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REVIEW

From the unfolded protein response to metabolic diseases – lipids under the spotlight

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ABSTRACT

The unfolded protein response (UPR) is classically viewed as a stress response pathway to maintain protein homeostasis at the endoplasmic reticulum (ER). However, it has recently emerged that the UPR can be directly activated by lipid perturbation, independently of misfolded proteins. Comprising primarily phospholipids, sphingolipids and sterols, individual membranes can contain hundreds of distinct lipids. Even with such complexity, lipid distribution in a cell is tightly regulated by mechanisms that remain incompletely understood. It is therefore unsurprising that lipid dysregulation can be a key factor in disease development. Recent advances in analysis of lipids and their regulators have revealed remarkable mechanisms and connections to other cellular pathways including the UPR. In this Review, we summarize the current understanding in UPR transducers functioning as lipid sensors and the interplay between lipid metabolism and ER homeostasis in the context of metabolic diseases. We attempt to provide a framework consisting of a few key principles to integrate the different lines of evidence and explain this rather complicated mechanism.

KEY WORDS: Endoplasmic reticulum stress, Unfolded protein response, Lipid perturbation, Phospholipids, Metabolic diseases

Introduction

The key factors in the unfolded protein response (UPR) were identified during the search for factors required for endoplasmic reticulum (ER)-to-nucleus communication (Mori et al., 1992; Kohno et al., 1993; Mori et al., 1993; Cox et al., 1993; Cox and Walter, 1996). The establishment of the UPR field was rather serendipitous, but nevertheless it surprised the scientific community by setting up a paradigm of unconventional signal transduction. Since then, the field has been ever expanding, just like any other fundamental discovery. As a means of coping with misfolded proteins and restoring ER homeostasis, it is unsurprising that the UPR is directly linked to protein-misfolding disorders, such as Alzheimer's disease and Parkinson's disease (Hetz and Saxena, 2017). Cancer cells exploit the UPR to sustain their high demand for protein production, which is a prerequisite for unconditional proliferation (Urra et al., 2016). Current evidence also shows a clear association between the UPR and the hallmarks of metabolic syndrome, dyslipidemia and obesity (Basseri and Austin, 2012; Cnop et al., 2012). Dyslipidemia, characterized as an increase in fasting and postprandial serum triglyceride (TAG) and cholesterol levels, is a common feature observed in obese patients (Klop et al., 2013; Nguyen et al., 2008). Obesity is now a global health pandemic afflicting individuals of both developing and developed countries (NCD Risk Factor Collaboration, 2016). Obesity and dyslipidemia are contributing risk factors for metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D). NAFLD is the most prevalent liver disease worldwide that is marked by the abnormal accumulation of fat in the liver (Younossi et al., 2016). The progression of NAFLD to severe non-alcoholic steatohepatitis (NASH) results in chronic inflammation of hepatocytes, cirrhosis of hepatic tissue and hepatocellular carcinoma, culminating in terminal liver damage (Michelotti et al., 2013). Recent studies suggest that elevated levels of serum free saturated fatty acids, a consequence of dyslipidemia, play a role in the damage caused to hepatocytes during NAFLD (Li et al., 2009). Similarly, T2D has been associated with increased levels of triglycerides and free fatty acids, eventually leading to β-cell dysfunction and insulin resistance (Eto et al., 2002; Kelpe et al., 2002; Briaud et al., 2002). Contrary to the well-established roles of the UPR in resolving protein misfolding, the UPR as a contributor of lipotoxic cell death in the liver and pancreatic β-cells are poorly characterized (Alkhouri et al., 2009; DeFronzo, 2004). This Review will briefly describe the classical functions of the UPR in maintaining ER protein homeostasis, before discussing the importance of lipid species in ER membrane homeostasis. The different types of membrane irregularities affecting ER homeostasis mentioned here will be referred to collectively as 'lipid bilayer stress'. We will discuss insights that have been derived from the yeast *Saccharomyces cerevisiae* and substantiated with evidence from mammalian *in vitro* and *in vivo* models. We will also examine the intricacy between lipid bilayer stress and the UPR and emphasize the implications that are relevant for disease progression.

The unfolded protein response sensors

As an ancient counter-stress programme to ease the damaging effects in the ER, extensive efforts have been made to better understand the UPR. Typically, the accumulation of unfolded proteins in the ER activates the three ER-stress transducers, inositol-requiring enzyme 1α (Ire1α; also known as ERN1 in mammals), PRKR-like endoplasmic reticulum kinase (PERK; also known as EIF2AK3) and activating transcription factor 6 (ATF6), resulting in translational control and transcriptional reprogramming of UPR target genes to elicit global cellular changes (Walter and Ron, 2011) (Fig. 1).

Ire1α is the most evolutionarily conserved UPR sensor and it is also solely present in lower eukaryotes such as *S. cerevisiae* and *Schizosaccharomyces pombe* (referred to as ScIre1 and SpIre1, respectively, for clarity). The luminal domain of Ire1α directly binds to misfolded proteins through a dimeric interface peptide binding groove (Zhou et al., 2006; Gardner and Walter, 2011; Carrara et al., 2015), which triggers formation of dimers and higher-ordered oligomers leading to trans-autophosphorylation (Gardner and Walter, 2011). Phosphorylated Ire1α/ScIre1 possesses a ribonuclease activity that

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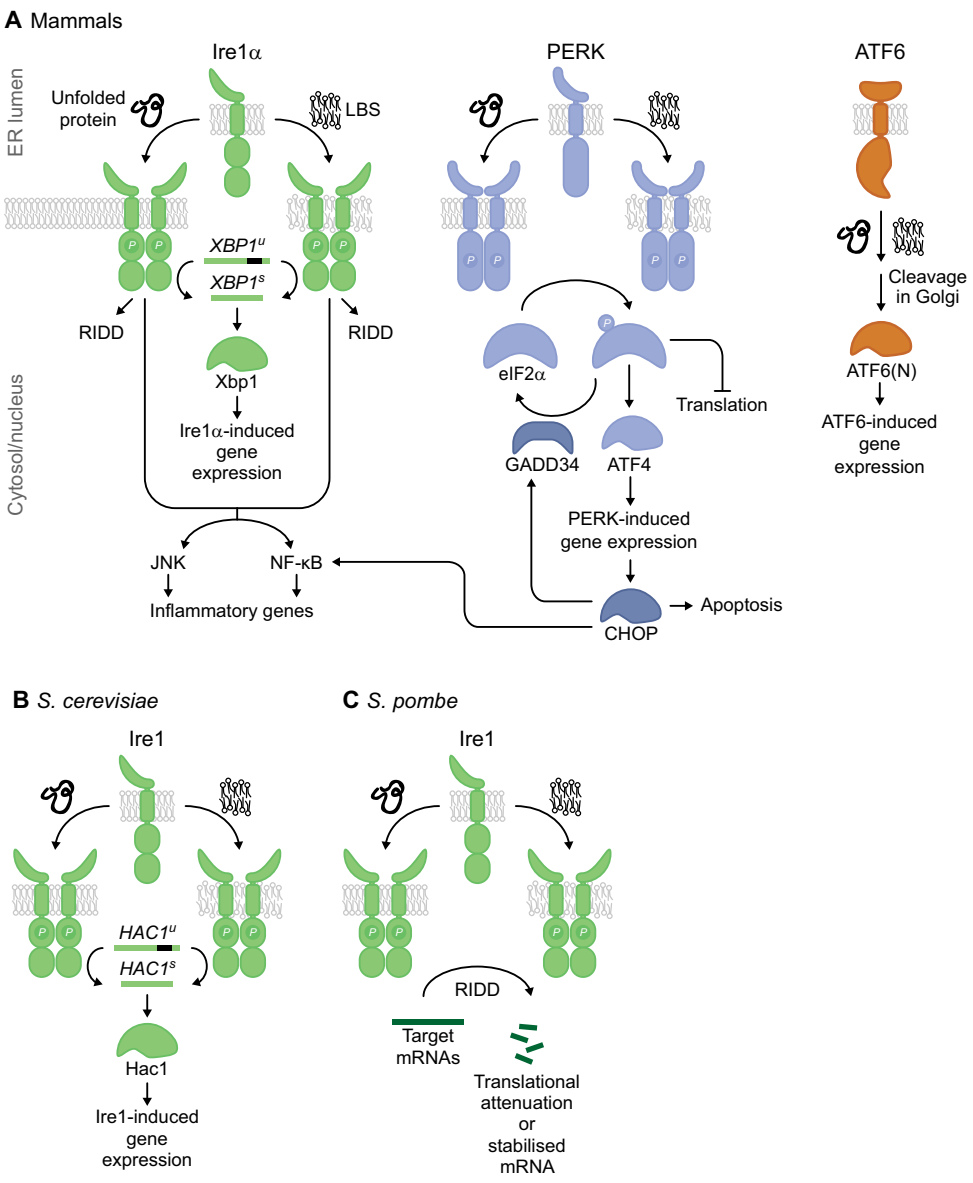

