Cell. Mol. Life Sci. (2009) 66:2835–2850
DOI 10.1007/s00018-009-0049-8

Cellular and Molecular Life Sciences

REVIEW

Intersection of the unfolded protein response and hepatic lipid metabolism

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Received: 29 March 2009 / Revised: 5 May 2009 / Accepted: 6 May 2009 / Published online: 26 May 2009

Abstract The liver plays a central role in whole-body lipid metabolism by governing the synthesis, oxidization, transport and excretion of lipids. The unfolded protein response (UPR) was identified as a signal transduction system that is activated by ER stress. Recent studies revealed a critical role of the UPR in hepatic lipid metabolism. The IRE1/XBP1 branch of the UPR is activated by high dietary carbohydrates and controls the expression of genes involved in fatty acid and cholesterol biosynthesis. PERK mediated eIF2α phosphorylation is also required for the expression of lipogenic genes and the development of hepatic steatosis, likely by activating C/EBP and PPARγ transcription factors. Further studies to define the molecular pathways that lead to the activation of the UPR by nutritional cues in the liver, and their contribution to human metabolic disorders such as hepatic steatosis, atherosclerosis and type 2 diabetes that are associated with dysregulation of lipid homeostasis, are warranted.

Keywords Unfolded protein response · Lipogenesis · Gene expression · XBP1 · PERK

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Introduction

In mammals, the liver plays a crucial role in the control of whole body energy homeostasis. Carbohydrates in the diet are partly consumed for normal bodily functions and metabolized to glycogen or fatty acids in the liver, if in excess [1]. Fatty acids are esterified to triacylglyceride (TG) and then transported to adipose tissue for long-term energy storage. Conversion of carbohydrates to TG involves two steps; glycolysis which generates acetyl CoA from glucose, and lipogenesis which converts acetyl CoA to fatty acids (Fig. 1). Enzymes involved in glycolytic and lipogenic pathways are dynamically regulated at both transcriptional and post-translational levels by various factors such as substrate concentrations and hormones. Pancreatic hormones, insulin and glucagon, play critical roles in the transcriptional regulation of these enzymes. For example, insulin activates the sterol regulatory element binding protein-1c (SREBP-1c) transcription factor which governs the expression of lipogenic genes including fatty acid synthase (FASN) (reviewed in [2, 3]). During fasting, glucagon activates the protein kinase A (PKA), which phosphorylates carbohydrate response element-binding protein (ChREBP), preventing its translocation to the nucleus and the subsequent activation of its target genes involved in glycolysis and lipogenesis (reviewed in [4, 5]).

The unfolded protein response (UPR) is a signaling system emanating from the endoplasmic reticulum (ER) that is activated when ER protein folding is disturbed [6, 7]. In normal animal physiology, the UPR is critically required for the development and/or secretory function of highly secretory cells in exocrine and endocrine glands. Recent studies revealed novel diverse functions of the mammalian UPR including its role in hepatic lipid metabolism [8–11]. The UPR plays essential roles in

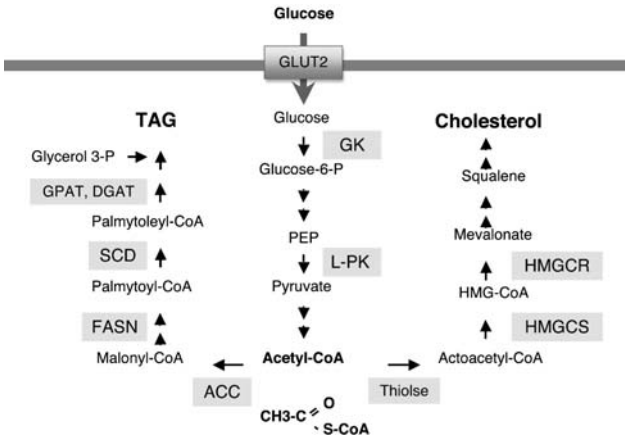

Fig. 1 Biochemical pathways leading to the synthesis of TAG and cholesterol. Glycolytic pathway generates Acetyl CoA from glucose imported into the cell through GLUT2. TAG and cholesterol are synthesized from Acetyl CoA by sequential enzymatic actions. *GLUT2* Glucose transporter 2, *GK* glucokinase, *L-PK* liver pyruvate kinase, *PEP* phosphoenolpyruvate, *ACC* acetyl-CoA carboxylase, *FASN* fatty acid synthase, *SCD* stearoyl-CoA desaturase, *GPAT* glycerol-3-phosphate acyltransferase, *DGAT* diacylglycerol acyltransferase, *HMG-CoA* 3-hydroxy-3-methylglutaryl-coenzyme A, *HMGCS* HMG-CoA synthase, *HMGCR* HMG-CoA reductase

hepatic lipogenesis by controlling the expression of genes in the lipogenic pathway. UPR activation has been observed in fatty liver diseases, suggesting the induction of ER stress in these pathological conditions (reviewed in [12]). This review summarizes the physiological functions of the UPR, focusing on studies analyzing the phenotype of mice mutant in each of the critical UPR regulatory genes. It also addresses recent findings regarding the role of the UPR in hepatic lipogenesis and fatty liver diseases (Table 1).

Transcriptional regulation of hepatic lipogenesis

Ingestion of dietary carbohydrates not only increases the plasma glucose concentration but also promotes the secretion of insulin from pancreatic β cells. These metabolic and hormonal cues activate glycolysis and de novo lipid synthesis in the liver to convert glucose into TG [13], which is carried to adipose tissue in very low-density lipoprotein (VLDL) particles [14]. Several key enzymes catalyzing glycolysis and de novo lipid synthesis such as glucokinase (GK), liver-pyruvate kinase (LPK), acetyl CoA carboxylase (ACC), FAS, and stearyl CoA desaturase-1 (SCD1) are transcriptionally activated in the liver upon ingestion of high carbohydrates [13, 15, 16]. Transcription factors SREBP-1c and ChREBP have been considered as major players in the transcriptional regulation of these glycolytic and lipogenic genes (Fig. 2) [2, 5, 15].

