). ATF4 heterozygous animals are viable and phenotypically normal, whereas only 30% of ATF4 knockout animals survive until adulthood (51). ATF4 deficiency leads to hematologic defects (52) and drastic alterations in eye-lens formation and postnatal hair growth, in addition to pancreatic hypotrophy and growth retardation (43,49–51,72). The severe microphthalmia observed in these animals is due to altered eye lens development, involving a specific perturbation in the survival of the anterior epithelial cells of the lens that give rise to secondary lens fibers (Fig. 1; refs. 50 and 51).

Although the etiology of the partial perinatal lethality triggered by ATF4 deficiency is not yet fully elucidated, several observations point to systemic and metabolic problems associated with poor viability of embryos. ATF4 knockout mice are severely anemic during fetal development, apparently because of insufficient hematopoiesis. It may be feasible that a defect in cell proliferation is involved in these phenotypes, because fetal liver, embryonic lens, and hair follicle are all sites of rapid proliferation (52). Interestingly, deletion of p53 reduces some of the developmental problems observed in ATF4-deficient eye lens (51). ATF4 mutant mice are less susceptible

to diet-induced and age-dependent obesity and diabetes (72). Furthermore, tissues from these animals contain lower amino acid levels. Overall, extensive reports support the concept that ATF4 regulates several aspects of mammalian metabolism, including fat storage, energy expenditure, and glycemic control (73).

Additionally, ablation of ATF4 in mice leads to severe skeletal defects, including delayed ossification and low bone mass, short stature and short limbs (49). ATF4 is expressed in proliferative and prehypertrophic growth plate chondrocytes, suggesting an autonomous function of ATF4 in chondrocytes during endochondral ossification (49). Using osteoblast-specific ATF4-deficient mice, it was demonstrated that this transcription factor regulates terminal differentiation of osteoblasts and many processes related to the control of bone mass (71). ATF4 favors the expression of key genes that regulate the bioactivity of osteocalcin in osteoblasts (71). In addition, ATF4 induces the expression of the trophic factor Indian Hedgehog in chondrocytes, which is essential for skeletal development (49).

The functional role of ATF4 in neural tissue has also been addressed and, surprisingly, it is mostly associated with antioxidant responses rather than control of protein folding-related genes. We reported that ATF4 deletion reduces locomotor recovery after experimental spinal cord injury (SCI; ref. 74). In this pathological context, the effects of ATF4 inactivation are associated with a significant increase in the number of damaged neurons and reduced amount of oligodendrocytes surrounding the injured neurons. In addition, altered microglial activation and proinflammatory cytokine expression were observed in ATF4-deficient mice (74). ATF4 has also been linked to apoptosis in response to oxidative stress in neurons. Gene expression profile analysis revealed that ATF4 regulates a subset of genes that are induced in response to oxidative stress and controls antioxidant response (20). The oxidative stress condition induced by ATF4 deficiency is mediated in part by depletion of the major antioxidant glutathione (20). In this context, ATF4-deficient mice are less susceptible to ischemic brain damage (20), amyotrophic lateral sclerosis (ALS; ref. 75), experience significantly smaller infarcts, and have improved behavioral recovery in models of stroke (20). Thus, these selected studies support the concept of a pleiotropic function of ATF4 in diverse tissues possibly due to its function in modulating cell cycle, metabolism, the redox balance, apoptosis, and protein folding.

CHOP

CHOP is a well-described downstream target of the PERK signaling branch. Ablation of *Chop* in mice does not affect animal survival and do not generate evident developmental defects. CHOP has been extensively studied in the control of apoptosis because it regulates the expression of many cell death regulators under ER stress (22). CHOP enhances the expression of GADD34, which reestablishes protein synthesis on a terminally stressed cell, increasing ROS production and apoptosis. The literature using CHOP-deficient animals in the context of disease

models is very large, and here we highlight only some studies for discussion.

CHOP knockout mice show decreased lung apoptosis in response to intraperitoneal injection of lipopolysaccharide (76). Similarly, these animals are more resistant to apoptosis in the kidney induced by the exposure of animals to the ER stress agent tunicamycin. Interestingly, sublethal intraperitoneal injection of tunicamycin results in severe renal insufficiency (21). CHOP has also been described in artherosclerotic syndrome where targeting CHOP substantially diminishes plaque necrosis and apoptosis (77).

A recent study suggested that ER stress might be involved in hepatocyte injury caused by cholestasis and ethanol consumption (78,79). Acute liver injury and liver fibrosis were assessed in CHOP-deficient mice following bile duct ligation, an experimental model of cholestasis. Liver fibrosis and cell death were attenuated in CHOP knockout mice (78). In addition, ablation of *Chop* triggers remarkable inhibition of hepatocellular apoptosis in response to alcohol feeding but no protection against hyperhomocysteinemia or fatty liver (79).

Although obesity and insulin resistance are associated with ER stress and with the activation of the UPR in adipose tissue and pancreas, respectively, it is not well understood how UPR signaling affects this process. Several studies have been performed to explore the role of CHOP in adipocyte differentiation and function *in vivo* in mice. In a physiological context, CHOP deficiency leads to significantly greater body weight and adiposity than wild-type mice when subjected to a high-fat diet (80). However, in this experimental setting, caloric intake, thermogenesis, glucose tolerance, and insulin sensitivity did not differ between genotypes (80–82). In addition, CHOP deletion in genetically obese mice increased body fat mass, without altering adipocyte size (81). This evidence demonstrated that CHOP suppresses adipogenesis and limits expansion of fat mass *in vivo* in mice, without causing a severe metabolic disorder (82). In a mouse model of type II diabetes, *Chop* deletion increases the capacity of islets to produce insulin (83) and limits the progression of insulin resistance to diabetes, preventing β-cell apoptosis possibly due to attenuated oxidative stress (84,85).

CHOP has also been the focus of attention in pathological conditions affecting the nervous system. For example, pharmacologically induced cerebral ER stress, elicited by intracerebroventricular injection of tunicamycin, causes extensive cell death in hippocampal neurons. Surprisingly, CHOP knockout mice showed enhanced hippocampal cell apoptosis and reduced performance in memory-related behavioral tests (86). In the context of brain ischemia, CHOP deficiency provides significant neuroprotection (87), and also protects against SCI (88) and experimental Parkinson's disease (89). Finally, genetic model of demyelination in Schwann cells was tested, where mutation in myelin protein zero fails to be incorporated into myelin and the protein is retained in the ER. Surprisingly CHOP deficiency in this model restores motor function and reduces active demyelination (90). This study concluded that

the UPR has a pathogenic role in demyelinating peripheral nerves and that signaling through the PERK/eIF2α/CHOP axis of the UPR provokes demyelination before induction of cell death.