Fig. 1. Activation of the unfolded protein response (UPR). (A) In mammals, the accumulation of unfolded proteins or lipid bilayer stress (LBS) in the ER activates three distinct UPR sensors. Upon sensing of ER stress, monomeric Ire1α and PERK1 form dimers or higher oligomers, followed by trans-autophosphorylation. The Ire1α ribonuclease domain cleaves the intron of *XBP1* mRNAs, and newly synthesized XBP1 activates the transcription of UPR target genes. Ire1α may also initiate regulated Ire1-dependent decay (RIDD) of a specific subset of mRNA to decrease mRNA load. JNK and NF-κB are activated by Ire1 and initiate the transcription of proinflammatory genes. Activated PERK phosphorylates eIF2α, resulting in the temporary attenuation of general translation and the activation of the transcription factor ATF4, which subsequently activates the transcription of specific UPR target genes such as CHOP and GADD34 (shown in dark blue). CHOP is a pro-apoptotic factor that initiates cell death and is also linked to NF-κB activation. Subsequently, GADD34 promotes the dephosphorylation of eIF2α, resuming translation. When sensing unfolded proteins, the ATF6 luminal domain stimulates its relocalisation to the Golgi where the transcription factor is released by intramembrane proteolysis. Soluble ATF6 then translocates to the nucleus (N) where it upregulates the expression of a subset of UPR target genes. (B) *S. cerevisiae* Ire1 (ScIre1) undergoes dimerization or higher oligomerization and trans-autophosphorylation resulting in the splicing of *HAC1* mRNA by its RNase domain. Transcription factor Hac1 upregulates UPR target genes to restore homeostasis. (C) Activated *S. pombe* Ire1 (SpIre1) triggers downstream RIDD activity in a similar manner to Ire1α. CHOP, C/EBP-homologous protein; RIDD, IRE1-dependent decay; ^{s}, spliced; ^{u}, unspliced.

cleaves the introns of target mRNAs, such as the pre-mRNA of the transcription factor X-box binding protein 1 (*XBP1*) or orthologous to Atf/Creb1 (*HAC1*) in budding yeast (Prischi et al., 2014; Korennykh et al., 2011) (Fig. 1A,B). Importantly, its activation initiates a spliceosome-independent mRNA splicing event. Ire1α unconventionally splices *XBP1* (*XBP1*^{s}) at a dual stem loop to remove a 26 nucleotide segment (Lu et al., 2014). The remaining fragments are subsequently ligated by the tRNA splicing ligase RtcB, generating a product with a frameshift that allows *XBP1s* to be translated into a transcription factor to activate downstream cytoprotective genes (Calfon et al., 2002; Lu et al., 2014). The unconventional splicing of *HAC1* is initiated by the docking of its 3′ UTR to the cytosolic domain of oligomerized ScIre1 (van Anken et al., 2014). Only when the intron is removed by ScIre1 upon ER stress activation, will HAC1 spliced (*HAC1*^{s}) mRNA be translated and ligated by the tRNA ligase Rlg1 to function as a potent bZIP transcription factor (Fig. 1B). Hac1 recognizes the *cis*-acting element *UPRE* in the promoter regions and initiates a transcriptional programme, which consists of approximately 381 genes that are involved in many cellular processes, including the phospholipid metabolic pathway, ER-associated protein degradation (ERAD), protein translocation across the ER membrane, vesicular trafficking, glycosylation, cell wall biogenesis and vacuolar protein sorting (Travers et al., 2000). Genes regulated by both XBP1 and Hac1 collectively resolve ER stress by increasing the volume of the ER through membrane expansion (Schuck et al., 2009; Kim et al., 2015), enhanced protein folding and degradation pathways, as well as via repression of translation (Harding et al., 2002).

To further reduce protein load on the ER, Ire1α cleaves ER-localized mRNAs by an additional mechanism termed regulated Ire1-dependent decay of mRNA (RIDD) (Fig. 1A). Even though ScIre1 does not possess RIDD activity, its counterpart in *S. pombe* exclusively relies on RIDD to decrease mRNA load on the ER, because of the lack of *HAC1* or *XBP1* to initiate transcriptional responses (Fig. 1C). A group of 471 mRNAs were identified as substrates of SpIre1. They are cleaved at specific UG/C sites in the coding region and the 3′ UTR mRNA region (Kimmig et al., 2012; Guydosh et al., 2017). Most of these RIDD mRNAs encode secretory pathway proteins containing either a signal sequence or a transmembrane domain. Importantly, a subset of mRNAs cleaved

by SpIre1 is stabilized instead of being degraded, contributing to ER homeostasis. In metazoans, because Ire1α has dual activities for both unconventional splicing and RIDD, *XBP1* and RIDD substrates can be distinguished by the oligomerisation state of Ire1α (Tam et al., 2014). While splicing of *XBP1* or *HAC1* requires oligomerized Ire1α/ScIre1, RIDD activity is dependent only on monomeric Ire1α (Coelho and Domingos, 2014; Tam et al., 2014).

The other two UPR branches have also been well characterized. The major responding strategies of these branches are conceptually similar to Ire1α in that they also reduce ER load and/or induce UPR targets. Like Ire1α, PERK dimerizes and auto-phosphorylates upon the sensing of unfolded proteins by its luminal domain (Carrara et al., 2015). Once activated, PERK phosphorylates the eukaryotic translation initiation factor 2α (eIF2α), resulting in the temporary attenuation of overall protein translation, except for the mRNA of the transcription factor *ATF4*. ATF4 then activates the transcription of specific UPR target genes, such as CCAAT-enhancer-binding protein homologous protein (CHOP) and the growth arrest and DNA damage-inducible protein (GADD34) (Fig. 1A). CHOP is a transcription factor that triggers cell death by inducing genes involved in apoptosis in the presence of persistent stress (Nishitoh, 2012). GADD34, by contrast, counteracts the PERK pathway by dephosphorylating eIF2α to reverse translation attenuation and re-establish the balance (Novoa et al., 2001).

ATF6 is activated by regulated intramembrane proteolysis. Upon ER stress, ATF6 is packed into coat protein complex II (COPII) vesicles and transported to the Golgi (Schindler and Schekman, 2009). Cleavage of the luminal and transmembrane domain of ATF6 occurs by membrane-bound transcription factor site-1 protease (MBTPS1) and MBTPS2 in the Golgi, after which the N-terminal cytosolic fragment ATF6(N) localizes into the nucleus to activate UPR target genes (Fig. 1A).

The emerging roles of the UPR in metabolic syndrome

In recent years, great efforts have been undertaken to better understand the roles of cellular stress responses in metabolic syndromes. Surprisingly, the UPR, initially perceived as a means of counteracting protein misfolding, emerged as a key pathway (Shamu et al., 1994). In hindsight, the link should have been anticipated since the disruption of lipid homeostasis compromises ER function. As a stress-response pathway that senses ER stress and activates a transcription programme to control lipid biosynthetic genes, the UPR is ideally positioned to modulate any lipid disequilibrium. Indeed, either prolonged dysregulation of lipid synthesis or failure of UPR activation to resolve insults disrupt the homeostatic ER environment and leads to disease development.

The initial clue to such a role can be traced back prior to the inception of the UPR field. Ire1 was first identified in *S. cerevisiae* as a protein required for synthesis of inositol, the essential building block for phospholipids (Nikawa and Yamashita, 1992) (Fig. 2A), before it was later re-discovered as the master regulator of the UPR (Mori et al., 1993; Cox et al., 1993; Cox and Walter, 1996). Other evidence shows that induction of the UPR by ER-stress inducer drugs upregulates a series of lipid metabolic genes (Travers et al., 2000). Reciprocally, deletion of genes involved in lipid biosynthesis triggers activation of the UPR (Jonikas et al., 2009; Promlek et al., 2011; Thibault et al., 2012). These early insights appeared to be somewhat indirect until recent work provided solid evidence to justify the UPR as a lipid regulator (Volmer et al., 2013; Halbleib et al., 2017), as discussed below.