SREBP-1c

SREBP-1c is activated by insulin and plays a major role in the induction of lipogenic genes by insulin [16, 17]. SREBP-1c is a member of a transcription factor family that was originally identified as a mediator of sterol signaling [18]. The SREBP family comprises SREBP-1a, SREBP-1c and SREBP-2 that are encoded by two separate genes [2, 19]. Utilization of alternate promoters in a single gene gives rise to SREBP-1a and SREBP-1c, which are identical in most domains except for the N-terminal region which is derived from an alternate exon 1 [20]. Studies in transgenic and knock-out mice revealed the function of SREBP-1c and SREBP-2 to primarily control the expression of fatty acid and cholesterol biosynthetic enzymes, respectively [2, 15, 16]. SREBPs are produced as precursor proteins containing two transmembrane domains, which anchor the protein in the ER complexed with the SREBP cleavage activating protein (SCAP) and ER retention protein called Insig [21]. Low sterol triggers a conformational change of the sterol sensitive SCAP protein that dissociates the SCAP–SREBP complex from Insig [22]. The SCAP–SREBP complex migrates to the Golgi apparatus, where SREBPs are sequentially cleaved by site 1 (S1P) and site 2 (S2P) proteases to liberate the mature proteins that translocate to the nucleus and act as active transcription factors [23]. While SREBP-2 is primarily controlled at the post-translational level by sterols which regulates its ER-Golgi trafficking, SREBP-1c is controlled at the transcriptional level by insulin [21]. Hepatic SREBP-1c mRNA is downregulated in fasted animals, and dramatically induced upon high carbohydrate ingestion by the action of insulin [20, 24]. The PI(3)-kinase signaling pathway plays an important role in SREBP-1c expression induced by insulin [25, 26]. Liver X receptor (LXR) can also induce SREBP-1c mRNA [27, 28]. Induction of lipogenic genes by a synthetic LXR agonist was blunted in SREBP-1c deficient mice, suggesting that SREBP1-c is essential for the lipogenic activity of LXR [17]. Overexpression of SREBP-1c in the liver by using transgenic mice or adenoviral gene delivery dramatically induced lipogenic genes accompanied by increased hepatic TG content [29–31]. In contrast, mice lacking SREBP-1c in the liver displayed reduced plasma TG levels, and decreased expression of multiple lipogenic genes upon refeeding [17]. It is of interest to note that SREBP-1c deletion only partially suppressed the expression of several key lipogenic genes, such as glucokinase, Fas, and Acc, suggesting the presence of compensatory mechanisms [17].

ChREBP

ChREBP was identified as a transcription factor that binds to a carbohydrate response element (ChRE) in the LPK

Table 1 Physiological functions of the UPR: Lessons from genetic alterations of essential UPR genes in humans and mice

UPR branch	Gene	Alteration	Phenotype	Reference
IRE1/XBP1	Ern1 (IRE1α)	Germline deletion	Embryonic lethal (E10.5)	[181]
		Ern1$^{-/-}$ Rag2 chimera	Impaired early B cell development	[181]
		Conditional deletion in liver	Altered gene expression upon tunicamycin challenge	[11]
	Ern2 (IRE1β)	Germline deletion	Increased susceptibility to DSS colitis	[182]
		Germline deletion	Hyperlipidemia on high cholesterol/fat diet	[11]
	Xbp1	Germline deletion	Embryonic lethal (E14.5), liver hypoplasia	[87]
		Xbp1$^{-/-}$ Rag2 chimera	Absent plasma B cell differentiation	[85]
		Xbp1$^{-/-}$ Rag2 chimera	Impaired dendritic cell differentiation	[88]
		Germline deletion, transgene rescued	Exocrine pancreas malfunction	[84]
		Conditional deletion in GI tract	Inflammatory bowel diseases, Paneth cell loss	[83]
		Conditional deletion in liver	Hypolipidemia, decreased hepatic lipogenesis	[8]
ATF6	Atf6 (ATF6α)	Germline deletion	Increased liver toxicity by tunicamycin	[10]
	Atf6, Atf6b	Germline deletion of ATF6α and β	Embryonic lethal	[81]
PERK/eIF2α	Eif2k3 (PERK)	R587Q mutation in human PERK gene	Wolcott-Rallison syndrome, diabetes, skeletal dysplasias	[107]
		Germline or conditional deletion	Diabetes, skeletal dysplasias, exocrine pancreas insufficiency	[110–113]
		Conditional deletion in β cells	Diabetes, impaired embryonic pancreatic β cell differentiation	[114]
		Conditional deletion in mammary gland	Mammary gland lipogenesis defect	[136]
	Eif2a (eIF2α)	Homozygous S51A	Perinatal lethality due to defective gluconeogenesis	[129]
		Heterozygous S51A	Insulin resistance, defective insulin production on high fat diet	[130]
		Homozygous S51A in liver, transgene rescued	Altered gene expression upon tunicamycin challenge	[11]
	Atf4	Germline deletion	Fetal anemia, impaired eye lens fiber formation and osteoblast differentiation	[70, 183, 184]

gene promoter [32]. ChRE is a motif present within several gene promoters responsive to high glucose [13]. As a bZIP transcription factor, ChREBP forms a heterodimeric complex with another bZIP protein Max-like protein X (MLX), and directly binds to ChRE [33]. It was originally proposed that PKA phosphorylates ChREBP under low glucose conditions, preventing its translocation into the nucleus [34]. However, it is controversial if nuclear translocation is sufficient for the activation of ChREBP [35, 36]. Recently, Towle and colleagues demonstrated that the derepression of the transcriptional activity conferred by the N-terminal domain of ChREBP is critical for the activation of ChREBP [37]. ChREBP knock-out mice displayed reduced fat mass, low plasma free fatty acid (FFA), hyperglycemia, high liver glycogen and normal hepatic and plasma TG levels on chow diet [38]. When fed high starch diet, these mice displayed more profound phenotypes, including significant reduction in hepatic TG level, associated with decreased mRNA levels of select lipogenic genes. Fatty acid synthesis rate was reduced by $\sim$65%, indicating the essential role of ChREBP in hepatic lipogenesis [38]. ChREBP knock-out mice exhibited moderate insulin resistance, presumably due to an efficient disposal of glucose [38]. In contrast, downregulation of ChREBP in the liver by using shRNA delivery improved the insulin sensitivity of ob/ob mice [39]. It is conceivable that ChREBP knockdown lowered lipotoxicity by suppressing hepatic lipogenesis.