Taken together, all these studies suggest that at least in neural models, the function of CHOP may depend on the disease context, where it could play either a protective role (adaptive UPR) or it may enhance apoptosis of irreversible damaged cell.

ATF6 Family

ATF6 is also a bZIP transcription factor of the cyclic AMP-responsive element (CRE)-binding protein ATF/CREB family. ATF6 exist in two isoforms, ATF6α and β, both of which are ubiquitously expressed and localized to the ER membrane under resting conditions. On ER stress, ATF6f translocates to the nucleus where it directly regulates genes involved in ER homeostasis such as ER chaperones and ERAD components, in addition to XBP1 mRNA levels (24). Neither ATF6α nor ATF6β is essential for embryonic development as both ATF6α- and ATF6β-deficient mice are born at the expected Mendelian ratios. These knockout animals do not show evident growth defects and develop normally into adulthood (24,53). However, these animals are hypersensitive to experimental ER stress as revealed by the unexpected lethality observed after the intraperitoneal injection of tunicamycin associated with the generation of acute liver and kidney damage (24,53). Importantly, ATF6α/β double deficiency results in full embryonic lethality, suggesting that ATF6α and ATF6β have functional redundancy, with essential functions during mouse development (Fig. 2; ref. 24).

ATF6α plays an important role in glucose and lipid homeostasis under both pharmacologically induced and physiological ER stress. For example, unresolved ER stress in ATF6α knockout animals due to intraperitoneal tunicamycin injection fallout in insulin resistance and hypoglycemia (54). In addition, increased levels of hepatic triacylglycerol, fat deposits, and accumulation of lipid droplets are observed in ATF6α-deficient mice undergoing experimental ER stress, a phenotype indicative of liver microvesicular steatosis (54,55). This abnormal hepatic phenotype is possibly caused by defects in fatty acid oxidation and lipoprotein secretion due to ER stress-dependent suppression of relevant metabolic transcription factors, such as C/ebpα and Pparα (54,55). In agreement with this concept, genetic ablation of any of the three UPR branches leads to the same phenotype, indicating that persistent ER stress suppresses metabolic transcription factors (54). On a more physiological setting, one study indicated that ATF6α knockout mice fed with a high-fat diet display decreased glucose tolerance but increased insulin sensitivity. These animals showed reduced insulin secretion probably caused by impaired β-cell function (56). What are the molecular mechanisms by which ATF6α regulates glucose and lipid metabolism? After exposure of mice to a high-fat diet, it has been shown that ATF6α interacts with the gluconeogenesis stimulator CREB-regulated transcription activator 2 (CRTC2), promoting the expression of ER
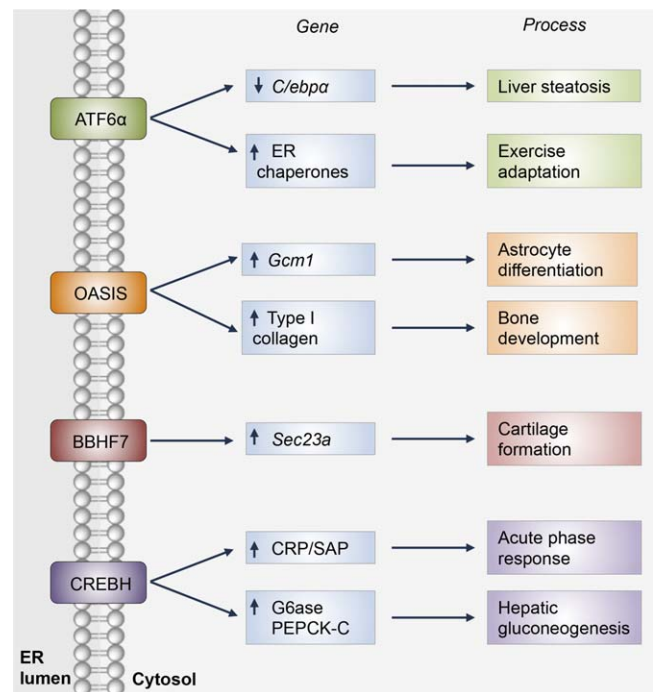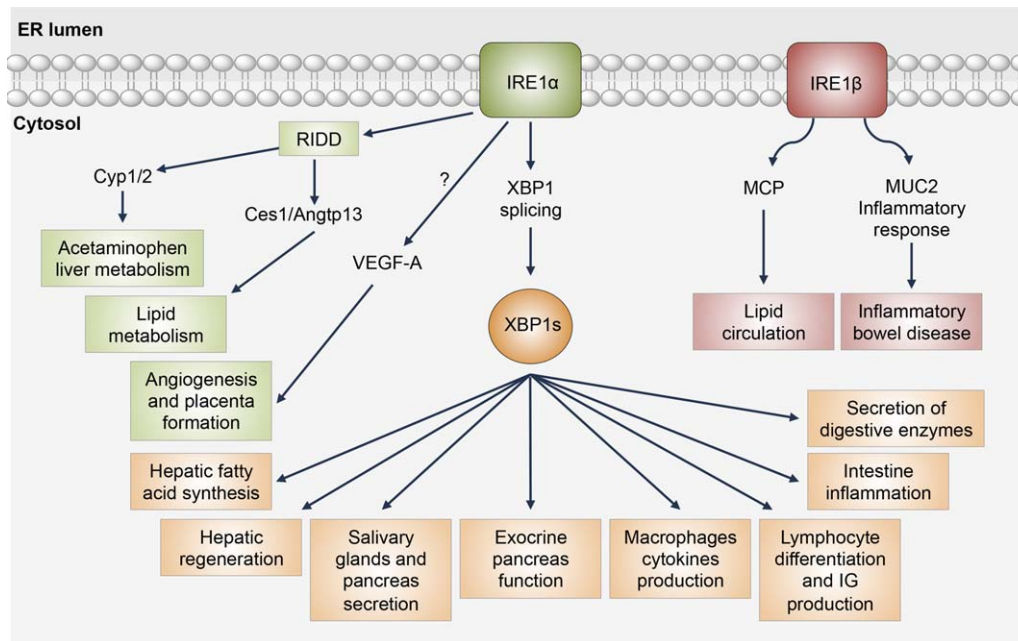

Fig 2 Physiological impact of the ATF6 family of proteins. ATF6 regulates liver fatty acid processing and prevent hepatic steatosis and also acts over muscle during exercise adaptation. OASIS controls astrocyte differentiation and bone development, similarly to BBF2H7. CREBH modulates the immunological acute phase response and hepatic gluconeogenesis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

stress-related genes. Simultaneously, ATF6α-CRTC2 interaction inhibits CRTC2 association with CREB, disrupting CRTC2 occupancy over gluconeogenic genes, thus reducing hepatic glucose output (91).

