Europe PMC Funders Group
Author Manuscript
Cold Spring Harb Perspect Biol. Author manuscript; available in PMC 2024 August 05.

Published in final edited form as:
Cold Spring Harb Perspect Biol. 2024 January 22; 16(8): . doi:10.1101/cshperspect.a041400.

Endoplasmic Reticulum Membrane Homeostasis and the Unfolded Protein Response

Robert Ernst[1,2], **Mike F. Renne**[1,2], **Aamna Jain**[1,2], **Alexander von der Malsburg**[1,2]
[1]Medical Biochemistry and Molecular Biology, Medical Faculty

[2]Preclinical Center for Molecular Signaling (PZMS), Medical Faculty, Saarland University, 66421 Homburg, Germany

Abstract

The endoplasmic reticulum (ER) is the key organelle for membrane biogenesis. Most lipids are synthesized in the ER, and most membrane proteins are first inserted into the ER membrane before they are transported to their target organelle. The composition and properties of the ER membrane must be carefully controlled to provide a suitable environment for the insertion and folding of membrane proteins. The unfolded protein response (UPR) is a powerful signaling pathway that balances protein and lipid production in the ER. Here, we summarize our current knowledge of how aberrant compositions of the ER membrane, referred to as lipid bilayer stress, trigger the UPR.

Diving into Biomembranes

Biomembranes are fascinating materials composed of lipids and proteins. Driven by the hydrophobic effect, lipids can self-assemble into liquid crystalline bilayers that are soft, semipermeable, and self-repairing. Both simple model membranes and complex biological membranes that are composed of hundreds of proteins and lipids feature remarkable heterogeneities along their thickness. An imaginary journey into a lipid bilayer would set off in an aqueous environment and cross a layer of high charge density from the lipid headgroups at the water–membrane interface before the hydrophobic core of the bilayer is reached. Heterogeneities in polarity are accompanied by dramatic changes of pressure and tension on the scale of hundreds of atmospheric pressures (Marsh 2007). The folding, function, and stability of membrane proteins are remarkably sensitive to their local environments and collective bilayer properties such as the bending rigidity, surface viscosity, and compressibility (Dowhan et al. 2019; Levental and Lyman 2023; Renne and Ernst 2023).

Membranes Organize Biochemistry and Maintain Organelle Identity

Eukaryotic cells organize biochemistry in a variety of organelles with characteristic membrane properties and compositions (van Meer et al. 2008; Harayama and Riezman

Correspondence to: Robert Ernst.
Correspondence: robert.ernst@uks.eu.
Editors: Robert G. Parton and Kai Simons

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

2018). Organelles exchange membrane material via vesicular traffic and lipid transfer proteins (Prinz 2010; Holthuis and Menon 2014), and they adjust their composition in response to metabolic and physical stimuli. Hence, cells invest substantial resources for maintaining characteristic organelle membrane compositions that ultimately establish membrane identity (Bigay and Antonny 2012).

Plasma membranes are thick, asymmetric, and feature low permeabilities due to their high content of cholesterol and saturated (glyco)sphingolipids in the extracellular membrane leaflet (Lorent et al. 2020). Anionic lipids such as phosphatidylserine (PS), phosphatidylinositol (PI), and phosphoinositides (PIPs) populate the cytosolic leaflet and serve, both through their negative charge and the recruitment of proteins via stereospecific interactions, as signposts for the plasma membrane and its subcompartments (Gassama-Diagne et al. 2006; Balla 2013; Posor et al. 2022). Lipid rafts are fluctuating nanoscale assemblies of sterols, sphingolipids, and proteins, which provide another layer of plasma membrane organization (Lingwood and Simons 2010). In the *trans*-Golgi network (TGN) they provide a basis for protein and lipid sorting, while at the plasma membrane they provide an organizational principle that orchestrates cellular signaling (Simons and van Meer 1988; Simons and Ikonen 1997; Castello-Serrano et al. 2023). The endoplasmic reticulum (ER) membrane, on the other hand, is less rigid and more compressible due to its low sterol content and the high abundance of loosely packing lipids with monounsaturated fatty acyl chains (Fig. 1; Antonny et al. 2015; Reinhard et al. 2022; Renne and Ernst 2023). Here, we discuss the role of the ER membrane in triggering and modulating ER stress.

The ER is a Key Organelle for Membrane Biogenesis

The ER forms a complex and dynamic network of interconnected sheets and tubules that originates from the nuclear envelope (NE). Membrane curvature in ER tubules and sheet edges are stabilized by proteins of the reticulon family featuring wedge-shaped transmembrane domains and sizable intrinsically disordered regions (Voeltz et al. 2006). A significant proportion of the ER is involved in membrane contact sites (MCSs) to other organelles for the exchange of ions and lipids (Valm et al. 2017). Intriguingly, the abundance and size of MCSs are dynamically adapted to metabolic cues and in response to ER stress (Elbaz-Alon et al. 2014; Gatta et al. 2015; Kwak et al. 2020; Liao et al. 2022).

The ER hosts important functions in Ca^{2+} homeostasis, lipid metabolism, and the folding of secretory and membrane proteins (Braakman and Bulleid 2011; Ernst et al. 2016; Carreras-Sureda et al. 2018; Wenzel et al. 2022). In mammalian cells, it handles at least 10,000 different proteins (roughly 30% of the proteome) adding up to ~0.1 to >2.0 million client proteins per minute depending on the cell type (Hibi and Dosch 1986). For example, 120,000 copies of each Semliki Forest virus protein are transiting the ER every minute in infected baby hamster kidney cells (Quinn et al. 1984), while hepatocytes fold and secrete ~1.5 million molecules of albumin at the same time (Schulze et al. 2019). Most proteins entering the secretory pathway in *Saccharomyces cerevisiae* are membrane proteins, which have more complex folding requirements. Their delivery into the ER membrane is facilitated by insertases that locally compress and distort the lipid bilayer (Wu et al. 2020; Wu and Rapoport 2021; Li et al. 2023). ER membrane thinning by compression lowers the

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

energetic penalty for moving hydrophilic amino acid residues across the hydrophobic core of the membrane. Insertion of membrane proteins is therefore lipid-dependent and efficiently stalled by increased cholesterol levels that reduce ER membrane compressibility (Nilsson et al. 2001; Brambillasca et al. 2006). Membrane protein production and lipid metabolism must be tightly coordinated for maintaining the ER membrane in a permissive state for insertion.

The Lipid Composition of the ER Membrane in Yeast and Mammalian Cells

The fundamental mechanisms of protein and lipid sorting along the secretory pathway are conserved from yeast to man (Conibear 2010; Surma et al. 2012; Muñiz and Zurzolo 2014). The ER marks the entry point to the secretory pathway and must provide a suitable environment for the insertion and folding of transmembrane proteins irrespective of their final subcellular destination. In yeast, the average hydrophobic length of a transmembrane helix is 20.6 hydrophobic amino acid residues for ER-resident proteins, while it is on average 27 for plasma membrane proteins (Sharpe et al. 2010). In vertebrates, the mean hydrophobic length of ER-resident transmembrane helices is 20.3 residues and 24.4 for plasma membrane proteins (Sharpe et al. 2010). Furthermore, transmembrane helices in the plasma membrane expose different amino acid residues at different depths in the plasma membrane. An archetypical transmembrane helix in the plasma membrane exposes bulky amino acid residues to the more loosely packed cytosolic leaflet, but smaller residues to the tightly packed extracellular leaflet (Sharpe et al. 2010; Quiroga et al. 2013; Lorent et al. 2017, 2020). Mutations that disrupt this shape asymmetry result in missorting of the respective proteins (Quiroga et al. 2013; Lorent et al. 2017).

To accommodate the entire spectrum of newly synthesized membrane proteins, the ER must locally deform and match the varying shapes and lengths of the transmembrane domains. The low sterol concentration in the ER (Fig. 1A), together with a high content of poorly packing monounsaturated lipids (Fig. 1B), provide sufficient flexibility at the molecular scale to lower the free energy penalties associated with hydrophobic mismatches between membrane proteins and the lipid bilayer.

The cholesterol concentration in the ER is efficiently maintained at a concentration above 5 mol% (Radhakrishnan et al. 2008) and ranges between 5 and 10 mol% for different cell types depending on the methods of ER isolation and lipid quantitation (Radhakrishnan et al. 2008; Vance 2015; Sokoya et al. 2022). Similarly, ergosterol, the most abundant sterol in yeast, is maintained at a low concentration between 8.5 and 9.7 mol% in the ER (Reinhard et al. 2022). Immunoisolation experiments coupled to mass spectrometry-based lipidomics suggest that the prominently discussed sterol gradient along the secretory pathway may be rather flat in the early secretory pathway (Klemm et al. 2009; Surma et al. 2011; Reinhard et al. 2022). Only in the late secretory pathway, where lipid rafts are functionalized for protein and lipid sorting, the ergosterol concentrations increase sharply from the TGN (9.8 mol%) via secretory vesicles (22.8 mol%) to the plasma membrane (~45 mol%), presumably through a combination of active forward transport of sterols and the clustering of lipid rafts (Klemm et al. 2009; Surma et al. 2011; Mesmin et al. 2013; Menon 2018). Notably, the absence of a steep cholesterol gradient in the early secretory pathway, does not preclude the possibility that local enrichments of cholesterol synergize with cargo receptors in the ER–

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Golgi intermediate compartment (ERGIC) to provide a length-based sorting of membrane proteins (Herzig et al. 2012; Rodriguez-Gallardo et al. 2020; Weigel et al. 2021).

A direct comparison of the ER membrane lipid composition from baker's yeast and mammalian cells reveals that they are remarkably distinct (Fig. 1). In the yeast ER, PI, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) lipids have similar abundances, each contributing ~20 to ~25 mol% to the lipidome (Fig. 1A; Reinhard et al. 2022). In mammalian cells, PC is by far the most abundant lipid class, contributing ~57 to 63 mol% to the ER lipidome (Vance 2015; Sokoya et al. 2022). This has important implications for the properties of the ER membrane, because PI lipids are negatively charged, while PC and PE lipids are zwitterionic. The total concentration of anionic lipids in the ER from *S. cerevisiae* of ~28 mol% compared to the ~6.5–13 mol% in the ER of mammalian cells, means that membrane protein folding occurs in membrane environments that differ in their negative charge densities (Fig. 1A; Vance 2015; Reinhard et al. 2022; Sokoya et al. 2022).

Even at the level of the ER lipid acyl chains, there are substantial differences between *S. cerevisiae* and mammalian cells (Fig. 1B,C). Unlike *S. cerevisiae* that can synthesize only monounsaturated fatty acids (Ballweg and Ernst 2017), mammalian cells can generate fatty acids with two or more unsaturations (Harayama and Riezman 2018). Monounsaturated fatty acyl chains are more abundant in the ER (Schneiter et al. 1999; Reinhard et al. 2022), while lipids with polyunsaturated acyl chains are normally enriched along the secretory pathway (Fig. 1B; Keenan and Morré 1970; Antonny et al. 2015). Despite that, the mammalian ER contains significant levels of lipids with polyunsaturated fatty acyl chains (Sokoya et al. 2022). The lipid acyl chains in the ER of *S. cerevisiae* are also shorter than the ones found in mammalian cells (Fig. 1C; Reinhard et al. 2022; Sokoya et al. 2022). Intriguingly, this is not reflected in the mean hydrophobic lengths of transmembrane proteins in the ER, which is 20.6 and 20.3 amino acid residues for *S. cerevisiae* and mammalian cells, respectively (Sharpe et al. 2010). Either the difference in lipid acyl chain composition compensates for differences in acyl chain lengths, or the evolutionary pressure to match the thickness of transmembrane domains with the ER lipid bilayer is low, because the ER is highly compressible.

It will be an interesting task for the upcoming years to decipher how the molecular machines that facilitate protein and lipid biogenesis have adapted to these vastly distinct membrane environments and whether the different features of membrane proteins reflect their coevolution with ER membrane lipids.