In mouse liver, Ire1α is also required for the assembly and secretion of very low-density lipoprotein (VLDL) and its secretion in hepatocytes (Wang et al., 2012). Upon deletion of *Ire1a*, excessive amounts of triglycerides accumulate in hepatocytes regardless of whether the animals have a fasting or high-fructose diet, whereas TAG biogenesis is unaffected. Downstream of Ire1α, XBP1 is required for normal fatty acid synthesis, and its deletion results in hypocholesterolemia and hypotriglyceridemia, in addition to activating the regulated Ire1-dependent decay (RIDD) activity of Ire1α (Lee et al., 2008). As targets of RIDD include mRNAs that encode proteins in lipogenesis and lipoprotein metabolism, diminishing the RIDD activity by knocking down *Ire1a* reverses hypolipidemia in *Xbp1*-deficent mice (So et al., 2012). In addition, XBP1 also serves as a regulator for lipogenesis and responds to high dietary carbohydrate levels by inducing genes that control fatty acid and cholesterol synthesis (Lee et al., 2008). Furthermore, *Xbp1*-deficient obese mice develop insulin resistance, suggesting that XBP1 has a role in protecting cells from metabolic disorders through the activation of UPR genes (Ozcan et al., 2004). Unexpectedly, XBP1 was shown to directly interact with the forkhead box O1 (FoxO1) transcription factor and target it for proteasomal degradation (Zhou et al., 2011). FoxO1 negatively regulates hepatic glucose metabolism by modulating expression and promoting degradation of hepatic gluconeogenic enzymes (Gross et al., 2008). This novel mechanism indicates that XBP1 has a dual role in modulating serum glucose levels, which is independent of the cytoprotective effects it exerts on the ER-folding capacity. These findings clearly undermine the protective roles of the Ire1 branch in maintaining hepatic lipid metabolism.

The PERK branch of the UPR is also involved in the metabolic response. CHOP, a downstream target of PERK, has been shown to activate the NF-κB pathway upon saturated free fatty acid (SFA)-induced lipotoxicity (Willy et al., 2015), suggesting a role in the pathogenesis of NASH and hepatic inflammation (Fig. 1A). CHOP-induced NF-κB promotes the secretion of proinflammatory cytokines, contributing to hepatic inflammation and the severity of NASH. SFA-induced lipotoxicity in apoptotic β-cells similarly induces the PERK–CHOP pathway and plays a role in development of NASH and T2D (Cunha et al., 2008). Rebalancing the PERK pathway by enforced expression of GADD34 to dephosphorylate hepatic Eif2α enhances glucose tolerance and attenuates hepatosteatosis in mice fed on a high-fat diet (Oyadomari et al., 2008) (Fig. 1A).

Although it is the least studied UPR activation pathway, ATF6 might also contribute to lipid homeostasis as it can be activated upon lipid bilayer stress (Maiuolo et al., 2011; Cunha et al., 2008). Ablation of *Atf6* in mice that are subjected to pharmacological ER stress leads to hepatosteatosis as a result of dysregulation of lipid metabolism, highlighting the critical role of ATF6 in maintaining phospholipid homeostasis (Yamamoto et al., 2010). Unlike Ire1α and PERK, evidence linking ATF6 to lipid homeostasis is still limited, perhaps due to a different activation mechanism. ATF6 is activated by COPII-mediated transport and cleaved in the Golgi. During lipid bilayer stress, altered lipids may affect the kinetics of COPII packaging and transport to the Golgi as well as proteolytic activity of MBTPS1 and MBTPS2.

ER membrane composition and UPR induction

The homeostatic environment of the ER relies on a fine-tuned balance between hundreds of membrane lipids and is essential in maintaining the stability of numerous membrane proteins and the proper folding equilibrium within the organelle (Thibault et al., 2012; Ng et al., 2017, preprint). Irregularities in lipid biosynthesis cause ER stress and trigger the activation of the UPR (Volmer and

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Ron, 2015) (Fig. 2). Here, both Ire1α and PERK can respond to lipid bilayer stress. Independent of sensing protein misfolding through their luminal domains, the transmembrane and proximal domains of Ire1α and PERK are positioned to monitor changes within the lipid bilayer. Importantly, Ire1α and PERK mutants that lack their luminal domains (ΔLD-Ire1α and ΔLD-PERK) and are thus unable to respond to the accumulation of unfolded proteins, still retained their activity in initiating the UPR, i.e. *XBP1* splicing and eIF2α phosphorylation, respectively, upon membrane saturation-induced lipid bilayer stress (Volmer et al., 2013) (Fig. 1A). Further work suggests that the N-terminal transmembrane domain of ScIre1 functions as an amphipathic helix to detect any biophysical changes at the ER membrane (Halbleib et al., 2017). Based on *in vivo*, *in vitro* and *in silico* evidence, the authors proposed that the transmembrane domain (TD) of Sclre1 adapts to lipid bilayer stress by dimerising to avoid a local membrane compression that is induced by the lumenal juxta-membrane amphipathic helix next to the ScIre1 TD. Reconstitution of this N-terminal amphipathic helix and the transmembrane helix into liposomes revealed a stabilising titled orientation assumed by the transmembrane helix in response to lipid bilayer stress conditions (increased lipid order) that allows for oligomerisation and Ire1 activation (Halbleib et al., 2017). PERK likely senses lipid disequilibrium through a similar mechanism, given that it has a high similarity to the Ire1α TD. Indeed, the Ire1α TD and PERK can be swapped without compromising the lipid-sensing activity (Volmer et al., 2013).

Saturated fatty acid palmitic acid is frequently added in large amounts to the growth medium to induce lipid bilayer stress in cell culture (Fig. 3). This excessive intake of SFA changes the metabolic flux of phospholipid synthesis and leads to an increased amount of saturated lipid species at the ER membrane (Borradaile et al., 2006). A high degree of fatty acid saturation at the biological membrane affects membrane fluidity by promoting the stiffening of the membrane (Quinn, 1981; Stubbs, 1983; Stubbs and Smith, 1984). It is likely that the dynamics of membrane–protein interactions are altered by the decrease in membrane fluidity, producing a signal that is eventually received by the UPR sensors and amplified in the initiation of the UPR programmes (Bogdanov et al., 2008). The SFA-induced UPR in INS-1E β-cells can be counteracted by supplementing growth medium with unsaturated oleic acids, bringing back the delicate balance between saturated and unsaturated FAs (Sommerweiss et al., 2013) (Fig. 3). Inhibiting the conversion of SFA to monounsaturated FA by targeting stearoyl-CoA desaturase genetically or pharmacologically leads to lipid-bilayer-stress-induced UPR, similar to results obtained when supplementing