The role of ATF6α in skeletal muscle recovery after acute exercise and in adaptation to persistent exercise was also uncovered. Transient activation of the UPR following exercise, mediated by the transcriptional coactivator peroxisome proliferators—activator receptor gamma coactivator 1α (PGC1α) and ATF6α, is necessary for muscle adaptation (57). Accordingly, ATF6α knockout mice do not recover from muscle damage after persistent exercise, correlating with altered expression of proinflammatory genes and the occurrence of global ER stress (57).

ATF6 forms part of a family of bZIP transcription factors, including old astrocyte-specifically induced substance (OASIS), LUMAN (also known as CREB3), BBF2 human homolog on chromosome 7 (BBF2H7; also known as CREB3L2), cyclic AMP-responsive element-binding protein hepatocyte (CREBH; also known as CREB3L3), and CREB4 (also known as CREB3L4; reviewed in ref. 92). These transcription factors share overall structural similarities with ATF6, and they are localized to the ER membrane and are regulated by

Biological impact of IRE1 signaling. The IRE1α pathway regulates physiological process involved in metabolism in the liver through RIDD activity. XBP1 also controls an extensive variety of processes in diverse tissues that include hepatic and pancreatic secretion, production of cytokines in inflammatory responses, and differentiation of lymphocytes and other cell types. IRE1β pathway regulates lipid metabolism in epithelial cells and inflammatory process in the intestine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

proteolysis. In many cases, these ATF6-related proteins are expressed in a tissue-specific manner (Fig. 2; ref. 92).

OASIS was first identified as a protein specifically induced in long-term cultured astrocytes (93). OASIS is also highly expressed in bone tissue, intestine, salivary glands, and prostate. Ablation of OASIS expression in mice leads to growth retardation and severe osteopenia due to impaired osteoblast type I collagen and matrix protein secretion (58). Osteoblast differentiation is a process accompanied by mild ER stress, resulting in cleavage and activation of OASIS (OASISp50). OASISp50 directly binds to an UPR-like sequence in the *Col1a1* gene promoter, increasing type I collagen expression and secretion (58). In contrast, although ATF6α is also activated in response to ER stress during osteoblast differentiation, it does not activate the transcription of *Col1a1* gene, and ATF6α knockout mice display no apparent defects in bone tissue development (58). A role of OASIS in astrocyte differentiation and maturation was also determined. OASIS deficiency results in reduced number of astrocytes and increased number of neurons in the cerebral cortex during embryonic development (59).

BBF2H7 is highly expressed in chondrocytes and is essential for cartilage development (59). Targeting BBF2H7 triggers severe chondrodysplasia, and the animals die by suffocation shortly after birth due to incomplete chest cavity formation. They also show reduced cartilage-specific matrix proteins, such as type II collagen and cartilage oligomeric matrix protein (59). CREBH was first identified as a liver-specific transcription factor induced during fetal liver development and by

systemic inflammatory reactions (61,94). CREBH knockdown mice showed no developmental defects; however, it presented a decreased expression of genes involved in the inflammatory acute phase response (61). Interestingly, the overexpression of the CREBH active form (CREBH-N) promotes hepatic gluconeogenesis through the upregulation of gluconeogenesis genes (60). Thus, in contrast to ATF6α, which functions as a gluconeogenic repressor through its interaction with CRTC2, CREBH operates as a gluconeogenic activator. This is a clear example of two related ER stress sensors having divergent functions in the same tissue. Finally, CREBH knockout mice show a slight decrease in hepatic lipid content, in addition to an increase in plasma triacylglycerides (95). CREBH directly regulates genes involved in lipid synthesis and metabolism (95). LUMAN and CREB4 are expressed in most tissues; however, no genetic manipulation studies *in vivo* have yet been conducted.

XBP1

XBP1 is probably the most studied UPR transcription factor. In adult tissue, unspliced *Xbp1* mRNA is ubiquitously expressed. Mice lacking XBP1 display hypoplastic fetal livers, with reduced hematopoiesis resulting in early death during development due to anemia (33). Postnatal XBP1 liver-specific knockout mice do not show any noticeable spontaneous abnormalities, and they have normal body weight and liver mass and present no evidence of liver damage (37). However, XBP1 liver deficiency results in a profound compromise of *de novo*

hepatic lipid synthesis, without causing hepatic steatosis (Fig. 3; ref. 37).

To rescue the deficiency of XBP1 in the liver, XBP1 expression was specifically restored in this tissue using a transgenic mouse model (96). Remarkably, this strategy fully bypasses the embryonic lethality observed in XBP1 knockout animals. However, animals lacking XBP1 in all organs except the liver died shortly after birth from a severe impairment in the production of pancreatic digestive enzymes leading to hypoglycemia and death. Expansion of the ER and the expression of certain ER chaperones were severely impaired in pancreatic exocrine cells and, to a lesser degree, in salivary gland acinar (96). This phenotype is likely due to the failure to handle the ER stress caused by increasing zymogen production during development (96). XBP1 conditional deletion in pancreatic β-cells caused hyperglycemia, glucose intolerance, and markedly decreased number of insulin granules, in addition to impaired proinsulin processing (38,39).

XBP1 was discovered by Laurie Glimcher's laboratory almost 20 years ago from the immunology field (97). Professional secretory immune cells lacking XBP1 display severe abnormalities in their development and function as initially reported in plasma B-cells (34,98,99), and dendritic cells (35). To address the function of XBP1 in lymphocytes, the RAG-2 complementation system was initially used to analyze XBP1-deficient lymphocytes from adult chimaeric animals. These lymphoid chimaeras displayed a severe defect in the generation of plasma cells and therefore reduced immunoglobulin levels in plasma (36). Additional studies then demonstrated that XBP1 is important for B-cell differentiation before the engagement of a highly secretory demand through signaling of the B-cell receptor (100). Although XBP1 regulates plasma cell differentiation, it did not impact the generation of memory B-cells (101). Remarkably, XBP1 deletion in intestinal epithelial cells results in spontaneous enteritis and increased susceptibility to colitis associated with a chronic inflammatory response (40). This is consistent with the discovery of polymorphisms in the *XBP1* gene in patients affected with inflammatory Crohn's disease (102). XBP1 has also been placed downstream of Toll-like receptors, where it modulates the production of proinflammatory cytokines in macrophages (103). Infection of XBP1 MxCre conditional knockout mice with the pathogen *F. tularensis* showed reduced ability to clear out the pathogen from tissues (103). Finally, overexpression of XBP1s in myeloid cells triggers spontaneous multiple myeloma (104,105). Consistent with this, analysis of the XBP1s gene regulatory network at a genomic scale revealed a key role of this UPR transcription factor in cell differentiation associated, for example, with the regulation *Mist1*, a master regulator of myoblast cell differentiation (8). In the same way, XBP1 deficiency in gastric epithelial cells led to altered differentiation of digestive enzyme–secreting zymogenic cells possibly due to *Mist1* deregulation (106).