Unfolded protein response

The ER is a continuous membrane network in the cell that has multiple important functions. First, the ER plays a crucial role in the protein secretory pathway [40–42]. All the nascent polypeptides destined to be secreted are imported into the ER lumen to attain proper three-dimensional protein structures and various post-translational

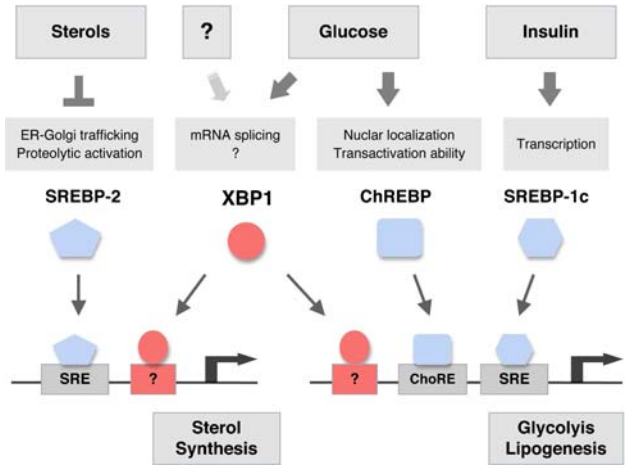

Fig. 2 Transcriptional control of hepatic lipogenesis. SREBP-2 is activated when cellular sterol levels are low, and controls the expression of genes involved in sterol biosynthesis. SREBP-1c and ChREBP are activated by insulin and glucose, respectively. Ingestion of carbohydrates increases circulating glucose and insulin levels, by inducing insulin production from pancreatic β cells. SREBP-1c and ChREBP bind to specific promoter elements to activate the transcription of genes in glycolytic and lipogenic pathways. XBP1 is required for the de novo synthesis of sterols and fatty acids in the liver and is activated in the presence of high glucose. The mechanism by which XBP1 controls sterol and fatty acid synthesis genes remains to be further investigated

modifications, such as disulfide bond formation and glycosylation, and then sorted for transport to the destined locations. ER resident chaperones, protein disulfide isomerases (PDIs), and various protein modifying enzymes are involved in this process. The ER also serves as a protein quality control check-point, so that misfolded proteins are screened out for retrotranslocation to the cytoplasm followed by proteasomal degradation [43, 44]. Second, the synthesis of neutral and phospholipids occurs in the ER [45–47]. The lipid bilayer expands in the ER and then is mobilized to the membranous organelles as needed. Third, the ER plays a crucial role in drug metabolism with major drug metabolizing enzymes including cytochrome P450 present in the ER [48]. Fourth, the ER is the major storage depot for calcium which is released upon appropriate stimulation to serve as signaling molecules [49].

ER stress can be defined as a condition that compromises the capacity of the ER to handle its client proteins [6, 7, 50]. Increased input of the client proteins into the ER during the development of highly secretory cells can induce ER stress. ER stress can also be induced by treatment of cells with pharmacological reagents that inhibit protein folding in the ER. These include protein glycosylation inhibitors (i.e., tunicamycin), reducing agents (i.e., dithiothreitol) that interfere with protein disulfide bond formation, and calcium channel blockers (thapsigargin) that deplete ER calcium stores. In response to ER stress, eukaryotic cells activate a signaling cascade called the UPR to protect the cell from stress. Mutation of genes in the UPR signaling cascade frequently results in the increased susceptibility of the cell to ER stress-induced cell death.

In yeast, the UPR is initiated by the ER stress sensor IRE1 and executed by the transcription factor HAC1 [51–54]. IRE1 is a type I transmembrane protein, which senses ER stress through its luminal domain [55, 56]. The cytosolic domain of IRE1 possesses dual enzymatic activities of a protein kinase and an endoribonuclease [51, 57–60]. Activated by trans-autophosphorylation, IRE1 specifically cleaves two sites in HAC1 mRNA, which is re-ligated by t-RNA ligase [57, 60, 61]. This unconventional splicing event generates HAC1p protein that activates a variety of genes involved in protein secretory pathways, including ER-resident chaperones and enzymes in phospholipid biosynthesis pathways, as well as those engaged in ER-associated protein degradation (ERAD) [62]. IRE1 or HAC1 mutant yeasts grow normally under standard conditions, but display increased sensitivity to ER stress inducing compounds, such as tunicamycin [53, 63].

Mammals have an IRE1/XBP1 UPR signaling system analogous to yeast IRE1/HAC1 (Fig. 3). IRE1-mediated

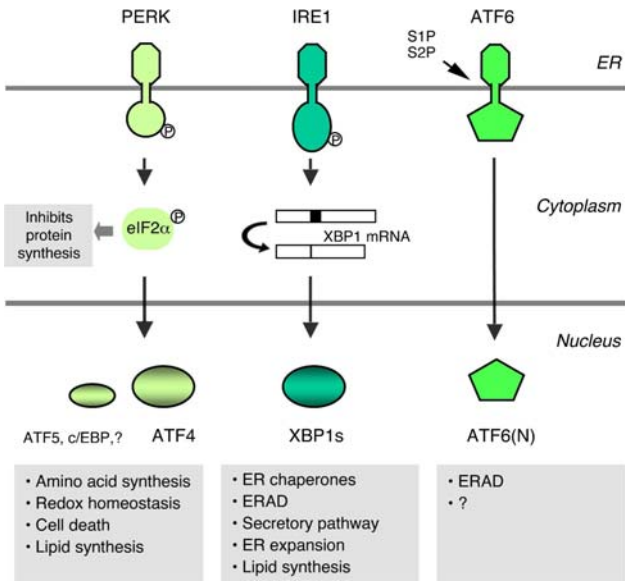

Fig. 3 Overview of mammalian UPR signaling pathways. *IRE1*, *PERK*, and *ATF6* are proximal ER stress sensors. *IRE1*, activated by autophosphorylation, removes 26 nucleotides from the *XBP1 mRNA* by using its cytosolic endoribonuclease activity. Spliced *XBP1s* protein is a potent transcription factor that induces diverse target genes. eIF2α phosphorylation by *PERK* impedes overall protein synthesis, while activating translation of some transcription factors. *ATF4* plays a major role in PERK-dependent UPR target gene expression. *ATF6* is a member of the family of ER transmembrane transcription factors, and normally resides in the ER associated with the ER chaperone BiP. Upon ER stress, *ATF6* moves to the Golgi apparatus where site 1 (*S1P*) and site 2 proteases (*S2P*) sequentially cleave *ATF6* to release the N-terminal part of the protein that translocates to the nucleus to activate its target genes