The Unfolded Protein Response (UPR) and its Three Transducers

The many functions of the ER in membrane protein insertion and folding, posttranslational modifications, and lipid metabolism are connected by the UPR (Mori 2022). When the protein folding machinery of the ER is overwhelmed or when aberrant lipid metabolism causes lipid bilayer stress, the UPR mounts a response that attenuates protein biosynthesis and up-regulates the expression of ER chaperones, ER-associated degradation, and de novo lipid biosynthesis (Travers et al. 2000; Sriburi et al. 2004; Schuck et al. 2009). In

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

mammalian cells, the UPR relies on three single-pass, transmembrane proteins in the ER: the inositol-requiring enzyme 1 (with two isoforms IRE1α/β), the double-stranded RNA-activated protein kinase (PKR)—like ER kinase (PERK), and the activating transcription factor 6 (with two isoforms ATF6α/β in mammals) (Fig. 2; Walter and Ron 2011). Whenever necessary, we will refer to IRE1 from *S. cerevisiae* as ScIRE1, and to IRE1 from mammalian cells as IRE1α for clarity.

UPR transducers relay information from the ER to the nucleus by different means. IRE1 has The Role of Lipid Bilayer Stress in UPR Activation kinase and endoribonuclease (RNase) activities, with the RNase domain being the main effector domain (Papa et al. 2003; Walter and Ron 2011). PERK uses a kinase domain for signal transmission, while ATF6 is a transcription factor that is released from its transmembrane anchor upon ER stress to initiate a transcriptional response (Yoshida et al. 1998). Each branch of the mammalian UPR generates a distinct basic leucine zipper (bZIP) transcription factor, which can form homo- or heterodimers with other members of the bZIP transcription factor family (Walter and Ron 2011).

Activation of IRE1 in mammalian cells leads to the expression of the X-box-binding protein 1 (XBP1) (Hac1 in *S. cerevisiae*). Upon ER stress, IRE1's RNase domain initiates the unconventional splicing of the *XBP1* mRNA (*HAC1* mRNA in *S. cerevisiae*) (Gonzalez et al. 1999; Yoshida et al. 2001), which is completed by RNA ligases (hRtcB in mammalian cells and tRNA ligase Rlg1 in *S. cerevisiae*) (Welihinda et al. 2000; Jurkin et al. 2014; Lu et al. 2014). Translation of the spliced mRNA yields a potent transcription factor regulating hundreds of UPR target genes (Travers et al. 2000) including those responsible for up-regulating de novo membrane lipid synthesis (Schuck et al. 2009; Moncan et al. 2021). Additionally, IRE1's RNase activity can reduce the flux of unfolded proteins into the ER by degrading ER-targeted mRNAs in a process referred to as regulated IRE1-dependent mRNA decay (RIDD) (Fig. 2; Moore and Hollien 2015). This alternative mode of signaling was first discovered in *Schizosaccharomyces pombe*, is absent in *S. cerevisiae*, but conserved in metazoans (Hollien et al. 2009; Li et al. 2021).

PERK attenuates protein translation via the integrated stress response by phosphorylating the α subunit of the eukaryotic translation initiation factor 2 (eIF2) (Harding et al. 1999). Specific mRNAs escape this global inhibition of translation and are selectively induced during ER stress (Harding et al. 2000). Among these are the mRNAs of the bZIP transcription factor ATF4 (Harding et al. 2000), which up-regulates gene expression for the transcription factor CHOP (GADD153) and XBP1 (Harding et al. 2000; Tsuru et al. 2016). CHOP has proapoptotic functions by up-regulating proteins of the BCL-2 family (Matsumoto et al. 1996). It also controls the expression of growth arrest and DNA-damaged protein 34 (GADD34) and provides negative feedback to PERK signaling by the dephosphorylation eIF2α (Novoa et al. 2001).

Unlike IRE1 and PERK, ATF6α lacks the ability to attenuate protein translation, but it potently promotes ER protein folding, ER-associated degradation, and ER membrane expansion as a bZIP transcription factor (Yoshida et al. 2001; Wu et al. 2007; Maiuolo et al. 2011). In the unstressed ER, ATF6α exists in different redox forms maintained by

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

the presence of highly conserved cysteines in the ER–luminal domains forming different types of disulfide bridges (Nadanaka et al. 2007). Upon ER stress, a specific ATF6α C467 dimer accumulates in the ER and is preferentially transported to the Golgi complex where the site-1 and site-2 proteases release the transcriptionally active ATF6p50 fragment (Fig. 2; Ye et al. 2000; Oka et al. 2022). Hence, ATF6α signaling relies on the same proteases that are required for the transcriptional regulation of lipid metabolism via the sterol response element-binding proteins SREBP1, SREBP1c, and SREBP2 (Horton et al. 2002, 2003; Brown et al. 2018). ATF6p50 binds ER stress response elements and activates specific UPR target genes (Yoshida et al. 1998; Bommiasamy et al. 2009; Maiuolo et al. 2011). Intriguingly, ATF6p50 can form heterodimers with SREBP2 to modulate cholesterol homeostasis (Zeng et al. 2004).

The coordinated activation of the three UPR branches aims at reestablishing ER homeostasis. Prolonged activation of the UPR, however, leads to cell death by necrosis, apoptosis, orothermechanisms (Tabas and Ron 2011; Lebeaupin et al. 2015; Iurlaro and Muñoz-Pinedo 2016; Hetz and Papa 2018). Although often represented as seemingly independent, parallel pathways, the three branches of the UPR affect each other. ATF6p50 induces the expression of XBP1 (Yoshida et al. 2001), while ATF4, which is downstream of PERK, up-regulates *ATF6* gene expression (Teske et al. 2011). The broad output functions of the UPR and their role in health and disease have been subject to excellent reviews (Hotamisligil 2010; Tabas and Ron 2011; Hetz et al. 2013; Iurlaro and Muñoz-Pinedo 2016; Urra et al. 2016; Wang and Kaufman 2016; Hetz and Papa 2018; Yong et al. 2021). Here, we focus on the molecular mechanisms by which aberrant membrane compositions, referred to as lipid bilayer stress, can contribute to UPR activation.

Controlling UPR Activity Via the Oligomeric State

All branches of the UPR are regulated by the oligomeric state of the UPR transducer (Fig. 2). Dimerization and oligomerization trigger PERK and IRE1 activation, while specific disulfide-linked dimers of ATF6 are preferentially transported to the Golgi complex (Sundaram et al. 2018). Aberrancies in protein folding modulate the oligomeric state of UPR transducers via their ER–luminal domains, while lipid bilayer stress is sensed by structural features in the transmembrane region (Ho et al. 2018; Radanović and Ernst 2021). Which of these complementary, yet fundamentally distinct, mechanisms provides the dominant signal for triggering the UPR remains largely unexplored for most ER stress conditions—especially in the pathophysiological context. Irrespective of the origin and molecular basis of stress, the juxtaposition of IRE1's cytosolic kinase and RNase domains triggers a *trans*-auto-phosphorylation and activation of the RNase domain (Liu et al. 2000).

Structures of the isolated, ER–luminal sensor domains of IRE1 from yeast and humans combined with mutational analyses have uncovered crucial interfaces for dimerization and oligomerization (Fig. 3A; Credle et al. 2005; Zhou et al. 2006; Karagöz et al. 2017). These have been instrumental to establish a firm link between IRE1 oligomerization and its signaling output. Single-molecule tracking experiments in cultured mammalian cells demonstrate that IRE1α molecules reside, under resting conditions, predominantly in dimers, which can assemble into higher oligomers upon ER stress (Belyy et al. 2022).

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Signaling-active clusters of IRE1 have been observed by confocal microscopy in both *S. cerevisiae* and mammalian cells and are stable for minutes (Kimata et al. 2007; Aragón et al. 2009; Väth et al. 2021). Recently, it was suggested that flexible loops extending from the ER–luminal domain of IRE1α can support condensation as a mechanism to support clustering (Kettel et al. 2023). Correlated light and electron microscopy combined with electron cryotomography revealed that mature IRE1αclusters form helical filaments that localize to narrow, anastomosing ER membrane tubes with complex topologies (Fig. 3B; Tran et al. 2021). Herein, dimeric ER–luminal sensor domains form a proteinaceous zipper that pulls together two opposing ER membranes to form membrane tubules with a diameter of ~28 nm (Tran et al. 2021). Hence, clustering of IRE1α drives ER membrane remodeling and establishes a highly curved ER subdomain.

Nonconventional modes of UPR signaling are facilitated by modulatory interactions of IRE1α with the translocon (Plumb et al. 2015; Sundaram et al. 2018; Li et al. 2020), the signal recognition particle, and ribosomes (Acosta-Alvear et al. 2018). These include a preemptive mode, which limits the influx of unfolded nascent chains into the ER lumen upon ER stress via RIDD, and a surveillance mode that selectively degrades ER-targeted mRNAs, whose cotranslational folding encounters substantial problems or delays (Plumb et al. 2015; Acosta-Alvear et al. 2018). Even the energy status of the cell may contribute to an “anticipatory” mode of UPR signaling: ScIRE1’s RNase can be activated by adenosine–diphosphate (ADP) binding to the kinase domain (Papa et al. 2003; Korennykh et al. 2009; Rubio et al. 2011) such that increased cellular ADP levels can activate UPR signaling (Le et al. 2021). How precisely different oligomeric states and interaction partners bias the UPR toward distinct signaling outputs in different cell types remains a major open question in the field (Han et al. 2009; Hetz and Glimcher 2009; Hetz and Papa 2018; Sundaram et al. 2018).

How UPR Transducers Sense Proteotoxic Stress

Several models of how IRE1 senses proteotoxic ER stress have been put forward (Fig. 3C; Adams et al. 2019; Karagöz et al. 2019; Preissler and Ron 2019). The “direct model” suggests an interaction between unfolded proteins and IRE1, which stabilizes dimeric and higher oligomeric assemblies of the UPR transducer (Gardner and Walter 2011; Karagöz et al. 2017, 2019). According to the “competition model,” IRE1 and unfolded proteins compete for the binding to the ER chaperone BiP/Kar2 (Bertolotti et al. 2000; Okamura et al. 2000; Preissler et al. 2015). The dissociation of BiP/Kar2 from IRE1 during ER stress would facilitate the dimerization and/or oligomerization of IRE1α. The “chaperone inhibition model,” on the other hand, suggests that BiP activity, rather than its stable association with IRE1α, is required to counteract IRE1α’s inherent tendency to oligomerize (Amin-Wetzel et al. 2017, 2019). Again, accumulating unfolded proteins would trigger the UPR, but also insufficient concentrations of cochaperones, ATP or Ca^{2+} in the ER lumen, which are required for full chaperone activity, would favor IRE1α clustering and activation even before unfolded proteins accumulate (Amin-Wetzel et al. 2017; Preissler et al. 2020). For as long as the level of unfolded proteins in the ER lumen cannot be quantified directly, it will be challenging to establish the relative contribution of these alternative modes of sensing (Rutkowski 2019; Radanović and Ernst 2021).

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Consistent with a direct interaction of unfolded proteins with IRE1, structural biology revealed that the dimeric ER–luminal domain of IRE1 from *S. cerevisiae* and mammals contains a hydrophobicgroove, which resembles the peptide-binding groove of antigen-presenting major histocompatibility complex (MHC) molecules (Zhou et al. 2006; Hollien 2013). Unfolded model peptides bind to the ER–luminal domain in vitro, and control its oligomeric state (Gardner and Walter 2011; Karagöz et al. 2017). In situ, the peptide-binding groove of IRE1α is oriented toward the inner surface membrane in highly curved ER tubules (Tran et al. 2021). It is tempting to speculate that this topological constraint renders IRE1α particularly sensitive to aberrantly folded membrane proteins. However, whether misfolded transmembrane proteins can partition into these highly curved ER tubules or if they trigger IRE1α oligomerization by changing ER membrane properties, as previously suggested (Covino et al. 2018), remains to be tested. Intriguingly, a mutant variant of the abundant plasma membrane proton pump Pma1 in *S. cerevisiae* accumulates in the ER and triggers apotent UPR (Phuong et al. 2023). Likewise, over-produced flaviviral transmembrane proteins lacking sizable ER–lumenal domains trigger the UPR via the IRE1-XBP1 axis (Yu et al. 2006). Hence, the contribution of membrane proteins to UPR activation is important to study.

PERK is structurally related and functionally equivalent to IRE1 (Liu et al. 2000). It also shares with IRE1 the dual sensitivity to proteotoxic and lipid bilayer stress (Ariyama et al. 2010; Volmer and Ron 2015). Like IRE1α, PERK interacts with BiP in the ER lumen (Bertolotti et al. 2000). The inverse correlation between BiP binding and PERK activity suggested that BiP may act as a sensor for unfolded proteins (Bertolotti et al. 2000) and has fueled the idea of a competition between UPR transducers and unfolded proteins for the binding of BiP. Based on crystal structures and biochemical reconstitutions, it was suggested that BiP binding to the dimeric ER–luminal domain of PERK locks the access to a peptide-binding groove for direct binding, thereby preventing the formation of PERK tetramers in resting cells but facilitating PERK oligomerization when unfolded proteins accumulate during ER stress (Carrara et al. 2015a,b). Hence, the different proposed modes of ER stress sensing can synergize, rather than exclude each other.