A novel function of XBP1 was also uncovered in the liver in the regulation of normal fatty acid synthesis (37). XBP1 reg-ulates key genes involved in fatty acid synthesis, and targeting XBP1 in the liver triggered hypocholesterolemia and hypotriglyceridemia, secondary to a decreased production of lipids from the liver in the absence of hepatic steatosis (37). Another study also showed that ablation of XBP1 ameliorates hepatosteatosis, liver damage, and hypercholesterolemia in an animal model of dyslipidemia (107).

Studies in neurons have uncovered unexpected results where XBP1s target genes may not be involved to protein folding stress in this specific cell type (108). Conditional deletion of XBP1 in the central nervous system has been performed to test its contribution to neurodegeneration. For example, targeting XBP1 in the brain results in autophagy enhancement and protection against experimental Huntington's disease and ALS (109–111). The opposite effect was reported in a model of SCI observing reduced locomotor recovery (74), whereas in the context of optic nerve degeneration, no effects were detected (112). In contrast, the pathology of a prion disease model was not affected by deletion of XBP1 despite evident ER stress in the model (113). Other studies have shown that XBP1 may also have an important activity in the hypothalamus, where it may control energy metabolism through the body and leptin resistance (114). More studies are needed to address the functional impact of XBP1 in the physiology of the nervous system. In summary, all these studies indicate that XBP1 function is important to sustain the function of a subset of specialized secretory cells and their differentiation and has an important activity in the control of energy, lipid, and cholesterol metabolism.

IRE1α and IRE1β

IRE1α is the most conserved sensor of the UPR across species and probably the best described UPR component at the biochemical level. IRE1α knockout embryos develop drastic defects, leading to embryonic death after 12.5 days of gestation in mice (Fig. 3; ref. 26). The cause of this embryonic lethality is partially understood by studies that argue an extra-embryonic activity of IRE1α that is essential for embryonic survival. Loss of IRE1α led to reduction in vascular endothelial growth factor and severe dysfunction of blood vessels in the placenta (27). Surprisingly, a conditional knockout strategy to target IRE1α but to reconstitute its expression in the placenta rescued the embryonic lethality (27). Unexpectedly, these animals were born and alive without developing fetal liver hypoplasia (27). This rescue allowed exploring the possible function of IRE1α in adult tissue and organs. Unexpectedly, and in contrast to the phenotypes described for XBP1-deficient cells, IRE1α deletion caused only mild abnormalities of exocrine tissues, increased blood glucose level, and slightly decreased serum immunoglobulin levels (28). IRE1α is essential for early lymphopoiesis at the stage of pro-B cells and at a late stage in B-cell lymphopoiesis modulating the terminal differentiation into plasma cells (26). Unexpectedly, in this study, a novel function of IRE1α was proposed in the recombination of immunoglobulin genes (26).

The role of RIDD in cell physiology is starting to be uncovered. Recent studies in XBP1-deficient tissue had revealed that a feedback loop can induce the constitutive activation of IRE1α, enhancing RIDD activity. For example, RIDD mediates the degradation of a subset of mRNA related with drug metabolism and the generation of hepatotoxic metabolites, which enhances the resistant to acetaminophen (115). A similar model was proposed in pancreatic β-cells, in which RIDD was responsible for the regulation of insulin expression (38). In the liver, IRE1α controls lipogenesis and lipoprotein metabolism through the RIDD pathway (107). These few studies highlight the need for the reevaluation of the phenotypes described in XBP1-deficient animals, which could be explained in part by the overactivation of RIDD.

IRE1β is also implicated in sensing and responding to ER stress signaling in specific tissues (Fig. 3). IRE1β is exclusively expressed in epithelial cells of the gastrointestinal tract and bronchial epithelia (29–31). IRE1β-deficient mice develop normally and are indistinguishable from wild-type littermates (29). Mice deficient in IRE1β did not show any spontaneous intestinal inflammation, but when challenged with dextran sodium sulfate, they develop severe colitis, increasing the kinetic of the inflammatory process and higher mortality than control animals (29).

IRE1β is also implicated in chylomicron secretion in response to high-cholesterol and high-fat diet (31). IRE1β mediates the post-transcriptional degradation of microsomal triglyceride transfer protein, a protein involved in the assembly of chylomicron in the ER (31). Moreover, the same idea was tested in a genetic background of artherosclerosis experimental mouse model APOE knockout (116). Double knockout mice for APOE and IRE1β developed hyperlipidemia and have higher atherosclerotic plaques. In conclusion, these studies indicated that IRE1β regulates intestinal lipid absorption and circulating lipids (116). Finally, two independent groups demonstrated that IRE1β is required for mucin production in bronchial epithelia and goblet cells (30,32). IRE1β deficiency led to aberrant mucin accumulation in the ER of goblet cells and this produced a distention of the ER and UPR activation (32). In the context of lung and intestine, it may be interesting to test in the future the impact of the double deficiency of IRE1α and β to assess possible complementary functions in tissue homeostasis.

As we mentioned previously, deletion of *Xbp1* produces the overactivation of IRE1α activity that decrease of some RIDD target genes (115). In this context, overactivation of the IRE1/TRAF2/JNK axis was proposed to contribute to the inflammatory disease observed when XBP1 was targeted in the intestine (40). In some experimental models, including ALS, a chronic ER stress is associated with activation of JNK and ASK1. ASK1 functions as an important molecular effector of apoptosis in many experimental systems in addition to ER stress (117). ASK1-deficient mice are born at the expected Mendelian ratio with normal histological features (118). However, in a pathological context, targeting *Ask1* can provide protection against ALS or Huntington's disease (11,119), two models where ER stress is observed (3). The impact of the IRE1α/JNK pathway in general is poorly studied *in vivo*.

Conclusion

Despite extensive studies addressing the consequences of ER stress in cell culture models, the biological impact of UPR signaling *in vivo* appears to be unclear for many relevant physiological processes. Accumulating evidence based on the genetic manipulation of key UPR components in mice revealed that the pathway has an important role in maintaining protein homeostasis during embryonic development and adult tissues. By comparing the phenotypes of different UPR mouse models, it is becoming clear that the pathway has some degree of overlap in terms of organs and cellular processes controlled by distinct ER stress signaling models. One of the major functions of the UPR is to maintain specialized secretory cell function as revealed in B-lymphocytes, salivary glands, gastric cells, exocrine and endocrine pancreas, and other tissues. Although the initial prediction indicated that activation of the UPR may be a consequence of the occurrence of basal and physiological stress due to a high demand of protein synthesis, it is also becoming clear that the UPR network impacts early differentiation stages before acquiring a specialized secretory phenotype (2,120).