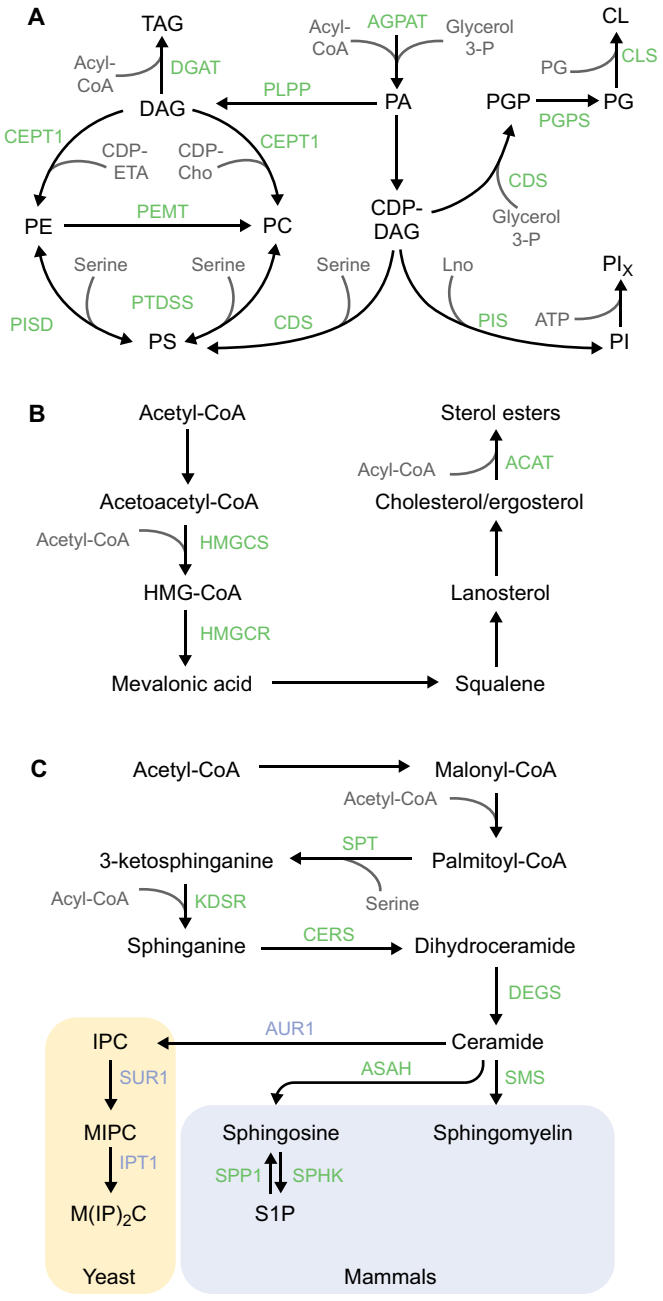

Fig. 2. Simplified schematic illustration of the major lipid metabolic pathways. (A) Phosphatidic acid (PA) is the product of two acyl-CoAs incorporated into glycerol 3-phosphate (glycerol 3-P). Converted from PA, diacylglycerol (DAG) is the precursor of phosphatidylethanolamine (PE), phosphatidylcholine (PC) and triacylglycerol (TAG) while cytidine diphosphate choline-DAG (CDP-DAG) is the precursor for phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylglycerol (PG). PI can be polyphosphorylated (PI_X) and PG can be dimerized to cardiolipin (CL). The *de novo* biosynthesis of PC from PE occurs in a three-step methylation reaction through the intermediates monomethyl-PE (MMPE) and dimethyl-PE (DMPE). PS is converted to PE via the *de novo* pathway. (B) Sterol ester biosynthesis pathway. Sterols are biosynthesized from a series of intermediate products including the rate-limiting step where 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is reduced into mevalonic acid. (C) Sphingolipid biosynthesis pathway. The condensation of serine to palmitoyl-CoA is the first rate-limiting step leading to ceramide through intermediate products. Ceramide can be further modified to form complex sphingolipids such as sphingosine-1-phosphate (S1P). Several sphingolipids and sterol intermediates are omitted in this figure for simplification. Proteins in green and blue refer to mammals and yeast, respectively. ACAT, acyl-coenzyme A:cholesterol acyltransferase; AGPAT, acylglycerolphosphate acyltransferase; ASAH, N-acylsphingosine amidohydrolase 1; AUR1, Inositol phosphorylceramide synthase; DGAT, diglyceride acyltransferase; CDP-Cho, cytidine diphosphate choline; CDP-ETA, cytidine diphosphate ethanolamine; CDS, CDP-diacylglycerol synthase; CEPT1, choline/ethanolamine phosphotransferase 1; CERS, ceramide synthase; coenzyme A, coenzyme A; CLS, cardiolipin synthase; HMGCR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HMGCS, 3-hydroxy-3-methyl-glutaryl-coenzyme A synthase; Ino, inositol; IPC, inositolphosphorylceramide; IPT1, inositolphosphotransferase 1; KDSR, 3-ketodihydrosphingosine reductase; MIPC, mannosylinositolphosphorylceramide; M(IP)2C, mannosyldinositolphosphorylceramide, PGP, phosphatidylglycerolphosphate; PGPS, phosphatidylglycerolphosphate synthase; PIS, phosphatidylinositol synthase; PLPP, Phospholipid phosphatase; SMS, sphingomyelin synthase; SPT, serine palmitoyltransferase; SPP1, sphingosine-1-phosphate phosphohydrolase; SPHK, sphingosine kinase; SUR1, mannosyl phosphorylinositol ceramide synthase SUR1.

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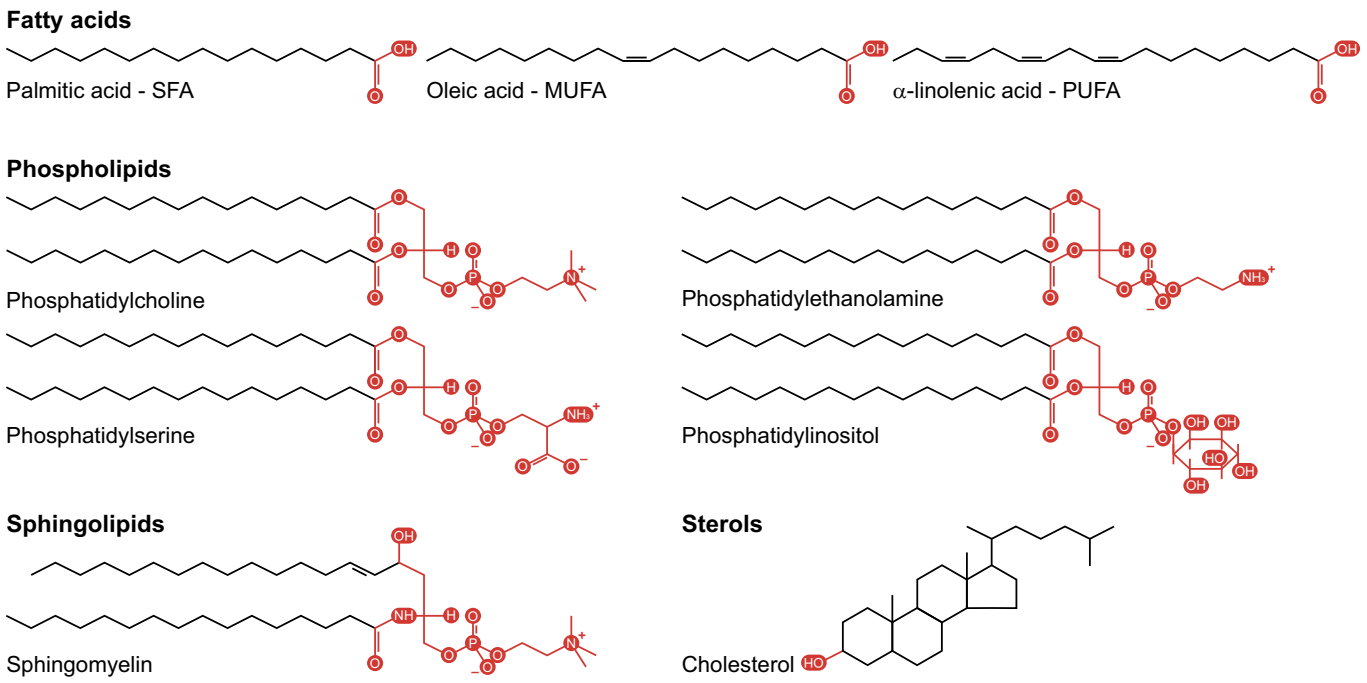

Fig. 3. Lipid classes and representatives. Structures in red and black highlight polar and nonpolar regions, respectively. Representative phospholipids are: phosphatidylcholine (PC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; phosphatidylethanolamine (PE), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; phosphatidylserine (PS), 1,2-dipalmitoyl-sn-glycero-3-phosphoserine and phosphatidylinositol (PI), 1,2-dipalmitoyl-sn-glycero-3-phospho-(1′-myo-inositol). Sphingomyelin is represented by N-palmitoyl-D-erythro-sphingosylphosphorylcholine and cholesterol by cholest-5-en-3β-ol. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

growth medium with SFA (Volmer et al., 2013; Ariyama et al., 2010). The mechanisms underlying these observations appear to be conserved throughout evolution. In *S. cerevisiae*, increases in membrane saturation owing to deletion of 5-aminolevulinate synthase *HEM1* or desaturase *OLE1* results in UPR activation (Pineau et al., 2009). Here, supplementation of monounsaturated fatty acids myristoleic acid (C14:1) and oleic acid (C18:1) to correct the unbalanced ratio between saturated and unsaturated phospholipids relieved ER stress, whereas SFA palmitic acid (C16:0) supplementation to *hem1*Δ cells further exacerbates the UPR activation (Pineau et al., 2009) (Fig. 3). Loss of *HEM1* leads to a defect in ergosterol and unsaturated FA synthesis (Pineau et al., 2008; Ferreira et al., 2004) (Fig. 2). Additionally, a yeast-based fatty-acid screening assay demonstrated that addition of the long-chain monounsaturated oleic acid is particularly effective to shut down UPR activation and overcome ER stress in *hem1*Δ cells (Deguil et al., 2011). Surprisingly, some long-chain polyunsaturated fatty acids that are absent in *S. cerevisiae* significantly improved the growth of *hem1*Δ cells. UPR induced by lipid bilayer stress can also be triggered by deletion of ubiquitin-like (UBX)-domain-containing protein (*UBX2*), an important component of the ERAD pathway. The absence of *UBX2* triggers the accumulation of saturated fatty acid, as the transcriptional control of the FA desaturase *OLE1* is impaired (Surma et al., 2013). The upregulation of *OLE1* is mediated through multicopy suppressor of Gam1 (*MGA2*) that is activated by the ERAD machinery. Interestingly, Mga2 was recently shown to be an important regulator of membrane fluidity by acting as a sensor of membrane lipid saturation and controlling unsaturated fatty acid synthesis mediated by the desaturase Ole1 (Covino et al., 2016). These findings suggest that membrane properties, such as those defined by the ratio between saturated and unsaturated phospholipids and acyl chain length, are constantly monitored by the UPR.