ATF6 is a single-pass type 2 transmembrane protein, with the amino-terminal transcription factor domain projecting into the cytosol and a carboxy-terminal sensor domain into the ER lumen (Haze et al. 1999). ATF6 is structurally unrelated to IREα and PERK, but also associates with BiP in nonstressed cells (Shen et al. 2002). The ER–luminal domain of ATF6α forms disulfide-linked and reduced dimers (Koba et al. 2020; Oka et al. 2022), which represent an inactive pool of ATF6α that can be mobilized on demand (Nadanaka et al. 2007; Koba et al. 2020; Oka et al. 2022). Hence, ATF6α is directly sensitive to reducing conditions that would interfere with the oxidative folding of secretory and membrane proteins. Upon ER stress, BiP dissociates from ATF6α, thereby unmasking Golgi localization signals and facilitating forward transport (Shen et al. 2002) by COPII vesicles (Schindler and Schekman 2009). While it is clear that ATF6α can be retained in the ER by the small protein disulfide isomerase (PDI) protein ERp18 to regulate ER-to-Golgi transport (Oka et al. 2019, 2022), less is known how the cleavage is regulated in the Golgi complex (Jheng et al. 2018; Tam et al. 2018). The selective activation of ATF6α by intermediates of sphingolipid metabolism and by the enforced production of cytochrome *b*5,

a transmembrane protein with an unusual transmembrane helix, underscores that membrane-based signals may contribute to UPR activation (Maiuolo et al. 2011; Tam et al. 2018).

The Sensitivity to Lipid Bilayer Stress is Evolutionarily Conserved

Despite its potent signaling outputs, the UPR often falls short in addressing metabolic challenges, as observed in obesity, the metabolic syndrome, and atherosclerosis (Fu et al. 2012; Rutkowski 2019). Chronic ER stress manifests when UPR signaling triggers only an insufficient adaptation or when it exacerbates the initial metabolic insult (Hotamisligil 2010). An excess of saturated fatty acids, for example, causes lipotoxicity, accompanied by UPR activation and characteristic changes of the ER membrane composition (Borradaile et al. 2006; Brookheart et al. 2009; Han and Kaufman 2016; Piccolis et al. 2019). UPR-driven up-regulation of de novo lipid biosynthesis, however, can further increase the incorporation of saturated fatty acids into membrane lipids, thereby establishing a vicious cycle that perpetuates ER stress (Covino et al. 2018). Consistently, complex metabolic conditions associated with ER stress such as diabetes and non-alcoholic fatty liver disease (NAFLD) display characteristic lipid signatures (Han et al. 2007; Fu et al. 2011, 2012; Kartsoli et al. 2020; Wigger et al. 2021). The mechanistic contributions of ER membrane aberrancies to disease, however, remain understudied.

Evidence for a membrane-based activation of the UPR has been uncovered in yeast (Cox et al. 1997; Pineau et al. 2009; Promlek et al. 2011; Surma et al. 2013), plants (Nguyen et al. 2019), worms (Hou et al. 2014; Garcia et al. 2023), and mammals (Ariyama et al. 2010; Volmer et al. 2013). Lipid bilayer stress activates UPR transducers directly, without requiring signals from misfolded proteins in the ER lumen. ScIRE1 can still mount a robust UPR even though (1) its ability to respond to unfolded proteins is impaired (Promlek et al. 2011; Halbleib et al. 2017), (2) the dimerization and oligomerization of the ER–luminal domain is disrupted (Väth et al. 2021), or (3) when the entire ER–luminal domain is removed (Ho et al. 2020). Similar observations have been made for the mammalian IRE1α and PERK (Ariyama et al. 2010; Volmer et al. 2013; Kono et al. 2017). Intriguingly, a naturally occurring IRE1 isoform in *Arabidopsis*, IRE1C, lacks an ER-luminal domain altogether, but still is functionally relevant (Pu et al. 2019). ATF6, on the other hand, can be activated by specific intermediates of sphingolipid metabolism that bind to the transmembrane helix of ATF6 to promote forward transport to the Golgi complex (Tam et al. 2018). Hence, all UPR transducers can be activated bysignals from within the membrane.

A Stunning Variety of Lipid Metabolic Perturbations Trigger the UPR

Aberrant lipid metabolism can trigger the UPR directly. Inositol-depletion, for example, causes lipid bilayer stress and triggers the UPR in *S. cerevisiae* (Cox et al. 1997; Promlek et al. 2011; Väth et al. 2021). The recently established Mem-Prep technology for isolating ER membranes from both stressed and unstressed cells provided a first glimpse into the molecular signatures of lipid bilayer stress (Reinhard et al. 2022). Inositol depletion results in a several-fold reduction of PI lipids in the ER (from 23.6 to 6.1 mol%) (Fig. 4A), while CDP-DAG and phosphatidic acid (PA) lipids, the precursors of PI lipid synthesis, accumulate (Reinhard et al. 2022). These changes are associated with a significant, overall

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

drop of anionic lipids from ~32 to 23 mol% and a markedly reduced negative charge density at the ER membrane. The levels of inositol-containing sphingolipids, however, are barely affected by inositol-depletion (Reinhard et al. 2022). These findings suggest distinct rates of PI and sphingolipid metabolism under these conditions. Intriguingly, a blockade of de novo sphingolipid biosynthesis during inositol depletion lowers UPR activity (Promlek et al. 2011). Hence, targeting and rewiring metabolism may provide new handles to counteract ER stress. Lipids in the stressed ER of inositol-depleted cells have—on average—slightly shorter and slightly more saturated lipid acyl chains compared to lipids in the unstressed ER. How precisely complex changes of the ER membrane affect the oligomeric state of UPR transducers and whether anionic lipids indeed modulate the oligomeric state of UPR transducers remains to be investigated in vivo and in vitro.

Mechanistically much better understood is the role of ER membrane compressibility, especially in the case of ScIRE1 (Halbleib et al. 2017; Renne and Ernst 2023). Both increased abundances of tightly packing, saturated lipids and the accumulation of sterols in the ER establish a powerful UPR-activating signal by increasing ER membrane thickness and decreasing membrane compressibility (Fig. 4A; Feng et al. 2003; Pineau et al. 2009; Deguil et al. 2011; Surma et al. 2013; Volmer et al. 2013). The accumulation of free cholesterol and saturated lipid acyl chains have been associated with chronic ER stress in hepatocytes (Wang et al. 2006; Fu et al. 2012), skeletal muscle cells (Lee et al. 2020), and insulin-producing β cells (Pineau and Ferreira 2010; Boslem et al. 2011; Fonseca et al. 2011). In fact, UPR activation is a major hallmark of the lipotoxicity triggered by saturated fatty acids (Piccolis et al. 2019). Increased concentrations of cholesterol in the ER of macrophages in advanced atherosclerotic lesions triggers the UPR and contributes to inflammation and apoptosis (Feng et al. 2003; Devries-Seimon et al. 2005; Duewell et al. 2010; Zhou and Tabas 2013). Both the inhibition of IRE1α by small molecules and the availability of the monounsaturated fatty acid palmitoleate alleviate inflammation and atherosclerosis and are modulated by the cytosolic fatty acid–binding protein aP2 (Erbay et al. 2009; Çimen et al. 2016, 2019; Tufanli et al. 2017). Thus, the intricate connection between lipid metabolism and UPR signaling opens up avenues for therapeutic, dietary interventions.

Notably, UPR signaling in macrophages induced by palmitate results in the unconventional maturation of microRNA (miR) 2137 (miR-2137) facilitated by IRE1α's RNase domain (Hamid et al. 2020). A direct target of miR-2137 is the lipid phosphatase inositol polyphosphate phosphatase like-1 converting phosphatidylinositol-3,4,5,-trisphosphate to phosphatidyl-inositol-3,4-bisphosphate to regulate cell growth via the mammalian target of rapamycin (mTOR) (Hamid et al. 2020).

Sterols and saturated lipids are chemically distinct, but act synergistically in lowering membrane compressibility and promoting ScIRE1 oligomerization in vitro via a hydrophobic mismatch based mechanism (Mouritsen and Bloom 1984; Kaiser et al. 2011; Halbleib et al. 2017; Renne and Ernst 2023). Likewise, the mammalian UPR transducers IRE1α and PERK are directly activated by saturated lipids (Ariyama et al. 2010; Volmer et al. 2013; Kono et al. 2017) even when their ER–luminal domain is removed or when the order of amino acid residues in the transmembrane helix is scrambled (Ariyama et al. 2010;

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Volmer et al. 2013; Kono et al. 2017). However, if mammalian UPR transducers also rely on a hydrophobic mismatch-based mechanism as demonstrated for ScIRE1, is still a matter of debate (Volmer and Ron 2015; Halbleib et al. 2017; Kono et al. 2017; Cho et al. 2019). We are convinced that lipid bilayer stress sensing relies on physicochemical features in IRE1α's and PERK's transmembrane regions, which includes not only their transmembrane helices, but also the juxtamembrane regions (Covino et al. 2018; Väth et al. 2021).

PC and PE are among the most abundant glycerophospholipids in both *S. cerevisiae* and mammalian cells (Vance 2015; Reinhard et al. 2022). Because PC has a larger hydrophilic head group than PE, the PC-to-PE ratio is an important determinant of the lipid bilayer structure, the lateral pressure profile and membrane fluidity (Fig. 4A; Marsh 2007; Dawaliby et al. 2016). A decreased hepatic PC-to-PE ratio has been linked to NAFLD and steatohepatitis (Li et al. 2006). Cells that lack a methyltransferase for PE-to-PC conversion (*opi3*Δ in *S. cerevisiae* and $PEMT^{-/-}$ in mammals) feature markedly reduced PC-to-PE ratios and a highly active UPR (Thibault et al. 2012; Gao et al. 2015). ScIRE1 responds to this type of lipid bilayer stress even when the ER–luminal domain is removed, thereby suggesting a direct role of the ER membrane (Thibault et al. 2012; Ho et al. 2020). Notably, *N*-monomethyl phosphatidylethanolamine (MMPE) lipids, which accumulate in methyltransferase-deficient cells, are likely to further modulate ER membrane properties and UPR signaling under these conditions (Shyu et al. 2019; Ishiwata-Kimata et al. 2022). PClipids can be produced not only by methylation of PE, but also via the Kennedy pathway (Kennedy and Weiss 1956). Consequently, the lipid bilayer stress in *OPI3* knockout cells can be rescued by choline supplementation and PC production via the Kennedy pathway (Thibault et al. 2012; Ho et al. 2020). Analogously, liver damage observed in $PEMT^{-/-}$ mice is reversed by choline-enriched diets (Waite et al. 2002). These examples further highlight the potency of lipid metabolites in modulating the UPR. Because the turnover of ER membrane proteins via ER-associated degradation is also perturbed by aberrantly low PC-to-PE ratios (Shyu et al. 2019), it would be interesting to study the impact of lipids on membrane protein insertion, topogenesis, and folding more systematically on a proteome-wide scale.