The function of the UPR had expanded in the recent years to several physiological processes. It is very clear that loss of function of key UPR components generates drastic metabolic changes at the physiological level in processes related to energy control and lipid and cholesterol metabolism. However, because of the analysis of tissue-specific knockouts, new features have been uncovered in various organs and tissues. The UPR is now emerging as a relevant signaling network with novel activities in the immune system, regulating cytokine production and the global handling of diverse types of pathogens. Finally, many recent drug screenings and studies in mouse models of disease have shown that the pathway represent a relevant target for disease intervention, with demonstrated roles in diseases such as cancer, neurodegeneration, diabetes, and ischemia (see review in ref. 3). More over, studies using *C. elegans* as a model organism suggests that UPR signals may propagate on a cell-nonautonomous manner through different tissues, having functional consequences in innate immunity and aging-related processes (121,122). In summary, most data discussed here place the ER and the stress signals emanating from this compartment as a central rheostat to integrate and fine-tune diverse homeostatic process, having a fundamental biological function in full organ physiology.

Acknowledgements

References

[1] Ron, D. and Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell Biol. 8, 519–529.

[2] Hetz, C. (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat. Rev. Mol. Cell Biol. 13, 89–102.

[3] Hetz, C., Chevet, E., and Harding, H. P. (2013) Targeting the unfolded protein response in disease. Nat. Rev. Drug Discov. 12, 703–719.

[4] Walter, P. and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334, 1081–1086.

[5] Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., et al. (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 415, 92–96.

[6] Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., et al. (2002) IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev. 16, 452–466.

[7] Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 107, 881–891.

[8] Acosta-Alvear, D., Zhou, Y., Blais, A., Tsikitis, M., Lents, N. H., et al. (2007) XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Mol. Cell 27, 53–66.

[9] Lee, A. H., Iwakoshi, N. N., and Glimcher, L. H. (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol. Cell. Biol. 23, 7448–7459.

[10] Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., et al. (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287, 664–666.

[11] Nishitoh, H., Matsuzawa, A., Tobiume, K., Saegusa, K., Takeda, K., et al. (2002) ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. Genes Dev. 16, 1345–1355.

[12] Hollien, J., Lin, J. H., Li, H., Stevens, N., Walter, P., et al. (2009) Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J. Cell Biol. 186, 323–331.

[13] Han, D., Lerner, A. G., Vande Walle, L., Upton, J. P., Xu, W., et al. (2009) IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 138, 562–575.

[14] Hollien, J. and Weissman, J. S. (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science 313, 104–107.

[15] Upton, J. P., Wang, L., Han, D., Wang, E. S., Huskey, N. E., et al. (2012) IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic caspase-2. Science 338, 818–822.

[16] Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., et al. (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol. Cell 6, 1099–1108.

[17] Harding, H. P., Zhang, Y., and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397, 271–274.

[18] Ameri, K. and Harris, A. L. (2008) Activating transcription factor 4. Int. J. Biochem. Cell Biol. 40, 14–21.

[19] Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., et al. (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol. Cell 11, 619–633.

[20] Lange, P. S., Chavez, J. C., Pinto, J. T., Coppola, G., Sun, C. W., et al. (2008) ATF4 is an oxidative stress-inducible, prodeath transcription factor in neurons in vitro and in vivo. J. Exp. Med. 205, 1227–1242.

[21] Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R. T., et al. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 12, 982–995.

[22] Tabas, I. and Ron, D. (2011) Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat. Cell Biol. 13, 184–190.

[23] Urra, H., Dufey, E., Lisbona, F., Rojas-Rivera, D., and Hetz, C. When ER stress reaches a dead end. Biochim. Biophys. Acta; doi:pii: S0167-4889(13)00311-X.10.1016/j.bbamcr.2013.07.024 (in press).

[24] Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., et al. (2007) Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1. Dev. Cell 13, 365–376.

[25] Shoulders, M. D., Ryno, L. M., Genereux, J. C., Moresco, J. J., Tu, P. G., et al. (2013) Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. Cell Rep. 3, 1279–1292.

[26] Zhang, K., Wong, H. N., Song, B., Miller, C. N., Scheuner, D., et al. (2005) The unfolded protein response sensor IRE1α is required at 2 distinct steps in B cell lymphopoiesis. J. Clin. Invest. 115, 268–281.

[27] Iwawaki, T., Akai, R., Yamanaka, S., and Kohno, K. (2009) Function of IRE1α in the placenta is essential for placental development and embryonic viability. Proc. Natl. Acad. Sci. USA 106, 16657–16662.

[28] Iwawaki, T., Akai, R., and Kohno, K. (2010) IRE1α disruption causes histological abnormality of exocrine tissues, increase of blood glucose level, and decrease of serum immunoglobulin level. PLoS One 5, e13052.

[29] Bertolotti, A., Wang, X., Novoa, I., Jungreis, R., Schlessinger, K., et al. (2001) Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. J. Clin. Invest. 107, 585–593.

[30] Martino, M. B., Jones, L., Brighton, B., Ehre, C., Abdulah, L., et al. (2013) The ER stress transducer IRE1β is required for airway epithelial mucin production. Mucosal Immunol. 6, 639–654.

[31] Iqbal, J., Dai, K., Seimon, T., Jungreis, R., Oyadomari, M., et al. (2008) IRE1β inhibits chylomicron production by selectively degrading MTP mRNA. Cell Metab. 7, 445–455.

[32] Tsuru, A., Fujimoto, N., Takahashi, S., Saito, M., Nakamura, D., et al. (2013) Negative feedback by IRE1β optimizes mucin production in goblet cells. Proc. Natl. Acad. Sci. USA 110, 2864–2869.

[33] Reimold, A. M., Etkin, A., Clauss, I., Perkins, A., Friend, D. S., et al. (2000) An essential role in liver development for transcription factor XBP-1. Genes Dev. 14, 152–157.

[34] Iwakoshi, N. N., Lee, A. H., Vallabhajosyula, P., Otipoby, K. L., Rajewsky, K., et al. (2003) Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat. Immunol. 4, 321–329.

[35] Iwakoshi, N. N., Pypaert, M., and Glimcher, L. H. (2007) The transcription factor XBP-1 is essential for the development and survival of dendritic cells. J. Exp. Med. 204, 2267–2275.