Another lipid feature important for ER homeostasis is the optimal ratio of phospholipids with different head groups (Lagace and Ridgway, 2013; Fagone and Jackowski, 2009). The ER membrane is composed predominantly of ∼40% phosphatidylcholine (PC) and 20% phosphatidylethanolamine (PE) in yeast, and in mammals of 55% PC and 30% PE, while phosphatidylinositol and phosphatidylserine are of lower abundance in both (van Meer et al., 2008; van Meer and de Kroon, 2011). An imbalance in the ratio between PC and PE has been shown to activate the UPR and to cause fatty liver (Fu et al., 2011; Gao et al., 2015). This can be explained by the distinct biophysical properties of these two classes: PC has a cylindrical shape allowing for better packing and bilayer forming propensity, whereas PE is conically shaped, thus lacking a bilayer-forming ability due to packing rigidity (Holthuis and Menon, 2014) (Fig. 3 and Fig. 4A,B). Thus, it is not surprising that changes in membrane integrity and rigidity that create imbalances between PC and PE levels are sensed by the UPR. The decrease in PE levels affects the trans-bilayer distribution of different PE species (Dawaliby et al., 2016; Williams et al., 2000) (Fig. 4B). The distribution of PE species amongst the membrane is critical for the function of membrane-associated processes, thus potentially affecting that of membrane proteins involved in UPR. Indeed, deletion of *CHO2* and *OPI3*, the major yeast genes involved in the biosynthesis of ER phospholipid PC, triggered hyperactivation of the UPR, which buffers the otherwise lethal defect this imbalance would have (Thibault et al., 2012). Ire1 activates the UPR directly from a decrease in PC independently of the Ire1 luminal domain, but the mechanism of activation is still unclear (Promlek et al., 2011). Mostly expressed in the liver, *PEMT*, the homologue of *OPI3*, is a methyltransferase required for *de novo* synthesis of PC from PE (Fig. 2A). *Pemt* deletion in mice (*Pemt*$^{-/-}$) causes liver failure after 3 days of choline-deficient diet (Walkey et al., 1998). The reduction of the PC/PE molar ratio in liver tissues

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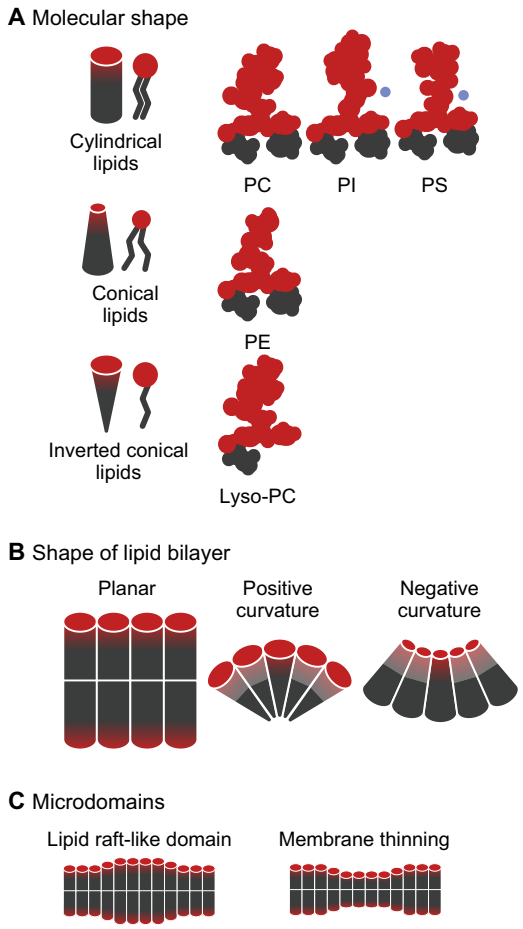

Fig. 4. Properties of a lipid bilayer affect its shape and fluidity. (A) Molecular shapes of lipids are defined by their polar headgroup (red) and fatty acid saturation states (grey). PC (phosphatidylcholine), PI (phosphatidylinositol) and PS (phosphatidylserine) form cylindrical-like shapes and PE resembles a cone, whereas phospholipids containing one instead of two acyl chain (lyso-PC) exhibit an inverted conical geometry. (B) Packaging of cylindrical lipids results in a planar-shaped lipid bilayer, whereas inverted-cone lipids and cylinders will be sorted into positively and negatively curved lipid bilayers, respectively. (C) Localized properties of lipids can result in a thickening or thinning of the lipid bilayer. For instance, clustering of cholesterol and glycosphingolipids will form a thicker microdomain, referred to as 'lipid raft-like' domains. Conversely, the concentration of phospholipids with short acyl chains will reduce the thickness of the lipid bilayer.

of both *Pemt*$^{-/-}$ mice and NASH human patients resulted in the loss of membrane integrity owing to the disproportional increase of the non-lamellar lipid-bilayer-forming PE, suggesting an increase in membrane curvature (Li et al., 2006; Beney and Gervais, 2001) (Fig. 4B). In addition to membrane integrity, aspects of membrane dynamics, including lipid–lipid and lipid–protein interactions, are also compromised by the loss of PC (Seu et al., 2006). In obese mice, the increased PC/PE ratio impairs the function of sarco-/endoplasmic reticulum calcium ATPase (SERCA) and activates the UPR (Fu et al., 2011). The impairment of the SERCA pump affects Ca^{2+} levels within the ER, thereby influencing chaperone functions and protein folding, which, consequently, activates the UPR. Furthermore, an imbalanced PC/PE ratio can also result in the aberrant activation of downstream signaling pathway and thus eventually might lead to disease manifestation. For example, the JNK signaling pathway is hyperactivated in cells with a dysregulated PC/PE ratio; this suppresses the insulin signaling pathway through phosphorylation of insulin receptor substrate-1 (IRS-1), which gives rise to insulin resistance, a hallmark of T2D (Ozcan et al., 2004). In addition, dysregulation of the PE biosynthetic pathway in hepatic steatosis was found to result in abnormal accumulation of neutral lipids, phospholipid remodeling and upregulation of lipogenic transcription factors, which all contributed to the development of the disease (Leonardi et al., 2009; Fullerton et al., 2009).

The ER membrane also contains sphingolipids, such as ceramide, sphingosine and sphingosine 1-phosphate (S1P), which are, however, only in low abundance compared with phospholipids (Fig. 2C and Fig. 3). Although it is unclear how alteration in sphingolipid biosynthesis is sensed by the UPR, it is evident that sphingolipids are necessary to maintain lipid homeostasis and prevent ER stress (Breslow, 2013). The first rate-limiting step in sphingolipid synthesis is carried out by the serine palmitoyltransferase complex (Spt), which is negatively regulated by the evolutionarily conserved proteins ORM1 and ORM2 (Futerman and Riezman, 2005) (Fig. 2C). Accordingly, deletion of *ORM1* or *ORM2* leads to lipid-mediated UPR activation (Han et al., 2010; Jonikas et al., 2009; Liu et al., 2012). Aberrant sphingolipid composition has also been found to be involved in the pathogenesis of obesity and insulin resistance. Indeed, ceramide accumulates in mice adipose and muscle tissues upon exposure to a high-fat diet (Shah et al., 2008). Furthermore, it has been shown that the accumulation of C16 ceramide upon UPR activation leads to cell death through an induction of pro-apoptotic factor Bax, which leads to cytochrome c release and mitochondrial apoptosis (Aflaki et al., 2012; Epstein et al., 2012; Pettus et al., 2002). In obese mice, cell death can be avoided by overexpression of sphingosine kinase 1 (SphK), which promotes the conversion of ceramide to S1P (Bruce et al., 2012) (Fig. 2C). S1P functions by attenuating the proinflammatory JNK pathway, thereby ameliorating high-fat-diet-induced insulin sensitivity partly by reducing inflammation of muscle cells. Importantly, it should be noted S1P-phosphohydrolase 1 (Spp1), a phosphatase of S1P, plays a role in maintaining ER homeostasis (Lépine et al., 2011) (Fig. 2C). S1P accumulation upon Spp1 deletion stimulates autophagy and ER stress, suggesting that the intricate regulatory mechanisms that are involved in sphingolipid metabolism are also required to maintain ER homeostasis.

Taken together, aberrant changes in lipid bilayer composition are sensed by the UPR and these have been implicated in the onset of diseases, particularly obesity and diabetes. Future studies are needed to explore the possibility of averting these processes by taking advantage of the accumulating knowledge in the UPR field. In this regard, searching for or designing compounds that pharmacologically modulate the UPR sensors and their downstream targets is a promising avenue.