splicing removes 26 nucleotides in the XBP1 mRNA starting from the 184th codon, resulting in a shift of the translational reading frame [64–66]. XBP1s mRNA encodes a bZIP transcription factor that binds to the CRE-like promoter element to activate a group of UPR target genes [67–69]. While the yeast UPR is controlled solely by HAC1, XBP1 is responsible for the induction of only a subset of UPR target genes, indicating the presence of additional components [68, 70]. Indeed, mammals contain at least two additional UPR branches that are controlled by the PKR-like ER-localized eukaryotic initiation factor 2α (eIF2α) kinase (PERK) and the ER transmembrane transcription factor precursor ATF6 [71, 72]. Phosphorylation of eIF2α by PERK suppresses translational initiation, lowering the input of client proteins into the ER, to mitigate ER stress [73]. In contrast, translation of certain mRNAs (i.e., ATF4 and ATF5) that possess short open reading frames in the 5′ UTR region are facilitated by PERK-dependent eIF2α phosphorylation under ER stress conditions [73, 74]. ATF4 plays a major role in the transcriptional control of PERK-dependent UPR target genes [75]. The ATF6 transcription factor family comprises two transmembrane bZIP transcription factors, ATF6α and ATF6β, that reside in the ER under basal conditions [72, 76–78]. ATF6α and ATF6β display limited amino acid sequence homology only in the bZIP and the ER transmembrane domains, suggesting that they recognize similar cis-acting promoter elements, but may differentially regulate their target genes [76, 79, 80]. Indeed, both ATF6α and ATF6β can bind to the CCAAT-(N_9)-CCACG sequences, called the ER stress response element (ERSE) which mediates the transcriptional induction of many ER chaperone genes [67, 69, 81]. It is noteworthy that ATF6 and XBP1 recognize similar DNA target sequences and can form a hetero-dimer, suggesting that they may have similar roles in the ER stress response [67, 82]. ER stress triggers the migration of ATF6 from the ER to the Golgi apparatus, where it is sequentially processed by S1P and S2P proteases to release the N-terminal cytosolic part of the protein, which then enters the nucleus to transactivate gene transcription [77].

Physiological functions of the UPR

IRE1/XBP1

Microarray analyses of XBP1-deficient mouse embryonic fibroblast (MEF) cells and primary B cells stimulated with lipopolysaccharides identified a series of genes involved in ER translocation, protein folding, secretory pathways, and degradation of misfolded proteins as XBP1 target genes [68, 70]. These XBP1 target genes are expressed at basal levels under normal conditions and further induced in an XBP1 dependent manner when cells are ER stressed or differentiate into antibody secreting plasma cells. Consistent with the relationship of its target genes to cellular secretory function, XBP1 was found to be required for the development and protein secretory function of secretory cells [83–85]. XBP1 is highly expressed in exocrine glands and osteoblasts in the skeletal system that produce large amount of secretory proteins [86]. XBP1-deficient mice die during embryonic development due to liver hypoplasia accompanied by defective erythropoiesis [87]. It is not known if embryonic liver development requires a robust UPR or whether XBP1 may have another function in embryonic liver unrelated to the UPR. XBP1 also contributes to the development of dendritic cells [88].

Analysis of recombination activating gene 2 (RAG2) chimeric mice possessing XBP1 deficient lymphoid cells revealed that XBP1 is essential for the terminal differentiation of mature B cells into antibody secreting plasma cells [85]. Similarly, the lack of XBP1 severely impairs the development of other highly secretory cells such as pancreatic acinar cells that secrete zymogens to the small intestine, and intestinal Paneth cells that produce antimicrobial peptides [83, 84]. Normal acinar cells contain tightly packed multilayers of ER in the basal area and zymogen granules at the apical side targeted to the lumen for secretion. Extensive ER is required for the maturation and transportation of zymogens. XBP1-deficient pancreatic acinar cells are completely devoid of mature zymogen granules. Their ER is minimally expanded and contains electron dense particles which appear to be zymogen aggregates that failed to mature into secretory granules [84]. Marked apoptosis of XBP1 deficient pancreatic acinar cells was observed during the embryonic developmental stage when cells dramatically increase zymogen production and expand the ER. Increased expression of a proapoptotic ER stress marker CHOP revealed heightened ER stress in XBP1-deficient pancreatic acinar cells. XBP1 deficiency exerts similar effects on Paneth cells, resulting in complete absence of zymogen granules, impaired ER expansion and apoptotic cell death associated with increased ER stress [83]. These data collectively suggest that XBP1 is required for the biogenesis of cellular secretory machinery and its absence results in apoptotic cell death due to increased ER stress.

XBP1 is absolutely required for only a subset of secretory cells. Moderately secretory cells such as adult hepatocytes, salivary gland acinar cells, pancreatic β cells are only partially dependent on XBP1, suggesting that the requirement of XBP1 correlates with the secretory load of any given cell [8, 84]. XBP1 is required not only for the development of secretory cells, but also to maintain the integrity of the secretory machinery [83]. Inducible deletion of XBP1 in already differentiated Paneth cells or pancreatic acinar cells causes the degeneration of the ER and malformation of the

secretory granule, as seen in XBP1 germline deleted mice [83] (Lee and Glimcher, unpublished data).

PERK/eIF2α

In response to various stress signals, mammalian cells control mRNA translation by regulating translational initiation [89]. Phosphorylation of the translational initiation component eIF2α is a central mechanism that regulates mRNA translation. eIF2α can be phosphorylated by different protein kinases, such as protein kinase RNA-activated (PKR), general control nonderepressible-2 (GCN2), PERK, and heme-regulated inhibitor kinase (HRI) under various stressful conditions [71, 89–94]. PKR is activated by double-stranded RNA during viral infection, GCN2 by amino acid starvation, PERK by unfolded proteins in the ER, and HRI by heme deficiency in erythroid cells. Phosphorylation of eIF2α at the Ser51 residue inhibits the translation of most mRNAs, suppressing global protein synthesis to mitigate cellular damage by stress [6]. It is believed that PERK-induced eIF2α phosphorylation lowers the input of cargo proteins into the ER, and thereby reduces ER stress. While global protein translation is suppressed upon eIF2α phosphorylation, the translation of select mRNAs is increased. Short open reading frames in the 5′ UPR of ATF4 mRNA allows translational initiation from the correct start codon, when eIF2α phosphorylation is increased [73]. ATF5 translation is also induced through a similar mechanism [74]. ATF4 is the major transcription factor that executes UPR gene regulation downstream of PERK signaling pathway [75, 95]. In response to ER stress, ATF4 induces genes largely involved in amino acid metabolism and cellular redox control. Therefore, $ATF4^{-/-}$ cells require supplementation with non-essential amino acids and reducing agents for their survival [75]. It has been suggested that oxidative protein folding in the ER generates reactive oxygen species [96], and that ATF4 plays an important role in the removal of reactive oxygen species under ER stress conditions.

The UPR signaling pathway controlled by PERK is important for cell survival under various ER stress conditions. Accumulation of free cholesterol in the ER induces ER stress and apoptosis of macrophages in advanced atherosclerosis [97]. $PERK^{-/-}$ macrophages are protected from free cholesterol induced apoptosis, indicating a cytoprotective role of PERK. Inactivation of the PERK signaling pathway impairs the survival of tumor cells under hypoxic conditions in tumor allograft models [98, 99].