Cells employ elegant mechanisms to maintain the PC-to-PE molar in a range acceptable for normal membrane functions (Cornell and Ridgway 2015; Haider et al. 2018). Not only low, but also aberrantly increased PC-to-PE molar ratios of ~2.0 in the ER of obese mice have been associated with chronic UPR activation underlying the progression of steatohepatitis (Fu et al. 2011). However, high PC-to-PE ratios activate UPR transducers only indirectly. It was suggested that an abnormally high PC-to-PE ratio impairs the ER-localized Ca^{2+} pump SERCA, thereby lowering the ER–luminal Ca^{2+} level and impairing the function of Ca^{2+}-dependent chaperones including BiP (Fu et al. 2011; Preissler et al. 2020). Yeast lacks the ER-localized Ca^{2+} pump SERCA. Consistently, metabolic challenges that increase the PC-to-PE ratio in the ER of *S. cerevisiae* from 1.1 to 2.4 does not lead to any detectable UPR activation (Reinhard et al. 2022). Hence, not every lipid metabolic perturbation that severely affects ER membrane composition and that correlates with UPR activation causes lipid bilayer stress. For designing suitable dietary interventions to counteract ER stress–related pathologies, it will be crucial to better understand the molecular and physical basis of lipid bilayer stress.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Sphingolipids form a large group of ceramide-containing lipids. Intermediates of the sphingolipid metabolic pathways, dihydrosphingosine and dihydroceramide, interact with the transmembrane helix of ATF6 and promote forward transport and proteolytic processing in the Golgi complex (Tam et al. 2018). Globally up-regulating sphingolipid biosynthesis in *S. cerevisiae* by the deletion of *ORM1* and *ORM2* causes a constitutive activation of the UPR, while suppression of sphingolipid biosynthesis by low doses of myriocin rescues the sensitivity of *orm1*Δ*orm2*Δ cells to ER stress–inducing agents (Breslow et al. 2010; Liu et al. 2012). There is a substantial cross talk between sphingolipid and glycerophospholipid bio-synthesis at the level of regulation because they share common metabolites (Carman and Han 2009). It is possible that a deregulated sphingolipid biosynthesis triggers the UPR indirectly (e.g., by reducing the level of PI lipids in the ER), which is certainly relevant under inositol-limiting conditions (Promlek et al. 2011; Reinhard et al. 2022) and potentially also in a yeast model of classic galactosemia (Pimentel et al. 2022). Important building blocks for sphingolipid biosynthesis are very long chain fatty acids (VLCFAs) with 22 or more carbon atoms, which are thought to contribute to the coupling of lipid dynamics in the two leaflets of cellular lipid bilayers. Compromising the utilization of VLCFAs by deleting *FAT1* in *S. cerevisiae* increases the levels of the sphingolipid metabolic intermediate phytosphingosine, results in mildly elevated levels of saturated glycerophospholipids, and sensitizes cells to ER stress (Micoogullari et al. 2020). Because VLCFAs are incorporated not only in sphingolipids, but also found in GPI anchors and PI, it will be important to study how precisely the UPR is triggered under this condition (Kajiwara et al. 2012; Erdbrügger and Fröhlich 2020).

From the above-mentioned examples, it should be clear that diverse perturbations of lipid metabolism targeting structurally distinct lipids cause lipid bilayer stress.

How UPR Transducers Sense Lipid Bilayer Stress

Most mechanistic understanding of how UPR transducers sense lipid bilayer stress stems from studies of ScIRE1 (Promlek et al. 2011; Halbleib et al. 2017; Ho et al. 2020; Väth et al. 2021). Withits short transmembrane helix adjacent to an ER-luminal amphipathic helix, ScIRE1 causes a local membrane thinning and acyl chain disordering (Fig. 4B; Halbleib et al. 2017; Väth et al. 2021). The free energy penalty for such membrane distortions is low in the unstressed ER, whose lipid composition is ideally suited to accommodate even extreme hydrophobic mismatches (Renne and Ernst 2023). However, when a lipid metabolic perturbation reduces ER membrane compressibility and thickens the ER membrane, the free energy penalty increases and provides a driving force for ScIRE1 dimerization and/or oligomerization (Fig. 4B; Halbleib et al. 2017). The hydro-phobic mismatch-based oligomerization relies solely on the overall architecture of ScIRE1's transmembrane domain and the resulting "footprint" in the membrane, but not on specific amino acid residues of the transmembrane helix (Fig. 4B; Halbleib et al. 2017; Väth et al. 2021). Consequently, all transmembrane helix residues of ScIRE1 can be mutated without disrupting membrane sensitivity (Väth et al. 2021). However, mutations that disrupt the integrity of the adjacent amphipathic helix and the architecture of the entire transmembrane region also reduce the size of ScIRE1's membrane footprint and, consequently, membrane sensing by ScIRE1 (Halbleib et al. 2017). Consequently, point mutations in the amphipathic helix of ScIRE1

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

disrupt the response to inositol depletion (Halbleib et al. 2017; Tran et al. 2019), ethanol treatments (Tran et al. 2019), reduced PC-to-PE ratios (Ho et al. 2020), prolonged exposures to proteotoxic agents (Väth et al. 2021), and mutations that accumulate plasma membrane proteins in the ER (Phuong et al. 2023).

The membrane footprint of ScIRE1 is unusually large for a single-pass transmembrane protein due to the amphipathic helix penetrating deep into the luminal leaflet of the ER membrane (Fig. 4B). Its size and ellipsoid shape might maximize the sensitivity of ScIRE1 to reduced ER membrane compressibility (Väth et al. 2021). The gain in free energy upon a membrane-driven dimerization and oligomerization is more substantial for ScIRE1 than for unrelated single-pass transmembrane proteins because a larger distorted membrane area can coalesce when ScIRE1 protomers approach each other (Fig. 4B).

Based on sequence analyses and secondary structure predictions, a similar transmembrane architecture has been proposed for the mammalian UPR transducers IRE1α and PERK (Halbleib et al. 2017; Covino et al. 2018). However, mutations introduced in the hydrophobic face of the predicted amphipathic helix in the transmembrane region of IRE1α did not significantly lower the response to palmitic acid–induced lipid bilayer stress (Kono et al. 2017). Because the predicted amphipathic helix region of IRE1α also contributes to a functionally relevant interaction with the Sec61 translocon (Plumb et al. 2015; Sundaram et al. 2017), it remains challenging to interpret the functional consequences in this region in a straightforward manner. It is possible, however, that the responsiveness of mammalian UPR transducers to lipid bilayer stress is further affected by lipid phase separation events in the ER, specific changes in the lipid headgroup regions, or an overcrowding of the membrane with proteins (Volmer and Ron 2015; Shen et al. 2017; Covino et al. 2018).

Concluding Remarks

The remarkable ability of all three mammalian UPR transducers to sense membrane aberrancies highlights an intricate cross talk between protein quality control and lipid metabolism. It will be important to dissect the relative contributions of proteotoxic and lipid bilayer stress to the pathogenesis and course of ER stress–related diseases. As shown by a number of examples from model organisms, cultured mammalian cells, and preclinical models, this might facilitate new dietary or pharmacological interventions targeting complex metabolic diseases and the associated pathologies.

Acknowledgments

Our work is supported by the EU Horizon 2020 research and innovation program (grant agreement no. 866011) and the Deutsche Forschungsgemeinschaft in the framework of the CRC1027. We apologize to all those colleagues whose contributions we could not sufficiently reference due to space limitations.

References

Acosta-Alvear D, Karagöz GE, Fröhlich F, Li H, Walther TC, Walter P. The unfolded protein response and endoplasmic reticulum protein targeting machineries converge on the stress sensor IRE1. eLife. 2018; 7 e43036 doi: 10.7554/eLife.43036 [PubMed: 30582518]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Adams CJ, Kopp MC, Larburu N, Nowak PR, Ali MMU. Structure and molecular mechanism of ER stress signaling by the unfolded protein response signal activator IRE1. Front Mol Biosci. 2019; 6: 11. doi: 10.3389/fmolb.2019.00011 [PubMed: 30931312]

Amin-Wetzel N, Saunders RA, Kamphuis MJ, Rato C, Preissler S, Harding HP, Ron D. A J-protein co-chaperone recruits BiP to monomerize IRE1 and repress the unfolded protein response. Cell. 2017; 171: 1625–1637. e13 doi: 10.1016/j.cell.2017.10.040 [PubMed: 29198525]

Amin-Wetzel N, Neidhardt L, Yan Y, Mayer MP, Ron D. Unstructured regions in IRE1α specify BiP-mediated destabilisation of the luminal domaindimerand repression of the UPR. eLife. 2019; 8 e50793 doi: 10.7554/eLife.50793 [PubMed: 31873072]

Antonny B, Vanni S, Shindou H, Ferreira T. From zero to six double bonds: phospholipid unsaturation and organelle function. Trends Cell Biol. 2015; 25: 427–436. DOI: 10.1016/j.tcb.2015.03.004 [PubMed: 25906908]

Aragón T, van Anken E, Pincus D, Serafimova IM, Korennykh AV, Rubio CA, Walter P. Messenger RNA targeting to endoplasmic reticulum stress signalling sites. Nature. 2009; 457: 736–740. DOI: 10.1038/nature07641 [PubMed: 19079237]

Ariyama H, Kono N, Matsuda S, Inoue T, Arai H. Decrease in membrane phospholipid unsaturation induces unfolded protein response. J Biol Chem. 2010; 285: 22027–22035. DOI: 10.1074/jbc.M110.126870 [PubMed: 20489212]

Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev. 2013; 93: 1019–1137. DOI: 10.1152/physrev.00028.2012 [PubMed: 23899561]

Ballweg S, Ernst R. Control of membrane fluidity: the OLE pathway in focus. Biol Chem. 2017; 398: 215–228. DOI: 10.1515/hsz-2016-0277 [PubMed: 27787227]

Belyy V, Zuazo-Gaztelu I, Alamban A, Ashkenazi A, Walter P. Endoplasmic reticulum stress activates human IRE1α through reversible assembly of inactive dimers into small oligomers. eLife. 2022; 11 e74342 doi: 10.7554/eLife.74342 [PubMed: 35730415]

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000; 2: 326–332. DOI: 10.1038/35014014 [PubMed: 10854322]

Bigay J, Antonny B. Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity. Dev Cell. 2012; 23: 886–895. DOI: 10.1016/j.devcel.2012.10.009 [PubMed: 23153485]

Bommiasamy H, Back SH, Fagone P, Lee K, Meshinchi S, Vink E, Sriburi R, Frank M, Jackowski S, Kaufman RJ, et al. ATF6α induces XBP1-independent expansion of the endoplasmic reticulum. J Cell Sci. 2009; 122: 1626–1636. DOI: 10.1242/jcs.045625 [PubMed: 19420237]

Borradaile NM, Han X, Harp JD, Gale SE, Ory DS, Schaffer JE. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. J Lipid Res. 2006; 47: 2726–2737. DOI: 10.1194/jlr.M600299-JLR200 [PubMed: 16960261]

Boslem E, MacIntosh G, Preston AM, Bartley C, Busch AK, Fuller M, Laybutt DR, Meikle PJ, Biden TJ. A lipidomic screen of palmitate-treated MIN6 β-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. Biochem J. 2011; 435: 267–276. DOI: 10.1042/BJ20101867 [PubMed: 21265737]

Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. Annu Rev Biochem. 2011; 80: 71–99. DOI: 10.1146/annurev-biochem-062209-093836 [PubMed: 21495850]

Brambillasca S, Yabal M, Makarow M, Borgese N. Unassisted translocation of large polypeptide domains across phospholipid bilayers. J Cell Biol. 2006; 175: 767–777. DOI: 10.1083/jcb.200608101 [PubMed: 17130291]

Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, Ejsing CS, Weissman JS. Orm family proteins mediate sphingolipid homeostasis. Nature. 2010; 463: 1048–1053. DOI: 10.1038/nature08787 [PubMed: 20182505]

Brookheart RT, Michel CI, Schaffer JE. As a matter of fat. Cell Metab. 2009; 10: 9–12. DOI: 10.1016/j.cmet.2009.03.011 [PubMed: 19583949]

Europe PMC Funders Author Manuscripts

Brown MS, Radhakrishnan A, Goldstein JL. Retrospective on cholesterol homeostasis: the central role of scap. Annu Rev Biochem. 2018; 87: 783–807. DOI: 10.1146/annurev-biochem-062917-011852 [PubMed: 28841344]

Carman GM, Han GS. Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis. J Biol Chem. 2009; 284: 2593–2597. DOI: 10.1074/jbc.R800059200 [PubMed: 18812320]

Carrara M, Prischi F, Nowak PR, Ali MM. Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling. EMBO J. 2015a; 34: 1589–1600. DOI: 10.15252/embj.201489183 [PubMed: 25925385]

Carrara M, Prischi F, Nowak PR, Kopp MC, Ali MMU. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. eLife. 2015b; 4 e03522 doi: 10.7554/eLife.03522 [PubMed: 25692299]

Carreras-Sureda A, Pihán P, Hetz C. Calcium signaling at the endoplasmic reticulum: fine-tuning stress responses. Cell Calcium. 2018; 70: 24–31. DOI: 10.1016/j.ceca.2017.08.004 [PubMed: 29054537]