[36] Reimold, A. M., Iwakoshi, N. N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., et al. (2001) Plasma cell differentiation requires the transcription factor XBP-1. Nature 412, 300–307.

[37] Lee, A. H., Scapa, E. F., Cohen, D. E., and Glimcher, L. H. (2008) Regulation of hepatic lipogenesis by the transcription factor XBP1. Science 320, 1492–1496.

[38] Lee, A. H., Heidtman, K., Hotamisligil, G. S., and Glimcher, L. H. (2011) Dual and opposing roles of the unfolded protein response regulated by IRE1α and XBP1 in proinsulin processing and insulin secretion. Proc. Natl. Acad. Sci. USA 108, 8885–8890.

[39] Akiyama, M., Liew, C. W., Lu, S., Hu, J., Martinez, R., et al. (2013) X-box binding protein 1 is essential for insulin regulation of pancreatic α cell function. Diabetes 62, 2439–2449.

[40] Kaser, A., Lee, A. H., Franke, A., Glickman, J. N., Zeissig, S., et al. (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 134, 743–756.

[41] Harding, H. P., Zeng, H., Zhang, Y., Jungries, R., Chung, P., et al. (2001) Diabetes mellitus and exocrine pancreatic dysfunction in PERK−/− mice reveals a role for translational control in secretory cell survival. Mol. Cell 7, 1153–1163.

[42] Zhang, P., McGrath, B., Li, S., Frank, A., Zambito, F., et al. (2002) The PERK eukaryotic initiation factor 2α kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. Mol. Cell. Biol. 22, 3864–3874.

[43] Iida, K., Li, Y., McGrath, B. C., Frank, A., and Cavener, D. R. (2007) PERK eIF2α kinase is required to regulate the viability of the exocrine pancreas in mice. BMC Cell Biol. 8, 38.

[44] Gao, Y., Sartori, D. J., Li, C., Yu, Q. C., Kushner, J. A., et al. (2012) PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. Mol. Cell. Biol. 32, 5129–5139.

[45] Trinh, M. A., Kaphzan, H., Wek, R. C., Pierre, P., Cavener, D. R., et al. (2012) Brain-specific disruption of the eIF2α kinase PERK decreases ATF4 expression and impairs behavioral flexibility. Cell Rep. 1, 676–688.

[46] Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., et al. (2001) Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol. Cell 7, 1165–1176.

[47] Scheuner, D., Vander Mierde, D., Song, B., Flamez, D., Creemers, J. W., et al. (2005) Control of mRNA translation preserves endoplasmic reticulum function in β-cells and maintains glucose homeostasis. Nat. Med. 11, 757–764.

[48] Back, S. H., Scheuner, D., Han, J., Song, B., Ribick, M., et al. (2009) Translation attenuation through eIF2α phosphorylation prevents oxidative stress and maintains the differentiated state in β-cells. Cell Metab. 10, 13–26.

[49] Wang, W., Lian, N., Li, L., Moss, H. E., Perrien, D. S., et al. (2009) Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription. Development 136, 4143–4153.

[50] Tanaka, T., Tsujimura, T., Takeda, K., Sugihara, A., Maekawa, A., et al. (1998) Targeted disruption of ATF4 discloses its essential role in the formation of eye lens fibres. Genes Cells 3, 801–810.

[51] Hettmann, T., Barton, K., and Leiden, J. M. (2000) Microphthalmia due to p53-mediated apoptosis of anterior lens epithelial cells in mice lacking the CREB-2 transcription factor. Dev. Biol. 222, 110–123.

[52] Masuoka, H. C. and Townes, T. M. (2002) Targeted disruption of the activating transcription factor 4 gene results in severe fetal anemia in mice. Blood 99, 736–745.

[53] Wu, J., Rutkowski, D. T., Dubois, M., Swathirajan, J., Saunders, T., et al. (2007) ATF6α optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev. Cell 13, 351–364.

[54] Rutkowski, D. T., Wu, J., Back, S. H., Callaghan, M. U., Ferris, S. P., et al. (2008) UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. Dev. Cell 15, 829–840.

[55] Yamamoto, K., Takahara, K., Oyadomari, S., Okada, T., Sato, T., et al. (2010) Induction of liver steatosis and lipid droplet formation in ATF6α-knockout mice burdened with pharmacological endoplasmic reticulum stress. Mol. Biol. Cell 21, 2975–2986.

[56] Usui, M., Yamaguchi, S., Tanji, Y., Tominaga, R., Ishigaki, Y., et al. (2012) Atf6α-null mice are glucose intolerant due to pancreatic β-cell failure on a high-fat diet but partially resistant to diet-induced insulin resistance. Metabolism 61, 1118–1128.

[57] Wu, J., Ruas, J. L., Estall, J. L., Rasbach, K. A., Choi, J. H., et al. (2011) The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1α/ATF6α complex. Cell Metab. 13, 160–169.

[58] Murakami, T., Saito, A., Hino, S., Kondo, S., Kanemoto, S., et al. (2009) Signalling mediated by the endoplasmic reticulum stress transducer OASIS is involved in bone formation. Nat. Cell Biol. 11, 1205–1211.

[59] Saito, A., Hino, S., Murakami, T., Kanemoto, S., Kondo, S., et al. (2009) Regulation of endoplasmic reticulum stress response by a BBF2H7-mediated Sec23a pathway is essential for chondrogenesis. Nat. Cell Biol. 11, 1197–1204.

[60] Lee, M. W., Chanda, D., Yang, J., Oh, H., Kim, S. S., et al. (2010) Regulation of hepatic gluconeogenesis by an ER-bound transcription factor, CREBH. Cell Metab. 11, 331–339.

[61] Zhang, K., Shen, X., Wu, J., Sakaki, K., Saunders, T., et al. (2006) Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell 124, 587–599.

[62] Gupta, S., McGrath, B., and Cavener, D. R. (2009) PERK regulates the proliferation and development of insulin-secreting β-cell tumors in the endocrine pancreas of mice. PLoS One 4, e8008.

[63] Cavener, D. R., Gupta, S., and McGrath, B. C. (2010) PERK in β-cell biology and insulin biogenesis. Trends Endocrinol. Metab. 21, 714–721.

[64] Wei, J., Sheng, X., Feng, D., McGrath, B., and Cavener, D. R. (2008) PERK is essential for neonatal skeletal development to regulate osteoblast proliferation and differentiation. J. Cell. Physiol. 217, 693–707.

[65] Saito, A., Ochiai, K., Kondo, S., Tsumagari, K., Murakami, T., et al. (2011) Endoplasmic reticulum stress response mediated by the PERK-eIF2(α)-ATF4 pathway is involved in osteoblast differentiation induced by BMP2. J. Biol. Chem. 286, 4809–4818.