ATF4 is directly upstream of CHOP, an important PERK-regulated UPR target gene [73, 75, 100, 101]. CHOP has long been considered as a proapoptotic transcription factor, as CHOP deficient cells were resistant to ER stress-induced apoptosis [102, 103]. For example, CHOP-deficient macrophages are protected from free cholesterol-induced apoptosis [97]. Similarly, CHOP deletion ameliorates pancreatic β cell loss in Akita mice that express mutant misfolded insulin protein that evokes ER stress [104]. CHOP induces GADD34 transcription, which dephosphorylates eIF2α to restore protein translation during prolonged ER stress [100]. In the absence of CHOP or GADD34, eIF2α phosphorylation is maintained during chronic ER stress, reducing protein synthesis and ER stress. Therefore, mice lacking CHOP or GADD34 were protected from kidney damage in a tunicamycin-induced ER stress model [100].

NF-E2-related factor-2 (Nrf2), a protein that transcriptionally induces enzymes that remove reactive oxygen species in response to oxidative and xenobiotic stress, is also activated by PERK mediated phosphorylation [105, 106]. It is conceivable that PERK maintains the cellular redox status by activating Nrf2 in response to the accumulation of reactive oxygen species that are generated during the unfolded protein response.

PERK is essential for the development of pancreatic β cells and osteoblasts in the skeletal system. Mutation of the PERK gene in humans is responsible for Wolcott-Rallison syndrome (WRS), a syndrome characterized by early onset diabetes due to β cell dysfunction, multiple skeletal dysplasia and growth retardation [107–109]. Germline deletion of the PERK gene in mice results in similar defects of insulin production and skeletal abnormalities [110, 111]. PERK deletion also compromises zymogen production from the exocrine pancreas, accompanied by acinar cell apoptosis [110–113]. These data suggest that PERK is essential for the development of these secretory cells, consistent with its molecular function in transcriptional and translational control in response to ER stress, and that its absence causes apoptotic cell death secondary to increased ER stress. Surprisingly, however, PERK is not required for the postnatal insulin secreting function of β cells [114]. Hence, post-natal deletion of PERK specifically in β cells by using the cre/lox system did not induce β cell death or diabetes, demonstrating a temporal-specific control of β cell development by this branch of the UPR [111]. Similarly, a recent report suggested that the loss of acinar cells in the exocrine pancreas of PERK deficient mice is not related to an impaired ER stress response [113]. Neither the ER structure of the acinar cells, nor the secretagogue-induced exocytosis of zymogens from the pancreatic lobules was compromised in $PERK^{-/-}$ mice. No evidence for increased ER stress in PERK-deficient pancreas was observed. Nonetheless, PERK-deficient mice displayed progressive pancreatic atrophy, losing acinar cells by oncosis, a non-apoptotic form of cell death, starting at ∼3 weeks of age. These observations led to the

conclusion that PERK is essential for the integrity of acinar cells in a manner which is unrelated to the ER stress response.

ATF6

ATF6 comprises two isoforms, ATF6α and ATF6β, which are encoded by separate genes [76, 79, 80]. ATF6 belongs to a group of transcription factors that are synthesized as precursor proteins with ER transmembrane domains and proteolytically activated by site 1 and site 2 proteases [115, 116]. ATF6 is normally present in the ER forming a stable complex with BiP [117]. ER stress dissociates ATF6 from BiP, which allows the mobilization of ATF6 to the Golgi apparatus for sequential proteolysis by S1P and S2P, releasing the N-terminal cytosolic part of the protein [77–79].

The DNA binding specificity of ATF6 and XBP1 is remarkably similar. Independent studies identified almost identical consensus sequences as binding sites of XBP1 and ATF6 [67, 82]. Clauss et al. demonstrated that recombinant XBP1 protein bound to CRE-like GAT-GACGTG(T/G) NNN(A/T)T sequences [67]. Wang et al. demonstrated that ATF6 bound to TGACGTG(G/A) consensus sequences [82]. The UPRE motif which refers to the ATF6 binding site can be activated by both XBP1 and ATF6 [68]. XBP1 and ATF6 can also form a hetero-dimer, suggesting that they might regulate common target genes [81]. Microarray analysis revealed ATF6α target genes are limited to select ER chaperones and ERAD pathway genes, suggesting that XBP1 has a broader universe of target genes [68, 118]. ATF6$\alpha^{-/-}$ MEF cells and animals show increased susceptibility to tunicamycin, suggesting a protective role of ATF6α in the ER stress response [10, 81]. The physiological functions of ATF6α and ATF6β remain to be determined, however, as mice with targeted disruption of these genes are grossly normal, although ATF6α/β double mutant mice die early during embryonic development [10, 81]. It will be interesting to test if ATF6α has a redundant or supplementary role with XBP1 in the development and/or function of secretory cells.

Role of the UPR in lipid metabolism

Role of XBP1 in hepatic lipogenesis

A crucial function of the liver is to coordinate whole body energy homeostasis by regulating carbohydrate and lipid metabolism. Fasting stimulates glucose production from the liver through glycogenolysis and gluconeogenesis to maintain optimal circulating glucose levels, while suppressing fatty acid synthesis. Hepatic lipogenesis is induced upon ingestion of excess carbohydrates to convert extra carbohydrates to triglyceride (TG) [13]. TG is secreted from the liver as VLDL lipoprotein particles, and then transported to adipose tissue for long-term energy storage. Hepatic lipogenesis is controlled by transcription factors such as SREBP and ChREBP, which are regulated by nutritional and hormonal conditions [2–5] (Fig. 2). SREBP-1c is strongly activated by insulin and increases the expression of genes controlling fatty acid synthesis [119–121]. SREBP-2 activation is controlled by cellular sterol levels, so that low sterol activates SREBP-2 to induce genes in the cholesterol biosynthesis pathway [30, 122]. ChREBP appears to be activated by high glucose to activate genes involved in glycolysis and fatty acid synthesis [34, 38].

Microarray analyses revealed that the majority of XBP1 target genes are involved in protein secretory pathways [68, 70]. XBP1 also activates the phospholipid biosynthesis pathway at least by increasing the level of choline cytidylyltransferase protein, a rate-limiting enzyme in the CDP-choline pathway [123, 124]. The molecular function of XBP1 is highly reminiscent of yeast HAC1 protein, which also controls the expression of genes in protein secretory pathways and lipid synthesis (INO1) [62]. Given that protein secretion requires ER expansion and the trafficking of secretory vesicles, it makes sense that the UPR also activates lipid synthesis to meet the demand of lipid bilayer formation [125]. Consistent with its target gene signature, XBP1 is critically required for the survival and the secretory functions of highly secretory cells [83–85]. As discussed above, however, XBP1 is not essential for the secretory function of hepatocytes [8]. The liver is the major source of plasma proteins but plasma protein levels were only slightly decreased in mice lacking XBP1 in the liver. The ER in XBP1-deficient hepatocytes displayed no morphological abnormality. The secretion and turnover of apolipoprotein B (ApoB) protein was minimally influenced by XBP1 deficiency, collectively suggesting that the basal expression of genes in secretory pathway independent of XBP1 is sufficient to accommodate the secretory load of postnatal hepatocytes.