Castello-Serrano I, Heberle FA, Diaz-Rohrer B, Ippolito R, Shurer CR, Lujan P, Campelo F, Levental KR, Levental I. Partitioning to ordered membrane domains regulates the kinetics of secretory traffic. bioRxiv. 2023; doi: 10.1101/2023.04.18.537395

Cho H, Stanzione F, Oak A, Kim GH, Yerneni S, Qi L, Sum AK, Chan C. Intrinsic structural features of the human IRE1α transmembrane domain sense membrane lipid saturation. Cell Rep. 2019; 27: 307–320. e5 doi: 10.1016/j.celrep.2019.03.017 [PubMed: 30943411]

Çimen I, Kocatürk B, Koyuncu S, Tufanlı Ö, Onat UI, Yıl-dırım AD, Apaydın O, Demirsoy Ş, Aykut ZG, Nguyen UT, et al. Prevention of atherosclerosis by bioactive palmitoleate through suppression of organelle stress and inflammasome activation. Sci Transl Med. 2016; 8 358ra126 doi: 10.1126/scitranslmed.aaf9087

Çimen I, Yildirim Z, Dogan AE, Yildirim AD, Tufanli O, Onat UI, Nguyen U, Watkins SM, Weber C, Erbay E. Double bond configuration of palmitoleate is critical for atheroprotection. Mol Metab. 2019; 28: 58–72. DOI: 10.1016/j.molmet.2019.08.004 [PubMed: 31422082]

Conibear E. Converging views of endocytosis in yeast and mammals. Curr Opin Cell Biol. 2010; 22: 513–518. DOI: 10.1016/j.ceb.2010.05.009 [PubMed: 20538447]

Cornell RB, Ridgway ND. CTP:phosphocholine cytidylyltransferase: function, regulation, and structure of an amphitropic enzyme required for membrane biogenesis. Prog Lipid Res. 2015; 59: 147–171. DOI: 10.1016/j.plipres.2015.07.001 [PubMed: 26165797]

Covino R, Hummer G, Ernst R. Integrated functions of membrane property sensors and a hidden side of the unfolded protein response. Mol Cell. 2018; 71: 458–467. DOI: 10.1016/j.molcel.2018.07.019 [PubMed: 30075144]

Cox JS, Chapman RE, Walter P. The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol Biol Cell. 1997; 8: 1805–1814. DOI: 10.1091/mbc.8.9.1805 [PubMed: 9307975]

Credle JJ, Finer-Moore JS, Papa FR, Stroud RM, Walter P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. Proc Natl Acad Sci. 2005; 102: 18773–18784. DOI: 10.1073/pnas.0509487102 [PubMed: 16365312]

Dawaliby R, Trubbia C, Delporte C, Noyon C, Ruysschaert JM, Van Antwerpen P, Govaerts C. Phosphatidyl-ethanolamine is a key regulator of membrane fluidity in eukaryotic cells. J Biol Chem. 2016; 291: 3658–3667. DOI: 10.1074/jbc.M115.706523 [PubMed: 26663081]

Deguil J, Pineau L, Rowland Snyder EC, Dupont S, Beney L, Gil A, Frapper G, Ferreira T. Modulation of lipid-induced ER stress by fatty acid shape. Traffic. 2011; 12: 349–362. DOI: 10.1111/j.1600-0854.2010.01150.x [PubMed: 21143717]

Devries-Seimon T, Li Y, Yao PM, Stone E, Wang Y, Davis RJ, Flavell R, Tabas I. Cholesterol-induced macrophage apoptosis requires ER stress pathways and engagement of the type a scavenger receptor. J Cell Biol. 2005; 171: 61–73. DOI: 10.1083/jcb.200502078 [PubMed: 16203857]

Dowhan W, Vitrac H, Bogdanov M. Lipid-assisted membrane protein folding and topogenesis. Protein J. 2019; 38: 274–288. DOI: 10.1007/s10930-019-09826-7 [PubMed: 30937648]

Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nuñez G, Schnurr M, et al. NLRP3 inflammasomes are required for atherogenesis and activated

Europe PMC Funders Author Manuscripts

by cholesterol crystals. Nature. 2010; 464: 1357–1361. DOI: 10.1038/nature08938 [PubMed: 20428172]

Elbaz-Alon Y, Rosenfeld-Gur E, Shinder V, Futerman AH, Geiger T, Schuldiner M. A dynamic interface between vacuoles and mitochondria in yeast. Dev Cell. 2014; 30: 95–102. DOI: 10.1016/j.devcel.2014.06.007 [PubMed: 25026036]

Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, Fazio S, Wiest MM, Watkins SM, Linton MF, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med. 2009; 15: 1383–1391. DOI: 10.1038/nm.2067 [PubMed: 19966778]

Erdbrügger P, Fröhlich F. The role of very long chain fatty acids in yeast physiology and human diseases. Biol Chem. 2020; 402: 25–38. DOI: 10.1515/hsz-2020-0234 [PubMed: 33544487]

Ernst R, Ejsing CS, Antonny B. Homeoviscous adaptation and the regulation of membrane lipids. J Mol Biol. 2016; 428: 4776–4791. DOI: 10.1016/j.jmb.2016.08.013 [PubMed: 27534816]

Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, et al. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol. 2003; 5: 781–792. DOI: 10.1038/ncb1035 [PubMed: 12907943]

Fonseca SG, Gromada J, Urano F. Endoplasmic reticulum stress and pancreatic β-cell death. Trends Endocrinol Metab. 2011; 22: 266–274. DOI: 10.1016/j.tem.2011.02.008 [PubMed: 21458293]

Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, Hotamisligil GS. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. Nature. 2011; 473: 528–531. DOI: 10.1038/nature09968 [PubMed: 21532591]

Fu S, Watkins SM, Hotamisligil GS. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. Cell Metab. 2012; 15: 623–634. DOI: 10.1016/j.cmet.2012.03.007 [PubMed: 22560215]

Gao X, van der Veen JN, Vance JE, Thiesen A, Vance DE, Jacobs RL. Lack of phosphatidylethanolamine *N*-methyltransferase alters hepatic phospholipid composition and induces endoplasmic reticulum stress. Biochim Biophys Acta. 2015; 1852: 2689–2699. DOI: 10.1016/j.bbadis.2015.09.006 [PubMed: 26391255]

Garcia G, Zhang H, Moreno S, Tsui CK, Webster BM, Higuchi-Sanabria R, Dillin A. Lipid homeostasis is essential for a maximal ER stress response. eLife. 2023; 12 e83884 doi: 10.7554/eLife.83884 [PubMed: 37489956]

Gardner BM, Walter P. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science. 2011; 333: 1891–1894. DOI: 10.1126/science.1209126 [PubMed: 21852455]

Gassama-Diagne A, Yu W, ter Beest M, Martin-Belmonte F, Kierbel A, Engel J, Mostov K. Phosphatidylinositol-3,4,5-trisphosphate regulates the formation of the basolateral plasma membrane in epithelial cells. Nat Cell Biol. 2006; 8: 963–970. DOI: 10.1038/ncb1461 [PubMed: 16921364]

Gatta AT, Wong LH, Sere YY, Calderón-Noreña DM, Cockcroft S, Menon AK, Levine TP. A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. eLife. 2015; 4 e07253 doi: 10.7554/eLife.07253 [PubMed: 26001273]

Gonzalez TN, Sidrauski C, Dörfler S, Walter P. Mechanism of non-spliceosomal mRNA splicing in the unfolded protein response pathway. EMBO J. 1999; 18: 3119–3132. DOI: 10.1093/emboj/18.11.3119 [PubMed: 10357823]

Haider A, Wei YC, Lim K, Barbosa AD, Liu C-H, Weber U, Mlodzik M, Oras K, Collier S, Hussain MM, et al. PCYT1A regulates phosphatidylcholine homeostasis from the inner nuclear membrane in response to membrane stored curvature elastic stress. Dev Cell. 2018; 45: 481–495. e8 doi: 10.1016/j.devcel.2018.04.012 [PubMed: 29754800]

Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, Hänelt I, Hummer G, Ernst R. Activation of the unfolded protein response by lipid bilayer stress. Mol Cell. 2017; 67: 673–684. e8 doi: 10.1016/j.molcel.2017.06.012 [PubMed: 28689662]

Hamid SM, Citir M, Terzi EM, Cimen I, Yildirim Z, Dogan AE, Kocaturk B, Onat UI, Arditi M, Weber C, et al. Inositol-requiring enzyme-1 regulates phosphoinositide signaling lipids and

Europe PMC Funders Author Manuscripts

macrophage growth. EMBO Rep. 2020; 21 e51462 doi: 10.15252/embr.202051462 [PubMed: 33140520]

Han J, Kaufman RJ. The role of ER stress in lipid metabolism and lipotoxicity. J Lipid Res. 2016; 57: 1329–1338. DOI: 10.1194/jlr.R067595 [PubMed: 27146479]

Han X, Yang J, Yang K, Zhao Z, Abendschein DR, Gross RW. Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. Biochemistry. 2007; 46: 6417–6428. DOI: 10.1021/bi7004015 [PubMed: 17487985]

Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, Papa FR. IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell. 2009; 138: 562–575. DOI: 10.1016/j.cell.2009.07.017 [PubMed: 19665977]

Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. Nat Rev Mol Cell Biol. 2018; 19: 281–296. DOI: 10.1038/nrm.2017.138 [PubMed: 29410529]

Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature. 1999; 397: 271–274. DOI: 10.1038/16729 [PubMed: 9930704]

Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell. 2000; 6: 1099–1108. DOI: 10.1016/S1097-2765(00)00108-8 [PubMed: 11106749]

Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell. 1999; 10: 3787–3799. DOI: 10.1091/mbc.10.11.3787 [PubMed: 10564271]

Herzig Y, Sharpe HJ, Elbaz Y, Munro S, Schuldiner M. A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14. PLoS Biol. 2012; 10 e1001329 doi: 10.1371/journal.pbio.1001329 [PubMed: 22629230]

Hetz C, Glimcher LH. Fine-tuning of the unfolded protein response: assembling the IRE1α interactome. Mol Cell. 2009; 35: 551–561. DOI: 10.1016/j.molcel.2009.08.021 [PubMed: 19748352]

Hetz C, Papa FR. The unfolded protein response and cell fate control. Mol Cell. 2018; 69: 169–181. DOI: 10.1016/j.molcel.2017.06.017 [PubMed: 29107536]

Hetz C, Chevet E, Harding HP. Targeting the unfolded protein response in disease. Nat Rev Drug Discov. 2013; 12: 703–719. DOI: 10.1038/nrd3976 [PubMed: 23989796]

Hibi T, Dosch HM. Limiting dilution analysis of the B cell compartment in human bone marrow. Eur J Immunol. 1986; 16: 139–145. DOI: 10.1002/eji.1830160206 [PubMed: 2869953]

Ho N, Xu C, Thibault G. From the unfolded protein response to metabolic diseases—lipids under the spotlight. J Cell Sci. 2018; 131 jcs199307 doi: 10.1242/jcs.199307 [PubMed: 29439157]

Ho N, Yap WS, Xu J, Wu H, Koh JH, Goh WWB, George B, Chong SC, Taubert S, Thibault G. Stress sensor Ire1 deploys a divergent transcriptional program in response to lipid bilayer stress. J Cell Biol. 2020; 219 e201909165 doi: 10.1083/jcb.201909165 [PubMed: 32349127]

Hollien J. Evolution of the unfolded protein response. Biochim Biophys Acta. 2013; 1833: 2458–2463. DOI: 10.1016/j.bbamcr.2013.01.016 [PubMed: 23369734]

Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol. 2009; 186: 323–331. DOI: 10.1083/jcb.200903014 [PubMed: 19651891]

Holthuis JCM, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. Nature. 2014; 510: 48–57. DOI: 10.1038/nature13474 [PubMed: 24899304]

Horton JD, Goldstein JL, Brown MS. SREBPs: transcriptional mediators of lipid homeostasis. Cold Spring Harb Symp Quant Biol. 2002; 67: 491–498. DOI: 10.1101/sqb.2002.67.491 [PubMed: 12858575]

Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, Goldstein JL. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci. 2003; 100: 12027–12032. DOI: 10.1073/pnas.1534923100 [PubMed: 14512514]

Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell. 2010; 140: 900–917. DOI: 10.1016/j.cell.2010.02.034 [PubMed: 20303879]

Europe PMC Funders Author Manuscripts

Hou NS, Gutschmidt A, Choi DY, Pather K, Shi X, Watts JL, Hoppe T, Taubert S. Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo. Proc Natl Acad Sci. 2014; 111: E2271–E2280. DOI: 10.1073/pnas.1308062111 [PubMed: 24843123]