[66] Galehdar, Z., Swan, P., Fuerth, B., Callaghan, S. M., Park, D. S., et al. (2010) Neuronal apoptosis induced by endoplasmic reticulum stress is regulated by ATF4-CHOP-mediated induction of the Bcl-2 homology 3-only member PUMA. J. Neurosci. 30, 16938–16948.

[67] Lin, W., Lin, Y., Li, J., Fenstermaker, A. G., Way, S. W., et al. (2013) Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis. J. Neurosci. 33, 5980–5991.

[68] Ma, T., Trinh, M. A., Wexler, A. J., Bourbon, C., Gatti, E., et al. (2013) Suppression of eIF2α kinases alleviates Alzheimer's disease-related plasticity and memory deficits. Nat. Neurosci. 16, 1299–1305.

[69] Dever, T. E. (2002) Gene-specific regulation by general translation factors. Cell 108, 545–556.

[70] Oyadomari, S., Harding, H. P., Zhang, Y., Oyadomari, M., and Ron, D. (2008) Dephosphorylation of translation initiation factor 2α enhances glucose tolerance and attenuates hepatosteatosis in mice. Cell Metab. 7, 520–532.

[71] Yoshizawa, T., Hinoi, E., Jung, D. Y., Kajimura, D., Ferron, M., et al. (2009) The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. J. Clin. Invest. 119, 2807–2817.

[72] Seo, J., Fortuno, E. S., III, Suh, J. M., Stenesen, D., Tang, W., et al. (2009) Atf4 regulates obesity, glucose homeostasis, and energy expenditure. Diabetes 58, 2565–2573.

[73] Xiao, G., Zhang, T., Yu, S., Lee, S., Calabuig-Navarro, V., et al. (2013) ATF4 protein deficiency protects against high fructose-induced hypertriglyceridemia in mice. J. Biol. Chem. 288, 25350–25361.

[74] Valenzuela, V., Collyer, E., Armentano, D., Parsons, G. B., Court, F. A., et al. (2012) Activation of the unfolded protein response enhances motor recovery after spinal cord injury. Cell Death Dis. 3, e272.

[75] Matus, S., Lopez, E., Valenzuela, V., Nassif, M., and Hetz, C. (2013) Functional contribution of the transcription factor ATF4 to the pathogenesis of amyotrophic lateral sclerosis. PLoS One 8, e66672.

[76] Endo, M., Oyadomari, S., Suga, M., Mori, M., and Gotoh, T. (2005) The ER stress pathway involving CHOP is activated in the lungs of LPS-treated mice. J. Biochem. 138, 501–507.

[77] Thorp, E., Li, G., Seimon, T. A., Kuriakose, G., Ron, D., et al. (2009) Reduced apoptosis and plaque necrosis in advanced atherosclerotic lesions of Apoe−/− and Ldlr−/− mice lacking CHOP. Cell Metab. 9, 474–481.

[78] Tamaki, N., Hatano, E., Taura, K., Tada, M., Kodama, Y., et al. (2008) CHOP deficiency attenuates cholestasis-induced liver fibrosis by reduction of hepatocyte injury. Am. J. Physiol. Gastrointest. Liver Physiol. 294, G498–G505.

[79] Ji, C., Mehrian-Shai, R., Chan, C., Hsu, Y. H., and Kaplowitz, N. (2005) Role of CHOP in hepatic apoptosis in the murine model of intragastric ethanol feeding. Alcohol Clin. Exp. Res. 29, 1496–1503.

[80] Ariyama, Y., Shimizu, H., Satoh, T., Tsuchiya, T., Okada, S., et al. (2007) Chop-deficient mice showed increased adiposity but no glucose intolerance. Obesity (Silver Spring) 15, 1647–1656.

[81] Han, J., Murthy, R., Wood, B., Song, B., Wang, S., et al. (2013) ER stress signalling through eIF2α and CHOP, but not IRE1α, attenuates adipogenesis in mice. Diabetologia 56, 911–924.

[82] Maris, M., Overbergh, L., Gysemans, C., Waget, A., Cardozo, A. K., et al. (2012) Deletion of C/EBP homologous protein (Chop) in C57Bl/6 mice dissociates obesity from insulin resistance. Diabetologia 55, 1167–1178.

[83] Oyadomari, S., Takeda, K., Takiguchi, M., Gotoh, T., Matsumoto, M., et al. (2001) Nitric oxide-induced apoptosis in pancreatic β-cells is mediated by the endoplasmic reticulum stress pathway. Proc. Natl. Acad. Sci. USA 98, 10845–10850.

Physiological Function of UPR in Organs and Tissues

[84] Song, B., Scheuner, D., Ron, D., Pennathur, S., and Kaufman, R. J. (2008) Chop deletion reduces oxidative stress, improves β-cell function, and promotes cell survival in multiple mouse models of diabetes. J. Clin. Invest. 118, 3378–3389.

[85] Oyadomari, S., Koizumi, A., Takeda, K., Gotoh, T., Akira, S., et al. (2002) Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. J. Clin. Invest. 109, 525–532.

[86] Chen, C. M., Wu, C. T., Chiang, C. K., Liao, B. W., and Liu, S. H. (2012) C/EBP homologous protein (CHOP) deficiency aggravates hippocampal cell apoptosis and impairs memory performance. PLoS One 7, e40801.

[87] Tajiri, S., Oyadomari, S., Yano, S., Morioka, M., Gotoh, T., et al. (2004) Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. Cell Death Differ. 11, 403–415.

[88] Ohri, S. S., Maddie, M. A., Zhang, Y., Shields, C. B., Hetman, M., et al. (2012) Deletion of the pro-apoptotic endoplasmic reticulum stress response effector CHOP does not result in improved locomotor function after severe contusive spinal cord injury. J. Neurotrauma 29, 579–588.

[89] Silva, R. M., Ries, V., Oo, T. F., Yarygina, O., Jackson-Lewis, V., et al. (2005) CHOP/GADD153 is a mediator of apoptotic death in substantia nigra dopamine neurons in an in vivo neurotoxin model of parkinsonism. J. Neurochem. 95, 974–986.

[90] Pennuto, M., Tinelli, E., Malaguti, M., Del Carro, U., D'Antonio, M., et al. (2008) Ablation of the UPR-mediator CHOP restores motor function and reduces demyelination in Charcot-Marie-Tooth 1B mice. Neuron 57, 393–405.

[91] Wang, Y., Vera, L., Fischer, W. H., and Montminy, M. (2009) The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. Nature 460, 534–537.

[92] Kondo, S., Saito, A., Asada, R., Kanemoto, S., and Imaizumi, K. (2011) Physiological unfolded protein response regulated by OASIS family members, transmembrane bZIP transcription factors. IUBMB Life 63, 233–239.