However, XBP1 deletion in the liver resulted in a dramatic reduction of plasma lipids [8]. Genes encoding key lipogenic enzymes such as Acc2, Dgat2 and Scd1 were dramatically downregulated in XBP1-deficient liver. These lipogenic enzymes were induced upon high carbohydrate diet feeding in WT, but not in XBP1-deficient mouse liver. Consistent with the gene expression profile, de novo synthesis of fatty acids is impaired in XBP1-deficient hepatocytes. Sterol synthesis was also downregulated in XBP1-deficient hepatocytes, although the underlying mechanism is unknown. Given the essential role of XBP1 in the ER stress response, one might ask whether Xbp1

deletion caused ER stress in the liver, and consequently hypodyslipidemia as an indirect effect. Indeed, a recent study demonstrated that acute ER stress induced hepatic steatosis, albeit expression of lipogenic genes was suppressed, suggesting that ER stress in the liver disrupts hepatic lipid metabolism [11]. It has been shown that ER stress inhibits ApoB100 folding and VLDL secretion, which might be responsible for the fat accumulation in the liver in tunicamycin-injected mice [126–128]. Unlike the tunicamycin-injected mice, XBP1-deficient mice did not develop hepatic steatosis, and their hepatocytes did not show any signs of ER stress. In addition to the normal turnover of ApoB protein, XBP1-independent UPR markers were not induced at baseline, and were normally induced upon tunicamycin challenge in the absence of XBP1. These data argue against the possibility that XBP1 deletion disturbed ER homeostasis, which then resulted in defective hepatic lipid metabolism. Instead, chromatin immunoprecipitation assays demonstrated that XBP1 can directly bind to the promoter region of lipogenic genes. Further, enforced expression of XBP1 induced the expression of Acc2 and Dgat2 genes, suggesting that XBP1 is a bona fide transcription factor that controls hepatic lipogenesis (Fig. 2). Active XBP1s protein is induced in mice fed a high fructose diet and in hepatocytes cultured in high glucose media, suggesting that increased glucose availability induces XBP1. The nature of the signals that leads to the activation of IRE1/XBP1 and the role of these proteins in the development of metabolic diseases associated with hyperdislipidemia remain to be further investigated.

PERK/eIF2α

Homozygous eIF2α S51A mutant mice die perinatally due to hypoglycemia caused by defective hepatic gluconeogenesis [129]. In contrast, heterozygous eIF2α S51A mice are prone to weight gain on a high fat diet and develop insulin resistance [130]. To investigate the function of the PERK/eIF2α UPR branch in the liver, Oyadomari et al. generated transgenic mice that overexpressed the c-terminal fragment of GADD34 protein in the liver [9]. GADD34 is a regulatory component of eIF2α phosphatase, induced by PERK signaling upon ER stress [131]. Phosphorylation of eIF2α both at baseline and after ER stress induction was dramatically reduced in GADD34 transgenic mice, which displayed markedly decreased hepatic lipogenesis. Expression of lipogenic enzymes such as Fasn, Acc2 and Scd1 was decreased, especially on high fat diet. Lipid accumulation in the transgenic liver was also reduced, suggesting that PERK/eIF2α signaling contributes to optimal hepatic lipogenesis. Enforced activation of PERK signaling can be achieved by using the chimeric PERK protein fused to a ligand-induced dimerization domain [9, 95]. Ligand-induced PERK activation increased the expression of lipogenic enzymes independent of ER stress, suggesting that PERK activation is sufficient for the activation of lipogenesis in the liver. PERK controls the expression of C/EBPα and C/EBPβ, which play important roles in hepatic lipogenesis [132, 133]. C/EBPα, C/EBPβ, and their downstream transcription factor, PPAR-γ, were significantly downregulated in high fat-diet fed GADD34 transgenic mouse liver. Interestingly, C/EBP gene expression is regulated at both the transcriptional and translational level by XBP1 and eIF2α [9, 134, 135]. Further, the upstream open reading frame (uORF) in C/EBP mRNA directs the production of a truncated form of the C/EBP protein in the absence of eIF2α phosphorylation [135]. eIF2α phosphorylation further increases the translation efficiency of the full-length C/EBP in a manner similar to ATF4 [73]. These data suggest an active role for the PERK-eIF2α signaling pathway in regulation of hepatic lipogenesis (Fig. 4). A recent study demonstrated that PERK is also critical for lipogenesis in the mammary gland. Bobrovnikova-Marjon et al. deleted the Perk gene specifically in mammary glands by using an MMTV-cre [136]. Pups nursed by PERK-deficient female mice showed growth retardation, and mammary glands from PERK-deficient mothers exhibited an immature phenotype with reduced alveolar expansion. Free fatty acid and TG content was significantly reduced in milk produced from these mothers, accounting for the growth retardation of the pups.

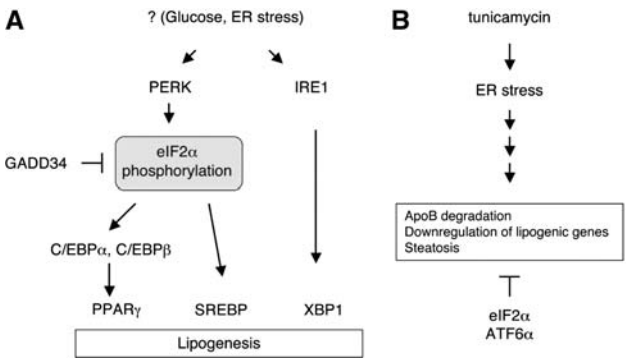

Fig. 4 Intersection of the UPR and hepatic lipid metabolism. **a** PERK/eIF2α and IRE1/XBP1 UPR signaling pathways control lipogenesis. eIF2α phosphorylation activates PPARγ and SREBP-1 in liver and mammary gland to increase the lipid synthesis. XBP1 activates the expression of the lipogenic genes, at least partly via direct interaction to target promoters. It is not fully understood what triggers UPR activation leading to increased lipogenesis. ER stress and high glucose may play a role. **b** Tunicamycin administration induces the ER stress and hepatic steatosis. Ironically, SREBP1 and lipogenic genes are downregulated by tunicamycin. Inhibition of ATF6α and eIF2α signaling pathways further increase fat accumulation, suggesting their protective roles in tunicamycin-induced steatosis. Tunicamycin induces degradation of ApoB protein, which is essential for VLDL secretion from the liver

The reduced lipid content in the milk was the direct consequence of low de novo lipid synthesis as SREBP1 and key lipogenic enzyme genes were significantly downregulated in PERK$^{-/-}$ mammary glands, suggesting that PERK transcriptionally regulates mammary gland lipogenesis via SREBP1. SREBP1 protein level was low in PERK-deficient mammary epithelial cells cultured under ER stress conditions, which activate SREBP processing, although the physiological significance of this observation is unknown [137, 138]. These data suggest that PERK controls mammary gland lipogenesis through controlling activation of SREBP.