Lipid-induced proteotoxicity

The dual function of the UPR in protein and lipid homeostasis suggests these two types of stress are interconnected. Indeed, in many cases, lipid disequilibrium induces proteotoxicity. For instance, SFA accumulation not only disrupts phospholipid homeostasis at the ER membrane, but also impacts on the morphology and integrity of the ER, thereby leading to disturbed ER proteostasis (Borradaile et al., 2006). In β-cells, supplementation of SFA activates all three UPR branches by affecting ER Ca^{2+} storage. Here, disturbance of the pump activity of SERCA decreases ER Ca^{2+} storage and thus compromises the function of the Ca^{2+}-dependent calnexin/calreticulin cycle that is required for glycoprotein folding and ER proteostasis (Cunha et al., 2008). Interestingly, treatment with the chemical chaperone

4-phenylbutyrate (4-PBA), which binds to ER misfolded proteins, rescued lipid bilayer stress-induced UPR, suggesting that proteotoxic stress is downstream of lipotoxicity (Pineau et al., 2009). In *S. cerevisiae*, lipid disequilibrium is also concomitant with the disruption of ER proteostasis (Borradaile et al., 2006; Deguil et al., 2011). Consequently, activation of the UPR is essential for proteome remodeling to counteract the global detrimental effects of lipid bilayer stress, such as the severe changes to global lipid levels, majorly affecting biological membranes (Thibault et al., 2012; Ng et al., 2017, preprint). In *C. elegans*, ablation of *mdt-15*, a regulator of lipid biosynthetic genes, leads to the activation of both Ire1α and PERK (Hou et al., 2014). Interestingly, in this instance, the activation of both UPR sensors was, however, found to be independent of proteotoxic stress (Hou et al., 2014). Because lipid bilayer stress caused by *mdt-15* is rather mild, this suggests the existence of a tipping point, beyond which any present lipid imbalance begins to exert additional burden on the proteostasis network. In either case however, the UPR is activated to cope with changes in protein and/or lipid homeostasis.

Conclusion and perspectives

As discussed above, there is now clear evidence for a link between the UPR and lipid homeostasis; in years to come, we anticipate a wave of new findings that will further reinforce this view. Unlike the classic roles of the UPR in maintaining protein homeostasis, its regulatory role in lipid metabolism appears to be more complex. Below, we attempt to provide some basic principles to integrate the different lines of evidence and consolidate this rather intricate mechanism.

First, the key factors in the UPR might have evolved additional features to cope with lipid bilayer stress. For example, XBP1 has been revealed to be a lipogenesis regulator, a function that is independent of its classical role in ER stress (Lee et al., 2008; Glimcher and Lee, 2009), and also controls the expression level of FoxO1 in the insulin signaling pathway through proteasomal degradation (Otoda et al., 2013). Second, the UPR sensors Ire1α/ScIre1 and PERK directly sense changes in ER lipid composition through their transmembrane domain – an activity that is independent of their luminal domains (Volmer et al., 2013; Halbleib et al., 2017).

It seems that the UPR, once activated, responds to lipid disequilibrium in a manner that is similar to its classic function. This includes transcriptional activation of lipid synthesis genes and other stress responsive factors (e.g. chaperones), controlling mRNAs of genes involved in lipogenesis and lipoprotein metabolism via RIDD activity, and tuning the overall translation through its downstream targets, such as CHOP and GADD34.

Even though we cannot exclude the possibility that new principles might emerge from future research, most of the current literature can largely fit into this framework, although our current understanding regarding the mechanistic roles of the UPR in lipid disequilibrium is far from complete. Nevertheless, with our current knowledge, it might already be feasible to design pharmacological interventions that modulate UPR activity under lipid disequilibrium. For example, chronic ER stress occurs in patients with obesity, NASH or T2D (Dara et al., 2011; Laybutt et al., 2007). Compounds that attenuate UPR activation or reverse the sustained phosphorylation of eIF2α, the transcription factor governing CHOP activity, should salvage hepatocytes or pancreatic cells that would otherwise undergo programmes leading to cell death (Hetz et al., 2013). Drugs that circumvent NASH and improve glucose tolerance by preventing ceramide accumulation are inhibitors of acid sphingomyelinase, called FIASMAs, as well as the marketed drug myriocin (FTY-720) for the treatment of multiple sclerosis, which inhibits serine palmitoyltransferase (SPT) (Bruce et al., 2013; Kasumov et al., 2015; Musso et al., 2016). Alternatively, direct targeting of ceramide for degradation with ceraminidase, which catalyses its degradation to sphingosine, has been shown to be effective in reducing high-fat-induced NASH and insulin resistance (Xia et al., 2015). The potential of newly developed therapeutic drugs should be able to circumvent inflammation that results from the hyperactivation of the UPR during conditions of altered lipid species, originating either from a high-fat diet or dysregulation of lipid metabolism and/or synthesis. With the ongoing research and explosion of technologies to study cellular lipids, we are optimistic that the UPR will become an excellent target for drug therapy and metabolic diseases, which will thus eventually be controllable, if not curable.

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Competing interests

The authors declare no competing or financial interests.

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References

Aflaki, E., Doddapattar, P., Radović, B., Povoden, S., Kolb, D., Vujić, N., Wegscheider, M., Koefeler, H., Hornemann, T., Graier, W. F. et al. (2012). C16 ceramide is crucial for triacylglycerol-induced apoptosis in macrophages. *Cell Death Dis.* **3**, e280.

Alkhouri, N., Dixon, L. J. and Feldstein, A. E. (2009). Lipotoxicity in nonalcoholic fatty liver disease: not all lipids are created equal. *Expert Rev. Gastroenterol. Hepatol.* **3**, 445-451.

Ariyama, H., Kono, N., Matsuda, S., Inoue, T. and Arai, H. (2010). Decrease in membrane phospholipid unsaturation induces unfolded protein response. *J. Biol. Chem.* **285**, 22027-22035.

Basseri, S. and Austin, R. C. (2012). Endoplasmic reticulum stress and lipid metabolism: mechanisms and therapeutic potential. *Biochem. Res. Int.* **2012**, 841362.

Beney, L. and Gervais, P. (2001). Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Appl. Microbiol. Biotechnol.* **57**, 34-42.

Bogdanov, M., Mileykovskaya, E. and Dowhan, W. (2008). Lipids in the assembly of membrane proteins and organization of protein supercomplexes: implications for lipid-linked disorders. *Subcell. Biochem.* **49**, 197-239.

Borradaile, N. M., Han, X., Harp, J. D., Gale, S. E., Ory, D. S. and Schaffer, J. E. (2006). Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J. Lipid Res.* **47**, 2726-2737.

Breslow, D. K. (2013). Sphingolipid homeostasis in the endoplasmic reticulum and beyond. *Cold Spring Harb. Perspect. Biol.* **5**, a013326.

Briaud, I., Kelpe, C. L., Johnson, L. M., Tran, P. O. T. and Poitout, V. (2002). Differential effects of hyperlipidemia on insulin secretion in islets of langerhans from hyperglycemic versus normoglycemic rats. *Diabetes* **51**, 662-668.

Bruce, C. R., Risis, S., Babb, J. R., Yang, C., Kowalski, G. M., Selathurai, A., LEE-Young, R. S., Weir, J. M., Yoshioka, K., Takuwa, Y. et al. (2012). Overexpression of sphingosine kinase 1 prevents ceramide accumulation and ameliorates muscle insulin resistance in high-fat diet-fed mice. *Diabetes* **61**, 3148-3155.

Bruce, C. R., Risis, S., Babb, J. R., Yang, C., LEE-Young, R. S., Henstridge, D. C. and Febbraio, M. A. (2013). The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. *Endocrinology* **154**, 65-76.

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G. and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415**, 92-96.

Carrara, M., Prischi, F., Nowak, P. R. and Ali, M. M. (2015). Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling. *EMBO J.* **34**, 1589-1600.

Cnop, M., Foufelle, F. and Velloso, L. A. (2012). Endoplasmic reticulum stress, obesity and diabetes. *Trends Mol. Med.* **18**, 59-68.