Ishiwata-Kimata Y, Le QG, Kimata Y. Induction and aggravation of the endoplasmic-reticulum stress by membrane-lipid metabolic intermediate phosphatidyl-*N*-monomethylethanolamine. Front Cell Dev Biol. 2022; 9 743018 doi: 10.3389/fcell.2021.743018 [PubMed: 35071223]

Iurlaro R, Muñoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. FEBS J. 2016; 283: 2640–2652. DOI: 10.1111/febs.13598 [PubMed: 26587781]

Jheng JR, Lau KS, Lan YW, Horng JT. A novel role of ER stress signal transducer ATF6 in regulating enterovirus A71 viral protein stability. J Biomed Sci. 2018; 25: 9. doi: 10.1186/s12929-018-0412-x [PubMed: 29386036]

Jurkin J, Henkel T, Nielsen AF, Minnich M, Popow J, Kaufmann T, Heindl K, Hoffmann T, Busslinger M, Martinez J. The mammalian tRNA ligase complex mediates splicing of *XBP1* mRNA and controls antibody secretion in plasma cells. EMBO J. 2014; 33: 2922–2936. DOI: 10.15252/embj.201490332 [PubMed: 25378478]

Kaiser HJ, Orłowski A, Róg T, Nyholm TKM, Chai W, Feizi T, Lingwood D, Vattulainen I, Simons K. Lateral sorting in model membranes by cholesterol-mediated hydrophobic matching. Proc Natl Acad Sci. 2011; 108: 16628–16633. DOI: 10.1073/pnas.1103742108 [PubMed: 21930944]

Kajiwara K, Muneoka T, Watanabe Y, Karashima T, Kitagaki H, Funato K. Perturbation of sphingolipid metabolism induces endoplasmic reticulum stress-mediated mitochondrial apoptosis in budding yeast. Mol Microbiol. 2012; 86: 1246–1261. DOI: 10.1111/mmi.12056 [PubMed: 23062268]

Karagöz GE, Acosta-Alvear D, Nguyen HT, Lee CP, Chu F, Walter P. An unfolded protein-induced conformational switch activates mammalian IRE1. eLife. 2017; 6 e30700 doi: 10.7554/eLife.30700 [PubMed: 28971800]

Karagöz GE, Acosta-Alvear D, Walter P. The unfolded protein response: detecting and responding to fluctuations in the protein-folding capacity of the endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2019; 11 a033886 doi: 10.1101/cshperspect.a033886 [PubMed: 30670466]

Kartsoli S, Kostara CE, Tsimihodimos V, Bairaktari ET, Christodoulou DK. Lipidomics in non-alcoholic fatty liver disease. World J Hepatol. 2020; 12: 436–450. DOI: 10.4254/wjh.v12.i8.436 [PubMed: 32952872]

Keenan TW, Morré DJ. Phospholipid class and fatty acid composition of Golgi apparatus isolated from rat liver and comparison with other cell fractions. Biochemistry. 1970; 9: 19–25. DOI: 10.1021/bi00803a003 [PubMed: 4312390]

Kennedy EP, Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem. 1956; 222: 193–214. DOI: 10.1016/S0021-9258(19)50785-2 [PubMed: 13366993]

Kettel P, Marosits L, Spinetti E, Rechberger M, Radler P, Niedermoser I, Fischer I, Versteeg GA, Loose M, Covino R, et al. Stress-induced clustering of the UPR sensor IRE1α is driven by disordered regions within its ER lumenal domain. bioRxiv. 2023; doi: 10.1101/2023.03.30.534746v1

Kimata Y, Ishiwata-Kimata Y, Ito T, Hirata A, Suzuki T, Oikawa D, Takeuchi M, Kohno K. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. J Cell Biol. 2007; 179: 75–86. DOI: 10.1083/jcb.200704166 [PubMed: 17923530]

Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, Sampaio JL, de Robillard Q, Ferguson C, Proszynski TJ, Shevchenko A, et al. Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J Cell Biol. 2009; 185: 601–612. DOI: 10.1083/jcb.200901145 [PubMed: 19433450]

Koba H, Jin S, Imada N, Ishikawa T, Ninagawa S, Okada T, Sakuma T, Yamamoto T, Mori K. Reinvestigation of disulfide-bonded oligomeric forms of the unfolded protein response transducer ATF6. Cell Struct Funct. 2020; 45: 9–21. DOI: 10.1247/csf.19030 [PubMed: 31852864]

Europe PMC Funders Author Manuscripts

Kono N, Amin-Wetzel N, Ron D. Generic membrane-spanning features endow IRE1α with responsiveness to membrane aberrancy. Mol Biol Cell. 2017; 28: 2318–2332. DOI: 10.1091/mbc.e17-03-0144 [PubMed: 28615323]

Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009; 457: 687–693. DOI: 10.1038/nature07661 [PubMed: 19079236]

Kwak C, Shin S, Park JS, Jung M, Nhung TTM, Kang MG, Lee C, Kwon TH, Park SK, Mun JY, et al. Contact-ID, a tool for profiling organelle contact sites, reveals regulatory proteins of mitochondrial-associated membrane formation. Proc Natl Acad Sci. 2020; 117: 12109–12120. DOI: 10.1073/pnas.1916584117 [PubMed: 32414919]

Le QG, Ishiwata-Kimata Y, Phuong TH, Fukunaka S, Kohno K, Kimata Y. The ADP-binding kinase region of Ire1 directly contributes to its responsiveness to endoplasmic reticulum stress. Sci Rep. 2021; 11: 4506. doi: 10.1038/s41598-021-83890-x [PubMed: 33627709]

Lebeaupin C, Proics E, de Bieville CHD, Rousseau D, Bonnafous S, Patouraux S, Adam G, Lavallard VJ, Rovere C, Le Thuc O, et al. ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis. 2015; 6 e1879 doi: 10.1038/cddis.2015.248 [PubMed: 26355342]

Lee SM, Lee SH, Jung Y, Lee Y, Yoon JH, Choi JY, Hwang CY, Son YH, Park SS, Hwang GS, et al. FABP3-mediated membrane lipid saturation alters fluidity and induces ER stress in skeletal muscle with aging. Nat Commun. 2020; 11: 5661. doi: 10.1038/s41467-020-19501-6 [PubMed: 33168829]

Levental I, Lyman E. Regulation of membrane protein structure and function by their lipid nano-environment. Nat Rev Mol Cell Biol. 2023; 24: 107–122. DOI: 10.1038/s41580-022-00524-4 [PubMed: 36056103]

Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab. 2006; 3: 321–331. DOI: 10.1016/j.cmet.2006.03.007 [PubMed: 16679290]

Li X, Sun S, Appathurai S, Sundaram A, Plumb R, Mariappan M. A molecular mechanism for turning off IRE1α signaling during endoplasmic reticulum stress. Cell Rep. 2020; 33 108563 doi: 10.1016/j.celrep.2020.108563 [PubMed: 33378667]

Li W, Crotty K, Garrido Ruiz D, Voorhies M, Rivera C, Sil A, Mullins RD, Jacobson MP, Peschek J, Walter P. Protomer alignment modulates specificity of RNA substrate recognition by Ire1. eLife. 2021; 10 e67425 doi: 10.7554/eLife.67425 [PubMed: 33904404]

Li D, Rocha-Roa C, Schilling MA, Reinisch KM, Vanni S. Lipid scrambling is a general feature of protein insertases. bioRxiv. 2023; doi: 10.1101/2023.09.01.555937

Liao PC, Yang EJ, Borgman T, Boldogh IR, Sing CN, Swayne TC, Pon LA. Touch and go: membrane contact sites between lipid droplets and otherorganelles. Front Cell Dev Biol. 2022; 10 852021 doi: 10.3389/fcell.2022.852021 [PubMed: 35281095]

Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010; 327: 46–50. DOI: 10.1126/science.1174621 [PubMed: 20044567]

Liu CY, Schröder M, Kaufman RJ. Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum. J Biol Chem. 2000; 275: 24881–24885. DOI: 10.1074/jbc.M004454200 [PubMed: 10835430]

Liu M, Huang C, Polu SR, Schneiter R, Chang A. Regulation of sphingolipid synthesis through Orm1 and Orm2 in yeast. J Cell Sci. 2012; 125: 2428–2435. DOI: 10.1242/jcs.10057 [PubMed: 22328531]

Lorent JH, Diaz-Rohrer B, Lin X, Spring K, Gorfe AA, Levental KR, Levental I. Structural determinants and functional consequences of protein affinity for membrane rafts. Nat Commun. 2017; 8: 1219. doi: 10.1038/s41467-017-01328-3 [PubMed: 29089556]

Lorent JH, Levental KR, Ganesan L, Rivera-Longsworth G, Sezgin E, Doktorova M, Lyman E, Levental I. Plasma membranes are asymmetric in lipid unsaturation, packing and protein shape. Nat Chem Biol. 2020; 16: 644–652. DOI: 10.1038/s41589-020-0529-6 [PubMed: 32367017]

Europe PMC Funders Author Manuscripts

Lu Y, Liang FX, Wang X. A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. Mol Cell. 2014; 55: 758–770. DOI: 10.1016/j.molcel.2014.06.032 [PubMed: 25087875]

Maiuolo J, Bulotta S, Verderio C, Benfante R, Borgese N. Selective activation of the transcription factor ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein. Proc Natl Acad Sci. 2011; 108: 7832–7837. DOI: 10.1073/pnas.1101379108 [PubMed: 21521793]

Marsh D. Lateral pressure profile, spontaneous curvature frustration, and the incorporation and conformation of proteins in membranes. Biophys J. 2007; 93: 3884–3899. DOI: 10.1529/biophysj.107.107938 [PubMed: 17704167]

Matsumoto M, Minami M, Takeda K, Sakao S, Akira S. Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastric leukemia cells. FEBS Lett. 1996; 21: 143–147. DOI: 10.1016/0014-5793(96)01016-2

Menon AK. Sterol gradients in cells. Curr Opin Cell Biol. 2018; 53: 37–43. DOI: 10.1016/j.ceb.2018.04.012 [PubMed: 29783105]

Mesmin B, Bigay J, Moser von Filseck J, Lacas-Gervais S, Drin G, Antonny B. A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP. Cell. 2013; 155: 830–843. DOI: 10.1016/j.cell.2013.09.056 [PubMed: 24209621]

Micoogullari Y, Basu SS, Ang J, Weisshaar N, Schmitt ND, Abdelmoula WM, Lopez B, Agar JN, Agar N, Hanna J. Dysregulation of very-long-chain fatty acid metabolism causes membrane saturation and induction of the unfolded protein response. Mol Biol Cell. 2020; 31: 7–17. DOI: 10.1091/mbc.E19-07-0392 [PubMed: 31746669]

Moncan M, Mnich K, Blomme A, Almanza A, Samali A, Gorman AM. Regulation of lipid metabolism by the unfolded protein response. J Cell Mol Med. 2021; 25: 1359–1370. DOI: 10.1111/jcmm.16255 [PubMed: 33398919]

Moore K, Hollien J. Ire1-mediated decay in mammalian cells relies on mRNA sequence, structure, and translational status. Mol Biol Cell. 2015; 26: 2873–2884. DOI: 10.1091/mbc.E15-02-0074 [PubMed: 26108623]

Mori K. Evolutionary aspects of the unfolded protein response. Cold Spring Harb Perspect Biol. 2022; 14 a041262 doi: 10.1101/cshperspect.a041262 [PubMed: 35940910]

Mouritsen OG, Bloom M. Mattress model of lipid-protein interactions in membranes. Biophys J. 1984; 46: 141–153. DOI: 10.1016/S0006-3495(84)84007-2 [PubMed: 6478029]

Muñiz M, Zurzolo C. Sorting of GPI-anchored proteins from yeast to mammals—common pathways at different sites? J Cell Sci. 2014; 127: 2793–2801. DOI: 10.1242/jcs.148056 [PubMed: 24906797]

Nadanaka S, Okada T, Yoshida H, Mori K. Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. Mol Cell Biol. 2007; 27: 1027–1043. DOI: 10.1128/MCB.00408-06 [PubMed: 17101776]