UPR in Yeast

In yeast, phospholipid biosynthesis is dynamically regulated by the availability of inositol in the media [139, 140]. When the inositol level is low, the master negative regulator of the yeast lipogenesis program, OPI1p, is attached to the ER membrane through its interaction with phosphatidic acid and Scs2p (Fig. 5). The addition of inositol consumes phosphatidic acid to produce phosphatidylinositol, resulting in the release of OPI1p from the ER membrane, which translocates to the nucleus and represses the expression of genes involved in phospholipid biosynthesis [141]. This repression occurs through the UAS_{INO} promoter element where OPI1p antagonizes the transcriptional activators, Ino2p and Ino4p [142, 143]. Microarray analyses revealed that inositol controls the expression of a group of genes including key lipogenic enzymes, transcription regulators, as well as inositol and choline transporters in an Ino-2p-Ino4p dependent manner [144, 145].

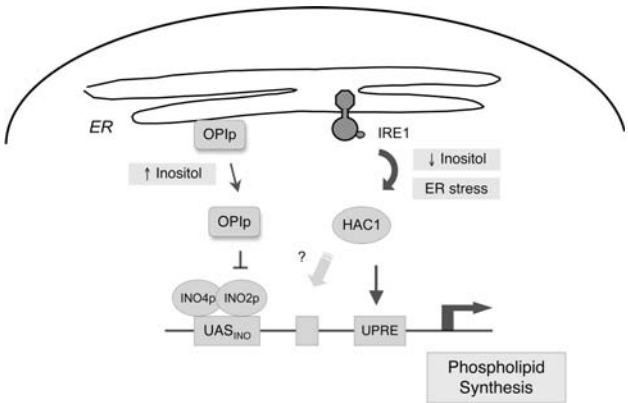

Fig. 5 Transcriptional regulation of lipid synthesis in yeast. OPI1p is a critical repressor of lipid synthesis in yeast. It is anchored in the ER membrane through its interaction with Scs2p (reviewed in [185]). Inositol starvation activates INO2p–INO4p lipogenic transcription factors by preventing the release of OPI1p from the ER which antagonizes INO2p. Inositol starvation also activates the UPR. INO1 expression is impaired in IRE1 or HAC1 mutant yeasts, which display inositol auxotrophy. The mechanism by which the UPR contributes to the expression of lipogenic genes is not fully understood

Interestingly, inositol deficiency also activates the UPR in yeast, inducing IRE1-mediated HAC1 mRNA splicing, although it is not known whether ER stress is induced under this condition [53]. Induction of UPR target genes including KAR2 by inositol deficiency is dependent on IRE1/HAC1. Notably, HAC1Δ and IRE1Δ strains of yeasts display inositol auxotrophy [146]. INO1 expression is impaired in these mutant yeast strains, and their inositol auxotrophy is rescued by OPI1 deletion or enforced expression of INO1, suggesting that IRE1/HAC1 is required for the induction of INO1 [125]. However, HAC1 is not sufficient for the expression of lipogenic genes that are regulated by OPI1, as constitutively active HAC1 is not sufficient to maintain the expression of these genes after inositol addition [144]. It has been suggested that HAC1 may de-repress OPI1p through heterodimerization to activate lipogenic genes [125]. However, other studies demonstrated the normal expression of INO1 in certain compound mutant yeast strains lacking HAC1, suggesting the minimal role of the UPR in INO1 expression under certain conditions [147, 148]. These data collectively suggest that the IRE1/HAC1 UPR pathway is required for the optimal expression of phospholipid biosynthesis genes in yeast. In addition, considering the role of XBP1 in membrane biogenesis and hepatic lipogenesis in mammals, lipid synthesis may be the evolutionarily conserved molecular function of the IRE1/HAC1/XBP1 pathway.

UPR activation by dysregulation of lipid homeostasis

UPR activation has been reported in the liver, adipose tissue, pancreatic islets, and brain under various genetic and nutritional conditions linked to obesity and insulin resistance [149–155]. Hotamisligil and colleagues first demonstrated the presence of ER stress in the liver of high fat diet-fed or leptin-deficient ob/ob mice [152]. Phosphorylation of PERK and its downstream target eIF2α was increased in the liver of these diet manipulated or genetically modified obese mice. ER stress inhibited insulin signaling by increasing the serine phosphorylation of insulin receptor substrate (IRS)-1, and this phosphorylation occurred via JNK activation by ER stress [156]. Induction of BiP in the liver of diabetic db/db mice was described as a marker of ER stress [151], although this finding was not reproduced in another study [157]. Wang and colleagues examined the effects of various diets on UPR activation in rat liver [150]. High sucrose or high levels of saturated fatty acids in the diet strongly activated the IRE1/XBP1 UPR pathway, as evidenced by increased XBP1 mRNA splicing, although the expression of XBP1 target genes was

not explored. In contrast, unsaturated fatty acids did not evoke the UPR, although hepatic TG levels were similarly increased. BiP and CHOP protein levels were ~2-fold increased by dietary sucrose or saturated fatty acids. UPR activation correlated with increased liver damage, suggesting that these diets induce ER stress that causes liver cell death. Several studies demonstrated that saturated fatty acids induced ER stress in cultured liver and pancreatic β cells, while unsaturated fatty acids reduced ER stress [158–161]. One recent study, however, demonstrated that monounsaturated oleic acids also induced ER stress and inhibited VLDL secretion in McA-RH7777 liver cells, suggesting that TG accumulation might induce ER stress in liver cells [128]. In contrast to these reports suggesting the induction of ER stress in fatty liver, others failed to detect the induction of ER stress markers in genetic models of steatosis [157, 162, 163]. Mice lacking microsomal triglyceride transfer protein (MTP) gene are unable to secrete VLDL and accumulate TG in the liver. Despite the TG accumulation, ER stress markers BiP and Grp94 were not induced in the liver of MTP deficient mice [163]. Dgat2 transgenic mice developed hepatic steatosis, but not insulin resistance [162]. JNK and PERK were not activated in the liver of a transgenenic mouse line that developed hepatic steatosis. It is unclear if ER stress is induced only under certain conditions of lipid accumulation in the liver. It is also possible that the limited specificity/sensitivity of the reagents to detect ER stress markers resulted in different outcomes.