[93] Kondo, S., Murakami, T., Tatsumi, K., Ogata, M., Kanemoto, S., et al. (2005) OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. Nat. Cell Biol. 7, 186–194.

[94] Omori, Y., Imai, J., Watanabe, M., Komatsu, T., Suzuki, Y., et al. (2001) CREB-H: a novel mammalian transcription factor belonging to the CREB/ATF family and functioning via the box-B element with a liver-specific expression. Nucleic Acids Res. 29, 2154–2162.

[95] Zhang, C., Wang, G., Zheng, Z., Maddipati, K. R., Zhang, X., et al. (2012) Endoplasmic reticulum-tethered transcription factor cAMP responsive element-binding protein, hepatocyte specific, regulates hepatic lipogenesis, fatty acid oxidation, and lipolysis upon metabolic stress in mice. Hepatology 55, 1070–1082.

[96] Lee, A. H., Chu, G. C., Iwakoshi, N. N., and Glimcher, L. H. (2005) XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. EMBO J. 24, 4368–4380.

[97] Liou, H. C., Boothby, M. R., Finn, P. W., Davidon, R., Nabavi, N., et al. (1990) A new member of the leucine zipper class of proteins that binds to the HLA DRα promoter. Science 247, 1581–1584.

[98] Shaffer, A. L., Shapiro-Shelef, M., Iwakoshi, N. N., Lee, A. H., Qian, S. B., et al. (2004) XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. Immunity 21, 81–93.

[99] Tirosh, B., Iwakoshi, N. N., Glimcher, L. H., and Ploegh, H. L. (2005) XBP-1 specifically promotes IgM synthesis and secretion, but is dispensable for degradation of glycoproteins in primary B cells. J. Exp. Med. 202, 505–516.

[100] Hu, C. C., Dougan, S. K., McGehee, A. M., Love, J. C., and Ploegh, H. L. (2009) XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells. EMBO J. 28, 1624–1636.

[101] Todd, D. J., McHeyzer-Williams, L. J., Kowal, C., Lee, A. H., Volpe, B. T., et al. (2009) XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. J. Exp. Med. 206, 2151–2159.

[102] Glas, J., Seiderer, J., Czamara, D., Pasciuto, G., Diegelmann, J., et al. (2012) PTGER4 expression-modulating polymorphisms in the 5p13.1 region predispose to Crohn's disease and affect NF-κB and XBP1 binding sites. PLoS One 7, e52873.

[103] Martinon, F., Chen, X., Lee, A. H., and Glimcher, L. H. (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat. Immunol. 11, 411–418.

[104] Carrasco, D. R., Sukhdeo, K., Protopopova, M., Sinha, R., Enos, M., et al. (2007) The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell 11, 349–360.

[105] Xu, G., Liu, K., Anderson, J., Patrene, K., Lentzsch, S., et al. (2012) Expression of XBP1s in bone marrow stromal cells is critical for myeloma cell growth and osteoclast formation. Blood 119, 4205–4214.

[106] Huh, W. J., Esen, E., Geahlen, J. H., Bredemeyer, A. J., Lee, A. H., et al. (2010) XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum. Gastroenterology 139, 2038–2049.

[107] So, J. S., Hur, K. Y., Tarrio, M., Ruda, V., Frank-Kamenetsky, M., et al. (2012) Silencing of lipid metabolism genes through IRE1α-mediated mRNA decay lowers plasma lipids in mice. Cell Metab. 16, 487–499.

[108] Hayashi, A., Kasahara, T., Kametani, M., and Kato, T. (2008) Attenuated BDNF-induced upregulation of GABAergic markers in neurons lacking Xbp1. Biochem. Biophys. Res. Commun. 376, 758–763.

[109] Hetz, C., Thielen, P., Matus, S., Nassif, M., Court, F., et al. (2009) XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev. 23, 2294–2306.

[110] Vidal, R. L., Figueroa, A., Court, F. A., Thielen, P., Molina, C., et al. (2012) Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. Hum. Mol. Genet. 21, 2245–2262.

[111] Zuleta, A., Vidal, R. L., Armentano, D., Parsons, G., and Hetz, C. (2012) AAV-mediated delivery of the transcription factor XBP1s into the striatum reduces mutant Huntingtin aggregation in a mouse model of Huntington's disease. Biochem. Biophys. Res. Commun. 420, 558–563.

[112] Hu, Y., Park, K. K., Yang, L., Wei, X., Yang, Q., et al. (2012) Differential effects of unfolded protein response pathways on axon injury-induced death of retinal ganglion cells. Neuron 73, 445–452.

[113] Hetz, C., Lee, A. H., Gonzalez-Romero, D., Thielen, P., Castilla, J., et al. (2008) Unfolded protein response transcription factor XBP-1 does not influence prion replication or pathogenesis. Proc. Natl. Acad. Sci. USA 105, 757–762.

[114] Ozcan, L., Ergin, A. S., Lu, A., Chung, J., Sarkar, S., et al. (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab. 9, 35–51.

[115] Hur, K. Y., So, J. S., Ruda, V., Frank-Kamenetsky, M., Fitzgerald, K., et al. (2012) IRE1α activation protects mice against acetaminophen-induced hepatotoxicity. J. Exp. Med. 209, 307–318.

[116] Iqbal, J., Queiroz, J., Li, Y., Jiang, X. C., Ron, D., et al. (2012) Increased intestinal lipid absorption caused by Ire1β deficiency contributes to hyperlipidemia and atherosclerosis in apolipoprotein E-deficient mice. Circ. Res. 110, 1575–1584.

[117] Matsuzawa, A., Nishitoh, H., Tobiume, K., Takeda, K., and Ichijo, H. (2002) Physiological roles of ASK1-mediated signal transduction in oxidative stress- and endoplasmic reticulum stress-induced apoptosis: advanced findings from ASK1 knockout mice. Antioxid. Redox Signal. 4, 415–425.

[118] Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitoh, H., Morita, K., et al. (2001) ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep. 2, 222–228.

[119] Nishitoh, H., Kadowaki, H., Nagai, A., Maruyama, T., Yokota, T., et al. (2008) ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. Genes Dev. 22, 1451–1464.

[120] Rutkowski, D. T. and Hegde, R. S. (2010) Regulation of basal cellular physiology by the homeostatic unfolded protein response. J. Cell Biol. 189, 783–794.

[121] Martinez, G. and Hetz, C. (2012) Cell-nonautonomous control of the UPR. EMBO Rep. 13, 767–768.

[122] Taylor, R. C. and Dillin, A. (2013) XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell 153, 1435–1447.