Coelho, D. S. and Domingos, P. M. (2014). Physiological roles of regulated Ire1 dependent decay. *Front. Genet.* **5**, 76.
Covino, R., Ballweg, S., Stordeur, C., Michaelis, J. B., Puth, K., Wernig, F., Bahrami, A., Ernst, A. M., Hummer, G. and Ernst, R. (2016). A eukaryotic sensor for membrane lipid saturation. *Mol. Cell* **63**, 49-59.
Cox, J. S. and Walter, P. (1996). A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **87**, 391-404.
Cox, J. S., Shamu, C. E. and Walter, P. (1993). Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* **73**, 1197-1206.
Cunha, D. A., Hekerman, P., Ladriere, L., Bazarra-Castro, A., Ortis, F., Wakeham, M. C., Moore, F., Rasschaert, J., Cardozo, A. K., Bellomo, E. et al. (2008). Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *J. Cell Sci.* **121**, 2308-2318.
Dara, L., Ji, C. and Kaplowitz, N. (2011). The contribution of endoplasmic reticulum stress to liver diseases. *Hepatology* **53**, 1752-1763.
Dawaliby, R., Trubbia, C., Delporte, C., Noyon, C., Ruysschaert, J.-M., Van Antwerpen, P. and Govaerts, C. (2016). Phosphatidylethanolamine is a key regulator of membrane fluidity in eukaryotic cells. *J. Biol. Chem.* **291**, 3658-3667.
Defronzo, R. A. (2004). Dysfunctional fat cells, lipotoxicity and type 2 diabetes. *Int. J. Clin. Pract. Suppl.* **58**, 9-21.
Deguil, J., Pineau, L., Rowland Snyder, E. C., Dupont, S., Beney, L., Gil, A., Frapper, G. and Ferreira, T. (2011). Modulation of lipid-induced ER stress by fatty acid shape. *Traffic* **12**, 349-362.
Epstein, S., Kirkpatrick, C. L., Castillon, G. A., Muñiz, M., Riezman, I., David, F. P. A., Wollheim, C. B. and Riezman, H. (2012). Activation of the unfolded protein response pathway causes ceramide accumulation in yeast and INS-1E insulinoma cells. *J. Lipid Res.* **53**, 412-420.
Eto, K., Yamashita, T., Matsui, J., Terauchi, Y., Noda, M. and Kadowaki, T. (2002). Genetic manipulations of fatty acid metabolism in beta-cells are associated with dysregulated insulin secretion. *Diabetes* **51** Suppl. 3, S414-S420.
Fagone, P. and Jackowski, S. (2009). Membrane phospholipid synthesis and endoplasmic reticulum function. *J. Lipid Res.* **50** Suppl., S311-S316.
Ferreira, T., Régnacq, M., Alimardani, P., Moreau-Vauzelle, C. and Bergès, T. (2004). Lipid dynamics in yeast under haem-induced unsaturated fatty acid and/or sterol depletion. *Biochem. J.* **378**, 899-908.
Fu, S., Yang, L., Li, P., Hofmann, O., Dicker, L., Hide, W., Lin, X., Watkins, S. M., Ivanov, A. R. and Hotamisligil, G. S. (2011). Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473**, 528-531.
Fullerton, M. D., Hakimuddin, F., Bonen, A. and Bakovic, M. (2009). The development of a metabolic disease phenotype in CTP: phosphoethanolamine cytidylyltransferase-deficient mice. *J. Biol. Chem.* **284**, 25704-25713.
Futerman, A. H. and Riezman, H. (2005). The ins and outs of sphingolipid synthesis. *Trends Cell Biol.* **15**, 312-318.
Gao, X., Van der Veen, J. N., Vance, J. E., Thiesen, A., Vance, D. E. and Jacobs, R. L. (2015). Lack of phosphatidylethanolamine N-methyltransferase alters hepatic phospholipid composition and induces endoplasmic reticulum stress. *Biochim. Biophys. Acta* **1852**, 2689-2699.
Gardner, B. M. and Walter, P. (2011). Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* **333**, 1891-1894.
Glimcher, L. H. and Lee, A.-H. (2009). From sugar to fat: how the transcription factor XBP1 regulates hepatic lipogenesis. *Ann. N. Y. Acad. Sci.* **1173** Suppl. 1, E2-E9.
Gross, D. N., van den Heuvel, A. P. J. and Birnbaum, M. J. (2008). The role of FoxO in the regulation of metabolism. *Oncogene* **27**, 2320-2336.
Guydosh, N. R., Kimmig, P., Walter, P. and Green, R. (2017). Regulated Ire1-dependent mRNA decay requires no-go mRNA degradation to maintain endoplasmic reticulum homeostasis in S. pombe. *Elife* **6**, e29216.
Halbleib, K., Pesek, K., Covino, R., Hofbauer, H. F., Wunnicke, D., Hänelt, I., Hummer, G. and Ernst, R. (2017). Activation of the unfolded protein response by lipid bilayer stress. *Mol. Cell* **67**, 673-684.e8.
Han, S., Lone, M. A., Schneiter, R. and Chang, A. (2010). Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. *Proc. Natl. Acad. Sci. USA* **107**, 5851-5856.
Harding, H. P., Calfon, M., Urano, F., Novoa, I. and Ron, D. (2002). Transcriptional and translational control in the Mammalian unfolded protein response. *Annu. Rev. Cell Dev. Biol.* **18**, 575-599.
Hetz, C. and Saxena, S. (2017). ER stress and the unfolded protein response in neurodegeneration. *Nat. Rev. Neurol.* **13**, 477-491.
Hetz, C., Chevet, E. and Harding, H. P. (2013). Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov.* **12**, 703-719.
Holthuis, J. C. M. and Menon, A. K. (2014). Lipid landscapes and pipelines in membrane homeostasis. *Nature* **510**, 48-57.
Hou, N. S., Gutschmidt, A., Choi, D. Y., Pather, K., Shi, X., Watts, J. L., Hoppe, T. and Taubert, S. (2014). Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo. *Proc. Natl. Acad. Sci. USA* **111**, E2271-E2280.
Jonikas, M. C., Collins, S. R., Denic, V., Oh, E., Quan, E. M., Schmid, V., Weibezahn, J., Schwappach, B., Walter, P., Weissman, J. S. et al. (2009). Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. *Science* **323**, 1693-1697.
Kasumov, T., Li, L., Li, M., Gulshan, K., Kirwan, J. P., Liu, X., Previs, S., Willard, B., Smith, J. D. and McCullough, A. (2015). Ceramide as a mediator of non-alcoholic Fatty liver disease and associated atherosclerosis. *PLoS ONE* **10**, e0126910.
Kelpe, C. L., Johnson, L. M. and Poitout, V. (2002). Increasing triglyceride synthesis inhibits glucose-induced insulin secretion in isolated rat islets of langerhans: a study using adenoviral expression of diacylglycerol acyltransferase. *Endocrinology* **143**, 3326-3332.
Kim, S. K., Oh, E., Yun, M., Lee, S.-B. and Chae, G. T. (2015). Palmitate induces cisternal ER expansion via the activation of XBP-1/CCTalpha-mediated phospholipid accumulation in RAW 264.7 cells. *Lipids Health Dis.* **14**, 73.
Kimmig, P., Diaz, M., Zheng, J., Williams, C. C., Lang, A., Aragón, T., Li, H. and Walter, P. (2012). The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *Elife* **1**, e00048.
Klop, B., Elte, J. W. and Cabezas, M. C. (2013). Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients* **5**, 1218-1240.
Kohno, K., Normington, K., Sambrook, J., Gething, M. J. and Mori, K. (1993). The promoter region of the yeast KAR2 (BiP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum. *Mol. Cell. Biol.* **13**, 877-890.
Korennykh, A. V., Korostelev, A. A., Egea, P. F., FINER-Moore, J., Stroud, R. M., Zhang, C., Shokat, K. M. and Walter, P. (2011). Structural and functional basis for RNA cleavage by Ire1. *BMC Biol.* **9**, 47.
Lagace, T. A. and Ridgway, N. D. (2013). The role of phospholipids in the biological activity and structure of the endoplasmic reticulum. *Biochim. Biophys. Acta* **1833**, 2499-2510.
Laybutt, D. R., Preston, A. M., Åkerfeldt, M. C., Kench, J. G., Busch, A. K., Biankin, A. V. and Biden, T. J. (2007). Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* **50**, 752-763.
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.
Leonardi, R., Frank, M. W., Jackson, P. D., Rock, C. O. and Jackowski, S. (2009). Elimination of the CDP-ethanolamine pathway disrupts hepatic lipid homeostasis. *J. Biol. Chem.* **284**, 27077-27089.
Lépine, S., Allegood, J. C., Park, M., Dent, P., Milstien, S. and Spiegel, S. (2011). Sphingosine-1-phosphate phosphohydrolase-1 regulates ER stress-induced autophagy. *Cell Death Differ.* **18**, 350-361.
Li, Z., Agellon, L. B., Allen, T. M., Umeda, M., Jewell, L., Mason, A. and Vance, D. E. (2006). The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab.* **3**, 321-331.
Li, Z. Z., Berk, M., Mcintyre, T. M. and Feldstein, A. E. (2009). Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. *J. Biol. Chem.* **284**, 5637-5644.
Liu, M., Huang, C., Polu, S. R., Schneiter, R. and Chang, A. (2012). Regulation of sphingolipid synthesis through Orm1 and Orm2 in yeast. *J. Cell Sci.* **125**, 2428-2435.
Lu, Y., Liang, F.-X. and Wang, X. (2014). A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. *Mol. Cell* **55**, 758-770.
Maiuolo, J., Bulotta, S., Verderio, C., Benfante, R. and Borgese, N. (2011). Selective activation of the transcription factor ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein. *Proc. Natl. Acad. Sci. USA* **108**, 7832-7837.
Michelotti, G. A., Machado, M. V. and Diehl, A. M. (2013). Nafld, NASH and liver cancer. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 656-665.
Mori, K., Sant, A., Kohno, K., Normington, K., Gething, M. J. and Sambrook, J. F. (1992). A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins. *EMBO J.* **11**, 2583-2593.
Mori, K., Ma, W., Gething, M.-J. and Sambrook, J. (1993). A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* **74**, 743-756.
Musso, G., Cassader, M. and Gambino, R. (2016). Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. *Nat. Rev. Drug Discov.* **15**, 249-274.
NCD Risk Factor Collaboration. (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* **387**, 1377-1396.
Ng, B. S. H., Shyu, P. T., Chaw, R., Seah, Y. L. and Thibault, G. (2017). Lipid perturbation compromises UPR-mediated ER homeostasis as a result of premature degradation of membrane proteins. *bioRxiv*. doi: https://doi.org/10.1101/178947.
Nguyen, N. T., Magno, C. P., Lane, K. T., Hinojosa, M. W. and Lane, J. S. (2008). Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to 2004. *J. Am. Coll. Surg.* **207**, 928-934.