ER stress was also reported in the hypothalamus of high fat diet-fed mice [154], although the direct cause of the ER stress was not described. ER stress inhibited leptin signaling in cultured cells, resulting in decreased STAT3 phosphorylation, linking ER stress to leptin resistance in the hypothalamus [154, 155].

Contrary to the induction of ER stress by lipids, recent studies suggested that ER stress can cause hepatic steatosis [10, 11]. Kaufman and colleagues demonstrated that tunicamycin, a potent ER stress inducer, induced hepatic steatosis. Surprisingly, however, tunicamycin markedly decreased the synthesis of lipogenic enzymes as well as SREBP1, suggesting that the tunicamycin-induced steatosis is unrelated to the increased lipogenesis [11]. It is not known why liver cells accumulate lipids under ER stress conditions. Given that ApoB protein folding and lipidation is highly sensitive to ER stress, tunicamycin might induce the accumulation of TG in the liver by inhibiting VLDL secretion, as seen in mice lacking ApoB or microsomal triglyceride transfer protein (MTTP) [126–128]. Indeed, plasma cholesterol and TG levels were markedly decreased in tunicamycin-injected mice, suggesting defective lipid secretion [11]. Interestingly, mice lacking ATF6α or harboring phosphorylation-defective eIF2α A/A mutation displayed further increase in lipid accumulation in the liver upon tuncamycin administration, suggesting a protective role of the UPR in tunicamycin-induced steatosis. Further studies are required to clarify the role of the UPR in this process, and its physiological relevance.

ER stress response in viral hepatitis

Hepatitis B (HBV) and hepatitis C (HCV) viruses are major pathogens causing acute and chronic hepatitis, cirrhosis and liver cancer in humans. Since the synthesis of viral structural proteins and the assembly of viral particles occur in the ER, one might expect that ER homeostasis is important for viral proliferation and disease pathogenesis. Indeed, numerous reports suggested the link between viral infection and the ER stress response. For example, it was reported that the HCV core protein induced ER stress by depleting the ER calcium storage, leading to apoptotic cell death [164]. Another study suggested that ER stress induced by core protein activated protein phosphatase 2A, which in turn inhibited interferon-γ signaling [165]. Activation of the UPR has also been observed in HCV-infected cells [166, 167] and in cells expressing viral proteins [168, 169], but not in another study [170]. Japanese encephalitis virus (JEV) and dengue viruses (DEN), which are flaviviruses like HCV, activated XBP1, which induced ER expansion and protected cells from virus-induced cytotoxicity, suggesting that flaviviruses may take advantage of the UPR for their propagation [171]. On the other hand, Siddiqui and colleagues demonstrated that HCV induced ER stress, but surprisingly, suppressed the transactivation ability of XBP1 and its downstream ERAD pathway, resulting in slow degradation of misfolded ER proteins [169]. Translation of HCV RNA utilizing the internal ribosome entry site was facilitated in IRE1-deficient cells, suggesting that HCV may promote its replication by suppressing the IRE1/XBP1 pathway [169]. Regarding HBV, large HBV surface antigens with deletions at the pre-S1 and pre-S2 regions, which are frequently detected in infected individuals, accumulated in the ER and activated GRP78 and GRP94 expression, suggesting the induction of ER stress [172, 173]. HBx, a regulatory protein encoded by HBV, activated XBP1 and ATF6 branches of the UPR [174]. Further studies are required to clarify the consequences of ER stress in viral infection, and the contribution of the UPR signaling pathways in viral propagation and host responses.

ER stress response in alcoholic liver disease

Alcohol consumption causes various types of liver damage including steatosis, hepatitis and cirrhosis. Interestingly, microarray analysis of liver samples from a murine model of intragastric ethanol feeding, which induced necrotic cell

death and steatosis, revealed the induction of genes related to the ER stress response [175]. Ethanol feeding increased the level of GRP78, GRP94, CHOP and caspase-12, as well as SREBP-1c, which may have contributed to the development of fatty liver [176]. It is not completely understood how ethanol consumption induces ER stress in the liver, but the increased level of homocysteine appears to play a role [177]. Plasma homocysteine level is increased upon alcohol consumption both in humans and mice [175, 178]. Homocysteine is known to induce ER stress and activate SREBP-1 processing in liver cells [179]. Betaine, which reduced the plasma homocysteine level in alcohol-fed mice, decreased the fat accumulation and the expression of ER stress response genes, suggesting the crucial role of homocysteine in alcoholic liver disease [175].

Concluding remarks

Recent studies have aroused great interest in the potential role of the UPR in energy metabolism in mammals. XBP1 plays a critical role in the synthesis of TG and cholesterol in the liver by controlling the expression of lipogenic genes. Deletion of XBP1 in the liver drastically lowered circulating TG and cholesterol levels. PERK also plays important role in both hepatic and mammary gland lipogenesis by regulating lipogenic transcription factors such as C/EBP, PPARγ, and SREBPs. It remained to be further investigated how the UPR is activated by nutritional and hormonal cues and interacts with other lipogenesis regulators, such as SREBP and ChREBP. Further studies are also required to define the role of the UPR in metabolic disorders accompanied by the dysregulation of lipid homeostasis.

UPR activation has been reported in the liver, adipose tissue, pancreatic β cells, skeletal muscle and brain under nutritional conditions associated with obesity and type 2 diabetes. Due to the technical difficulty of directly measuring actual ER stress as defined by an increase in unfolded protein species in the ER, or compromise of ER integrity, the UPR itself has been frequently used as a marker of ER stress. Given the active role of the UPR in cell metabolism and the lack of direct evidence of ER stress, one can ask if UPR activation is a causative factor in the development of metabolic dysregulation or is instead the consequence of ER stress. An assay system to measure actual ER stress in the cell [180] will be crucial to a better understanding of the role of the UPR in metabolic disorders. The mammalian UPR is comprised of at least three different signaling pathways, which are not necessarily activated simultaneously. A more thorough investigation of the activation status and function of each branch of the UPR will contribute to a better understanding of the UPR in metabolic disorders.

Acknowledgements Supported by the National Institutes of Health grants AI32412 and P01 AI56296 (L.H.G.), the Ellison Medical Foundation (L.H.G.), the American Heart Association, AHA0835610P (A.H.L.) and the Harvard University Technology Development Accelerator Fund (L.H.G.).

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