Nguyen VC, Nakamura Y, Kanehara K. Membrane lipid polyunsaturation mediated by *FATTY ACID DESA-TURASE 2 (FAD2)* is involved in endoplasmic reticulum stress tolerance *Arabidopsis thaliana*. Plant J. 2019; 99: 478–493. DOI: 10.1111/tpj.14338 [PubMed: 31001857]

Nilsson I, Ohvo-Rekilä H, Slotte JP, Johnson AE, von Heijne G. Inhibition of protein translocation across the endoplasmic reticulum membrane by sterols. J Biol Chem. 2001; 276: 41748–41754. DOI: 10.1074/jbc.M105823200 [PubMed: 11535595]

Novoa I, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by *GADD34*-mediated dephosphorylation of eIF2α. J Cell Biol. 2001; 153: 1011–1022. DOI: 10.1083/jcb.153.5.1011 [PubMed: 11381086]

Oka OB, van Lith M, Rudolf J, Tungkum W, Pringle MA, Bulleid NJ. ERp18 regulates activation of ATF6α during unfolded protein response. EMBO J. 2019; 38 e100990 doi: 10.15252/embj.2018100990 [PubMed: 31368601]

Oka OBV, Pierre AS, Pringle MA, Tungkum W, Cao Z, Fleming B, Bulleid NJ. Activation of the UPR sensor ATF6α is regulated by its redox-dependent dimerization and ER retention by ERp18. Proc Natl Acad Sci. 2022; 119 e2122657119 doi: 10.1073/pnas.2122657119 [PubMed: 35286189]

Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K. Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. Biochem Biophys Res Commun. 2000; 279: 445–450. DOI: 10.1006/bbrc.2000.3987 [PubMed: 11118306]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Papa FR, Zhang C, Shokat K, Walter P. Bypassing a kinase activity with an ATP-competitive drug. Science. 2003; 302: 1533–1537. DOI: 10.1126/science.1090031 [PubMed: 14564015]

Phuong HT, Ishiwata-Kimata Y, Kimata Y. An ER-accumulated mutant of yeast Pma1 causes membrane-related stress to induce the unfolded protein response. Biochem Biophys Res Commun. 2023; 667: 58–63. DOI: 10.1016/j.bbrc.2023.05.044 [PubMed: 37209563]

Piccolis M, Bond LM, Kampmann M, Pulimeno P, Chitraju C, Jayson CBK, Vaites LP, Boland S, Lai ZW, Gabriel KR, et al. Probing the global cellular responses to lipotoxicity caused by saturated fatty acids. Mol Cell. 2019; 74: 32–44. e8 doi: 10.1016/j.molcel.2019.01.036 [PubMed: 30846318]

Pimentel FSA, Machado CM, De-Souza EA, Fernandes CM, De-Queiroz ALFV, Silva GFS, Del Poeta M, Montero-Lomeli M, Masuda CA. Sphingolipid depletion suppresses UPR activation and promotes galactose hypersensitivity in yeast models of classic galactosemia. Biochim Biophys Acta Mol Basis Dis. 2022; 1868 166389 doi: 10.1016/j.bbadis.2022.166389 [PubMed: 35301088]

Pineau L, Ferreira T. Lipid-induced ER stress in yeast and β cells: parallel trails to acommon fate. FEMS Yeast Res. 2010; 10: 1035–1045. DOI: 10.1111/j.1567-1364.2010.00674.x [PubMed: 20738405]

Pineau L, Colas J, Dupont S, Beney L, Fleurat-Lessard P, Berjeaud JM, Bergès T, Ferreira T. Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. Traffic. 2009; 10: 673–690. DOI: 10.1111/j.1600-0854.2009.00903.x [PubMed: 19302420]

Plumb R, Zhang ZR, Appathurai S, Mariappan M. A functional link between the co-translational protein translocation pathway and the UPR. eLife. 2015; 4 e07426 doi: 10.7554/eLife.07426 [PubMed: 25993558]

Posor Y, Jang W, Haucke V. Phosphoinositides as membrane organizers. Nat Rev Mol Cell Biol. 2022; 23: 797–816. DOI: 10.1038/s41580-022-00490-x [PubMed: 35589852]

Preissler S, Ron D. Early events in the endoplasmic reticulum unfolded protein response. Cold Spring Harb Perspect Biol. 2019; 11 a033894 doi: 10.1101/cshperspect.a033894 [PubMed: 30396883]

Preissler S, Chambers JE, Crespillo-Casado A, Avezov E, Miranda E, Perez J, Hendershot LM, Harding HP, Ron D. Physiological modulation of BiP activity by trans-protomer engagement of the interdomain linker. eLife. 2015; 4 e08961 doi: 10.7554/eLife.08961 [PubMed: 26473973]

Preissler S, Rato C, Yan Y, Perera LA, Czako A, Ron D. Calcium depletion challenges endoplasmic reticulum proteostasis by destabilising BiP-substrate complexes. eLife. 2020; 9 e62601 doi: 10.7554/eLife.62601 [PubMed: 33295873]

Prinz WA. Lipid trafficking sans vesicles: where, why, how? Cell. 2010; 143: 870–874. DOI: 10.1016/j.cell.2010.11.031 [PubMed: 21145454]

Promlek T, Ishiwata-Kimata Y, Shido M, Sakuramoto M, Kohno K, Kimata Y. Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Mol Biol Cell. 2011; 22: 3520–3532. DOI: 10.1091/mbc.e11-04-0295 [PubMed: 21775630]

Pu Y, Ruberti C, Angelos ER, Brandizzi F. AtIRE1C, an unconventional isoform of the UPR master regulator AtIRE1, is functionally associated with AtIRE1B in *Arabidopsis* gametogenesis. Plant Direct. 2019; 3 e00187 doi: 10.1002/pld3.187 [PubMed: 31799493]

Quinn P, Griffiths G, Warren G. Density of newly synthesized plasma membrane proteins in intracellular membranes. II: Biochemical studies. J Cell Biol. 1984; 98: 2142–2147. DOI: 10.1083/jcb.98.6.2142 [PubMed: 6563038]

Quiroga R, Trenchi A, González Montoro A, Valdez Taubas J, Maccioni HJF. Short transmembrane domains with high-volume exoplasmic halves determine retention of type II membrane proteins in the Golgi complex. J Cell Sci. 2013; 126: 5344–5349. DOI: 10.1242/jcs.13065 [PubMed: 24105265]

Radanović T, Ernst R. The unfolded protein response as a guardian of the secretory pathway. Cells. 2021; 10: 2965. doi: 10.3390/cells10112965 [PubMed: 34831188]

Radhakrishnan A, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. Cell Metab. 2008; 8: 512–521. DOI: 10.1016/j.cmet.2008.10.008 [PubMed: 19041766]

Europe PMC Funders Author Manuscripts

Reinhard J, Starke L, Klose C, Haberkant P, Hammarén H, Stein F, Klein O, Berhorst C, Stumpf H, Sáenz JP, et al. A new technology for isolating organellar membranes provides fingerprints of lipid bilayer stress. bioRxiv. 2022; doi: 10.1101/2022.09.15.508072

Renne MF, Ernst R. Membrane homeostasis beyond fluidity: control of membrane compressibility. Trends Biochem Sci. 2023; 48: 963–977. DOI: 10.1016/j.tibs.2023.08.004 [PubMed: 37652754]

Rodriguez-Gallardo S, Kurokawa K, Sabido-Bozo S, Cortes-Gomez A, Ikeda A, Zoni V, Aguilera-Romero A, Perez-Linero AM, Lopez S, Waga M, et al. Ceramide chain length-dependent protein sorting into selective endoplasmic reticulum exit sites. Sci Adv. 2020; 6 eaba8237 doi: 10.1126/sciadv.aba8237 [PubMed: 33310842]

Rubio C, Pincus D, Korennykh A, Schuck S, El-Samad H, Walter P. Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. J Cell Biol. 2011; 193: 171–184. DOI: 10.1083/jcb.201007077 [PubMed: 21444684]

Rutkowski DT. Liver function and dysfunction—a unique window into the physiological reach of ER stress and the unfolded protein response. FEBS J. 2019; 286: 356–378. DOI: 10.1111/febs.14389 [PubMed: 29360258]

Schindler AJ, Schekman R. In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles. Proc Natl Acad Sci. 2009; 106: 17775–17780. DOI: 10.1073/pnas.0910342106 [PubMed: 19822759]

Schneiter R, Brügger B, Sandhoff R, Zellnig G, Leber A, Lampl M, Athenstaedt K, Hrastnik C, Eder S, Daum G, et al. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. J Cell Biol. 1999; 146: 741–754. DOI: 10.1083/jcb.146.4.741 [PubMed: 10459010]

Schuck S, Prinz WA, Thorn KS, Voss C, Walter P. Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. J Cell Biol. 2009; 187: 525–536. DOI: 10.1083/jcb.200907074 [PubMed: 19948500]

Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: a membrane trafficking machine. J Cell Biol. 2019; 218: 2096–2112. DOI: 10.1083/jcb.201903090 [PubMed: 31201265]

Sharpe HJ, Stevens TJ, Munro S. A comprehensive comparison of transmembrane domains reveals organelle-specific properties. Cell. 2010; 142: 158–169. DOI: 10.1016/j.cell.2010.05.037 [PubMed: 20603021]

Shen J, Chen X, Hendershot L, Prywes R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell. 2002; 3: 99–111. DOI: 10.1016/S1534-5807(02)00203-4 [PubMed: 12110171]

Shen Y, Zhao Z, Zhang L, Shi L, Shahriar S, Chan RB, Di Paolo G, Min W. Metabolic activity induces membrane phase separation in endoplasmic reticulum. Proc Natl Acad Sci. 2017; 114: 13394–13399. DOI: 10.1073/pnas.1712555114 [PubMed: 29196526]

Shyu P Jr, Ng BSH, Ho N, Chaw R, Seah YL, Marvalim C, Thibault G. Membrane phospholipid alteration causes chronic ER stress through early degradation of homeostatic ER-resident proteins. Sci Rep. 2019; 9: 8637. doi: 10.1038/s41598-019-45020-6 [PubMed: 31201345]

Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997; 387: 569–572. DOI: 10.1038/42408 [PubMed: 9177342]

Simons K, van Meer G. Lipid sorting in epithelial cells. Biochemistry. 1988; 27: 6197–6202. DOI: 10.1021/bi00417a001 [PubMed: 3064805]

Sokoya T, Parolek J, Foged MM, Danylchuk DI, Bozan M, Sarkar B, Hilderink A, Philippi M, Botto LD, Terhal PA, et al. Pathogenic variants of sphingomyelin synthase SMS2 disrupt lipid landscapes in the secretory pathway. eLife. 2022; 11 e79278 doi: 10.7554/eLife.79278 [PubMed: 36102623]

Sriburi R, Jackowski S, Mori K, Brewer JW. XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. J Cell Biol. 2004; 167: 35–41. DOI: 10.1083/jcb.200406136 [PubMed: 15466483]

Europe PMC Funders Author Manuscripts

Sundaram A, Plumb R, Appathurai S, Mariappan M. The Sec61 translocon limits IRE1α signaling during the unfolded protein response. eLife. 2017; 6 e27187 doi: 10.7554/eLife.27187 [PubMed: 28504640]

Sundaram A, Appathurai S, Plumb R, Mariappan M. Dynamic changes in complexes of IRE1α, PERK, and ATF6α during endoplasmic reticulum stress. Mol Biol Cell. 2018; 29: 1376–1388. DOI: 10.1091/mbc.E17-10-0594 [PubMed: 29851562]

Surma MA, Klose C, Klemm RW, Ejsing CS, Simons K. Generic sorting of raft lipids into secretory vesicles in yeast. Traffic. 2011; 12: 1139–1147. DOI: 10.1111/j.1600-0854.2011.01221.x [PubMed: 21575114]

Surma MA, Klose C, Simons K. Lipid-dependent protein sorting at the *trans*-Golgi network. Biochim Biophys Acta. 2012; 1821: 1059–1067. DOI: 10.1016/j.bbalip.2011.12.008 [PubMed: 22230596]

Surma MA, Klose C, Peng D, Shales M, Mrejen C, Stefanko A, Braberg H, Gordon DE, Vorkel D, Ejsing CS, et al. A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. Mol Cell. 2013; 51: 519–530. DOI: 10.1016/j.molcel.2013.06.014 [PubMed: 23891562]

Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol. 2011; 13: 184–190. DOI: 10.1038/ncb0311-184 [PubMed: 21364565]