Nikawa, J.-I. and Yamashita, S. (1992). IRE1 encodes a putative protein kinase containing a membrane-spanning domain and is required for inositol phototrophy in Saccharomyces cerevisiae. *Mol. Microbiol.* **6**, 1441-1446.
Nishitoh, H. (2012). CHOP is a multifunctional transcription factor in the ER stress response. *J. Biochem.* **151**, 217-219.
Novoa, I., Zeng, H., Harding, H. P. and Ron, D. (2001). Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J. Cell Biol.* **153**, 1011-1022.
Otoda, T., Takamura, T., Misu, H., Ota, T., Murata, S., Hayashi, H., Takayama, H., Kikuchi, A., Kanamori, T., Shima, K. R. et al. (2013). Proteasome dysfunction mediates obesity-induced endoplasmic reticulum stress and insulin resistance in the liver. *Diabetes* **62**, 811-824.
Oyadomari, S., Harding, H. P., Zhang, Y., Oyadomari, M. and Ron, D. (2008). Dephosphorylation of translation initiation factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice. *Cell Metab.* **7**, 520-532.
Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L. H. and Hotamisligil, G. S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**, 457-461.
Pettus, B. J., Chalfant, C. E. and Hannun, Y. A. (2002). Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta* **1585**, 114-125.
Pineau, L., Bonifait, L., Berjeaud, J.-M., Alimardani-Theuil, P., Berges, T. and Ferreira, T. (2008). A lipid-mediated quality control process in the Golgi apparatus in yeast. *Mol. Biol. Cell* **19**, 807-821.
Pineau, L., Colas, J., Dupont, S., Beney, L., Fleurat-Lessard, P., Berjeaud, J.-M., Bergès, T. and Ferreira, T. (2009). Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. *Traffic* **10**, 673-690.
Prischi, F., Nowak, P. R., Carrara, M. and Ali, M. M. U. (2014). Phosphoregulation of Ire1 RNase splicing activity. *Nat. Commun.* **5**, 3554.
Promlek, T., Ishiwata-Kimata, Y., Shido, M., Sakuramoto, M., Kohno, K. and Kimata, Y. (2011). Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. *Mol. Biol. Cell* **22**, 3520-3532.
Quinn, P. J. (1981). The fluidity of cell membranes and its regulation. *Prog. Biophys. Mol. Biol.* **38**, 1-104.
Schindler, A. J. and Schekman, R. (2009). In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles. *Proc. Natl. Acad. Sci. USA* **106**, 17775-17780.
Schuck, S., Prinz, W. A., Thorn, K. S., Voss, C. and Walter, P. (2009). Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. *J. Cell Biol.* **187**, 525-536.
Seu, K. J., Cambrea, L. R., Everly, R. M. and Hovis, J. S. (2006). Influence of lipid chemistry on membrane fluidity: tail and headgroup interactions. *Biophys. J.* **91**, 3727-3735.
Shah, C., Yang, G., Lee, I., Bielawski, J., Hannun, Y. A. and Samad, F. (2008). Protection from high fat diet-induced increase in ceramide in mice lacking plasminogen activator inhibitor 1. *J. Biol. Chem.* **283**, 13538-13548.
Shamu, C. E., Cox, J. S. and Walter, P. (1994). The unfolded-protein-response pathway in yeast. *Trends Cell Biol.* **4**, 56-60.
So, J.-S., Hur, K. Y., Tarrio, M., Ruda, V., Frank-Kamenetsky, M., Fitzgerald, K., Koteliansky, V., Lichtman, A. H., Iwawaki, T., Glimcher, L. H. et al. (2012). Silencing of lipid metabolism genes through IRE1alpha-mediated mRNA decay lowers plasma lipids in mice. *Cell Metab.* **16**, 487-499.
Sommerweiss, D., Gorski, T., Richter, S., Garten, A. and Kiess, W. (2013). Oleate rescues INS-1E beta-cells from palmitate-induced apoptosis by preventing activation of the unfolded protein response. *Biochem. Biophys. Res. Commun.* **441**, 770-776.
Stubbs, C. D. (1983). Membrane fluidity: structure and dynamics of membrane lipids. *Essays Biochem.* **19**, 1-39.
Stubbs, C. D. and Smith, A. D. (1984). The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **779**, 89-137.
Surma, M. A., Klose, C., Peng, D., Shales, M., Mrejen, C., Stefanko, A., Braberg, H., Gordon, D. E., Vorkel, D., Ejsing, C. S. et al. (2013). A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. *Mol. Cell* **51**, 519-530.
Tam, A. B., Koong, A. C. and Niwa, M. (2014). Ire1 has distinct catalytic mechanisms for XBP1/HAC1 splicing and RIDD. *Cell Rep.* **9**, 850-858.
Thibault, G., Shui, G., Kim, W., Mcalister, G. C., Ismail, N., Gygi, S. P., Wenk, M. R. and Ng, D. T. W. (2012). The membrane stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network. *Mol. Cell* **48**, 16-27.
Travers, K. J., Patil, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S. and Walter, P. (2000). Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* **101**, 249-258.
Urra, H., Dufey, E., Avril, T., Chevet, E. and Hetz, C. (2016). Endoplasmic reticulum stress and the hallmarks of cancer. *Trends Cancer* **2**, 252-262.
van Anken, E., Pincus, D., Coyle, S., Aragón, T., Osman, C., Lari, F., Gómez Puerta, S., Korennykh, A. V. and Walter, P. (2014). Specificity in endoplasmic reticulum-stress signaling in yeast entails a step-wise engagement of HAC1 mRNA to clusters of the stress sensor Ire1. *Elife* **3**, e05031.
van Meer, G. and de Kroon, A. I. P. M. (2011). Lipid map of the mammalian cell. *J. Cell Sci.* **124**, 5-8.
van Meer, G., Voelker, D. R. and Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**, 112-124.
Volmer, R. and Ron, D. (2015). Lipid-dependent regulation of the unfolded protein response. *Curr. Opin. Cell Biol.* **33**, 67-73.
Volmer, R., van der Ploeg, K. and Ron, D. (2013). Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. *Proc. Natl. Acad. Sci. USA* **110**, 4628-4633.
Walkey, C. J., Yu, L., Agellon, L. B. and Vance, D. E. (1998). Biochemical and evolutionary significance of phospholipid methylation. *J. Biol. Chem.* **273**, 27043-27046.
Walter, P. and Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081-1086.
Wang, S., Chen, Z., Lam, V., Han, J., Hassler, J., Finck, B. N., Davidson, N. O. and Kaufman, R. J. (2012). IRE1alpha-XBP1s induces PDI expression to increase MTP activity for hepatic VLDL assembly and lipid homeostasis. *Cell Metab.* **16**, 473-486.
Williams, E. E., Cooper, J. A., Stillwell, W. and Jenski, L. J. (2000). The curvature and cholesterol content of phospholipid bilayers alter the transbilayer distribution of specific molecular species of phosphatidylethanolamine. *Mol. Membr. Biol.* **17**, 157-164.
Willy, J. A., Young, S. K., Stevens, J. L., Masuoka, H. C. and Wek, R. C. (2015). CHOP links endoplasmic reticulum stress to NF-kappaB activation in the pathogenesis of nonalcoholic steatohepatitis. *Mol. Biol. Cell* **26**, 2190-2204.
Xia, J. Y., Holland, W. L., Kusminski, C. M., Sun, K., Sharma, A. X., Pearson, M. J., Sifuentes, A. J., Mcdonald, J. G., Gordillo, R. and Scherer, P. E. (2015). Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab.* **22**, 266-278.
Yamamoto, K., Takahara, K., Oyadomari, S., Okada, T., Sato, T., Harada, A. and Mori, K. (2010). Induction of liver steatosis and lipid droplet formation in ATF6alpha-knockout mice burdened with pharmacological endoplasmic reticulum stress. *Mol. Biol. Cell* **21**, 2975-2986.
Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L. and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64**, 73-84.
Zhou, J., Liu, C. Y., Back, S. H., Clark, R. L., Peisach, D., Xu, Z. and Kaufman, R. J. (2006). The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. *Proc. Natl. Acad. Sci. USA* **103**, 14343-14348.
Zhou, Y., Lee, J., Reno, C. M., Sun, C., Park, S. W., Chung, J., Lee, J., Fisher, S. J., White, M. F., Biddinger, S. B. et al. (2011). Regulation of glucose homeostasis through a XBP-1-FoxO1 interaction. *Nat. Med.* **17**, 356-365.