Tam AB, Roberts LS, Chandra V, Rivera IG, Nomura DK, Forbes DJ, Niwa M. The UPR activator ATF6 responds to proteotoxic and lipotoxic stress by distinct mechanisms. Dev Cell. 2018; 46: 327–343. e7 doi: 10.1016/j.devcel.2018.04.023 [PubMed: 30086303]

Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, Wek RC. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Mol Biol Cell. 2011; 22: 4390–4405. DOI: 10.1091/mbc.e11-06-0510 [PubMed: 21917591]

Thibault G, Shui G, Kim W, McAlister GC, Ismail N, Gygi SP, Wenk MR, Ng DTW. The membrane stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network. Mol Cell. 2012; 48: 16–27. DOI: 10.1016/j.molcel.2012.08.016 [PubMed: 23000174]

Tran DM, Takagi H, Kimata Y. Categorization of endoplasmic reticulum stress as accumulation of unfolded proteins or membrane lipid aberrancy using yeast Ire1 mutants. Biosci Biotechnol Biochem. 2019; 83: 326–329. DOI: 10.1080/09168451.2018.1530098 [PubMed: 30319071]

Tran NH, Carter SD, De Mazière A, Ashkenazi A, Klumperman J, Walter P, Jensen GJ. The stress-sensing domain of activated IRE1α forms helical filaments in narrow ER membrane tubes. Science. 2021; 374: 52–57. DOI: 10.1126/science.abh2474 [PubMed: 34591618]

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell. 2000; 101: 249–258. DOI: 10.1016/S0092-8674(00)80835-1 [PubMed: 10847680]

Tsuru A, Imai Y, Saito M, Kohno K. Novel mechanism of enhancing IRE1α-XBP1 signalling via the PERK-ATF4 pathway. Sci Rep. 2016; 6 24217 doi: 10.1038/srep24217 [PubMed: 27052593]

Tufanli O, Telkoparan Akillilar P, Acosta-Alvear D, Kocaturk B, Onat UI, Hamid SM, Çimen I, Walter P, Weber C, Erbay E. Targeting IRE1 with small molecules counteracts progression of atherosclerosis. Proc Natl Acad Sci. 2017; 114: E1395–E1404. DOI: 10.1073/pnas.1621188114 [PubMed: 28137856]

Urra H, Dufey E, Avril T, Chevet E, Hetz C. Endoplasmic reticulum stress and the hallmarks of cancer. Trends Cancer Res. 2016; 2: 252–262. DOI: 10.1016/j.trecan.2016.03.007

Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, Wait E, Cohen AR, Davidson MW, Betzig E, Lippincott-Schwartz J. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. Nature. 2017; 546: 162–167. DOI: 10.1038/nature22369 [PubMed: 28538724]

Vance JE. Phospholipid synthesis and transport in mammalian cells. Traffic. 2015; 16: 1–18. DOI: 10.1111/tra.12230 [PubMed: 25243850]

van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008; 9: 112–124. DOI: 10.1038/nrm2330 [PubMed: 18216768]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Väth K, Mattes C, Reinhard J, Covino R, Stumpf H, Hummer G, Ernst R. Cysteine cross-linking in native membranes establishesthe transmembrane architecture of Ire1. J Cell Biol. 2021; 220 e202011078 doi: 10.1083/jcb.202011078 [PubMed: 34196665]

Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell. 2006; 124: 573–586. DOI: 10.1016/j.cell.2005.11.047 [PubMed: 16469703]

Volmer R, Ron D. Lipid-dependent regulation of the unfolded protein response. Curr Opin Cell Biol. 2015; 33: 67–73. DOI: 10.1016/j.ceb.2014.12.002 [PubMed: 25543896]

Volmer R, van der Ploeg K, Ron D. Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc Natl Acad Sci. 2013; 110: 4628–4633. DOI: 10.1073/pnas.1217611110 [PubMed: 23487760]

Waite KA, Cabilio NR, Vance DE. Choline deficiency–induced liver damage is reversible in Pemt$^{-/-}$ mice. J Nutr. 2002; 132: 68–71. DOI: 10.1093/jn/132.1.68 [PubMed: 11773510]

Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011; 334: 1081–1086. DOI: 10.1126/science.1209038 [PubMed: 22116877]

Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016; 529: 326–335. DOI: 10.1038/nature17041 [PubMed: 26791723]

Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. Endocrinology. 2006; 147: 943–951. DOI: 10.1210/en.2005-0570 [PubMed: 16269465]

Weigel AV, Chang CL, Shtengel G, Xu CS, Hoffman DP, Freeman M, Iyer N, Aaron J, Khuon S, Bogovic J, et al. ER-to-Golgi protein delivery through an interwoven, tubular network extending from ER. Cell. 2021; 184: 2412–2429. e16 doi: 10.1016/j.cell.2021.03.035 [PubMed: 33852913]

Welihinda AA, Tirasophon W, Kaufman RJ. The transcriptional co-activator ADA5 is required for HAC1 mRNA processing in vivo. J Biol Chem. 2000; 275: 3377–3381. DOI: 10.1074/jbc.275.5.3377 [PubMed: 10652329]

Wenzel EM, Elfmark LA, Stenmark H, Raiborg C. ER as master regulator of membrane trafficking and organelle function. J Cell Biol. 2022; 221 e202205135 doi: 10.1083/jcb.202205135 [PubMed: 36108241]

Wigger L, Barovic M, Brunner AD, Marzetta F, Schöniger E, Mehl F, Kipke N, Friedland D, Burdet F, Kessler C, et al. Multi-omics profiling of living human pancreatic islet donors reveals heterogeneous β cell trajectories towards type 2 diabetes. Nat Metab. 2021; 3: 1017–1031. DOI: 10.1038/s42255-021-00420-9 [PubMed: 34183850]

Wu X, Rapoport TA. Translocation of proteins through a distorted lipid bilayer. Trends Cell Biol. 2021; 31: 473–484. DOI: 10.1016/j.tcb.2021.01.002 [PubMed: 33531207]

Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J, Song B, Yau GD-Y, Kaufman RJ. ATF6α optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell. 2007; 13: 351–364. DOI: 10.1016/j.devcel.2007.07.005 [PubMed: 17765679]

Wu X, Siggel M, Ovchinnikov S, Mi W, Svetlov V, Nudler E, Liao M, Hummer G, Rapoport TA. Structural basis of ER-associated protein degradation mediated by the Hrd1 ubiquitin ligase complex. Science. 2020; 368 eaaz2449 doi: 10.1126/science.aaz2449 [PubMed: 32327568]

Ye J, Rawson RB, Komuro R, Davé X, Prywes R, Brown MS, Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell. 2000; 6: 1355–1364. DOI: 10.1016/S1097-2765(00)00133-7 [PubMed: 11163209]

Yong J, Johnson JD, Arvan P, Han J, Kaufman RJ. Therapeutic opportunities for pancreatic β-cell ER stress in diabetes mellitus. Nat Rev Endocrinol. 2021; 17: 455–467. DOI: 10.1038/s41574-021-00510-4 [PubMed: 34163039]

Yoshida H, Haze K, Yanagi H, Yura T, Mori K. Identification of the *cis*-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. J Biol Chem. 1998; 273: 33741–33749. DOI: 10.1074/jbc.273.50.33741 [PubMed: 9837962]

Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001; 107: 881–891. DOI: 10.1016/S0092-8674(01)00611-0 [PubMed: 11779464]

Yu CY, Hsu YW, Liao CL, Lin YL. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. J Virol. 2006; 80: 11868–11880. DOI: 10.1128/JVI.00879-06 [PubMed: 16987981]

Zeng L, Lu M, Mori K, Luo S, Lee AS, Zhu Y, Shyy JYJ. ATF6 modulates SREBP2-mediated lipogenesis. EMBO J. 2004; 23: 950–958. DOI: 10.1038/sj.emboj.7600106 [PubMed: 14765107]

Zhou AX, Tabas I. The UPR in atherosclerosis. Semin Immunopathol. 2013; 35: 321–332. DOI: 10.1007/s00281-013-0372-x [PubMed: 23553213]

Zhou J, Liu CY, Back SH, Clark RL, Peisach D, Xu Z, Kaufman RJ. 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. 2006; 103: 14343–14348. DOI: 10.1073/pnas.0606480103 [PubMed: 16973740]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

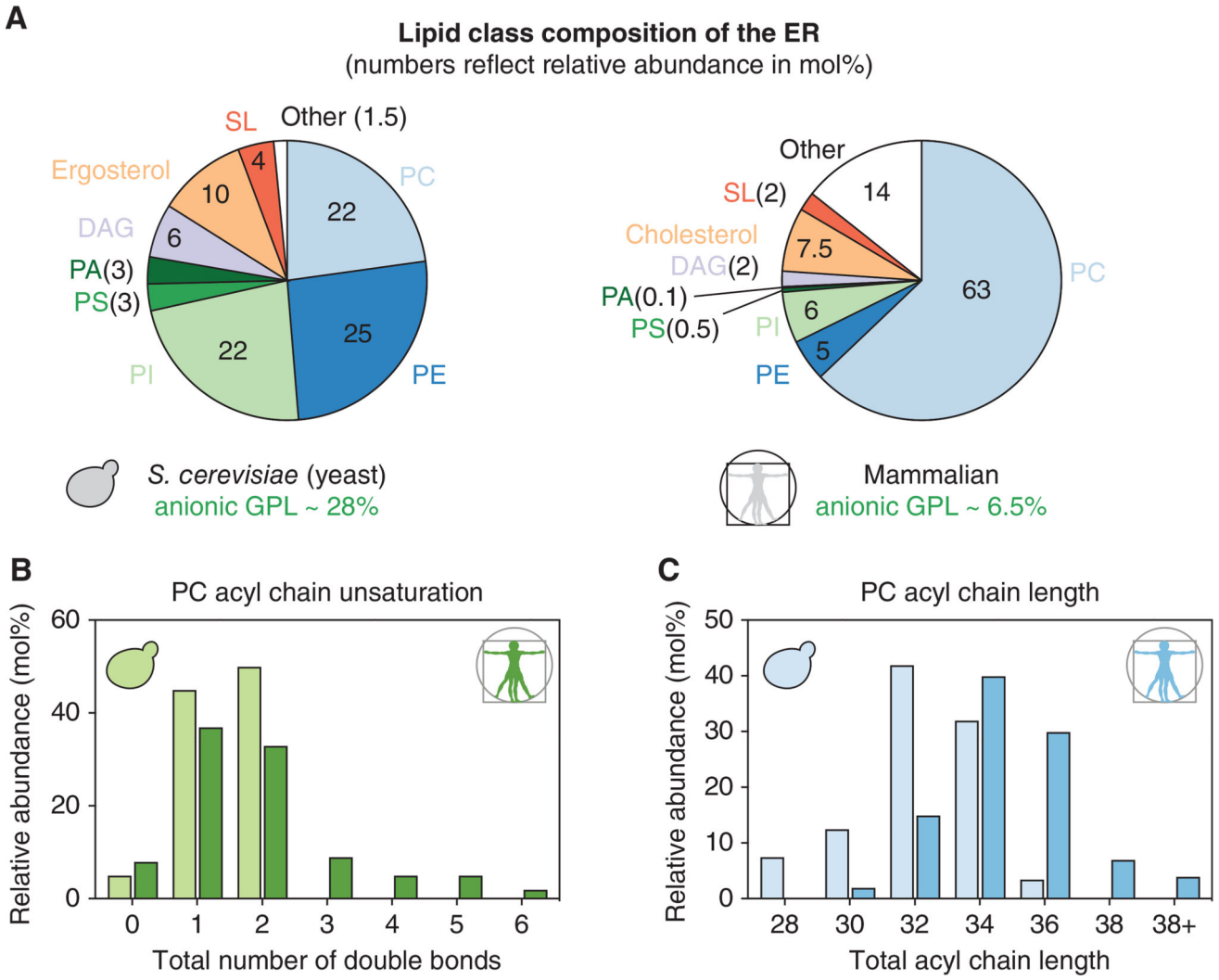

Figure 1.

Lipid composition of the endoplasmic reticulum (ER) from *Saccharomyces cerevisiae* and HeLa cells. The lipid class composition (*A*), the unsaturation of phosphatidylcholine (PC) species (*B*), and total acyl chain length of PC species as the sum of Catoms in both fatty acyl chains (*C*) are given as relative abundance (in mol%). (This figure is based on data in Reinhard et al. 2022 and Sokoya et al. 2022 for the yeast and mammalian ER, respectively.)

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

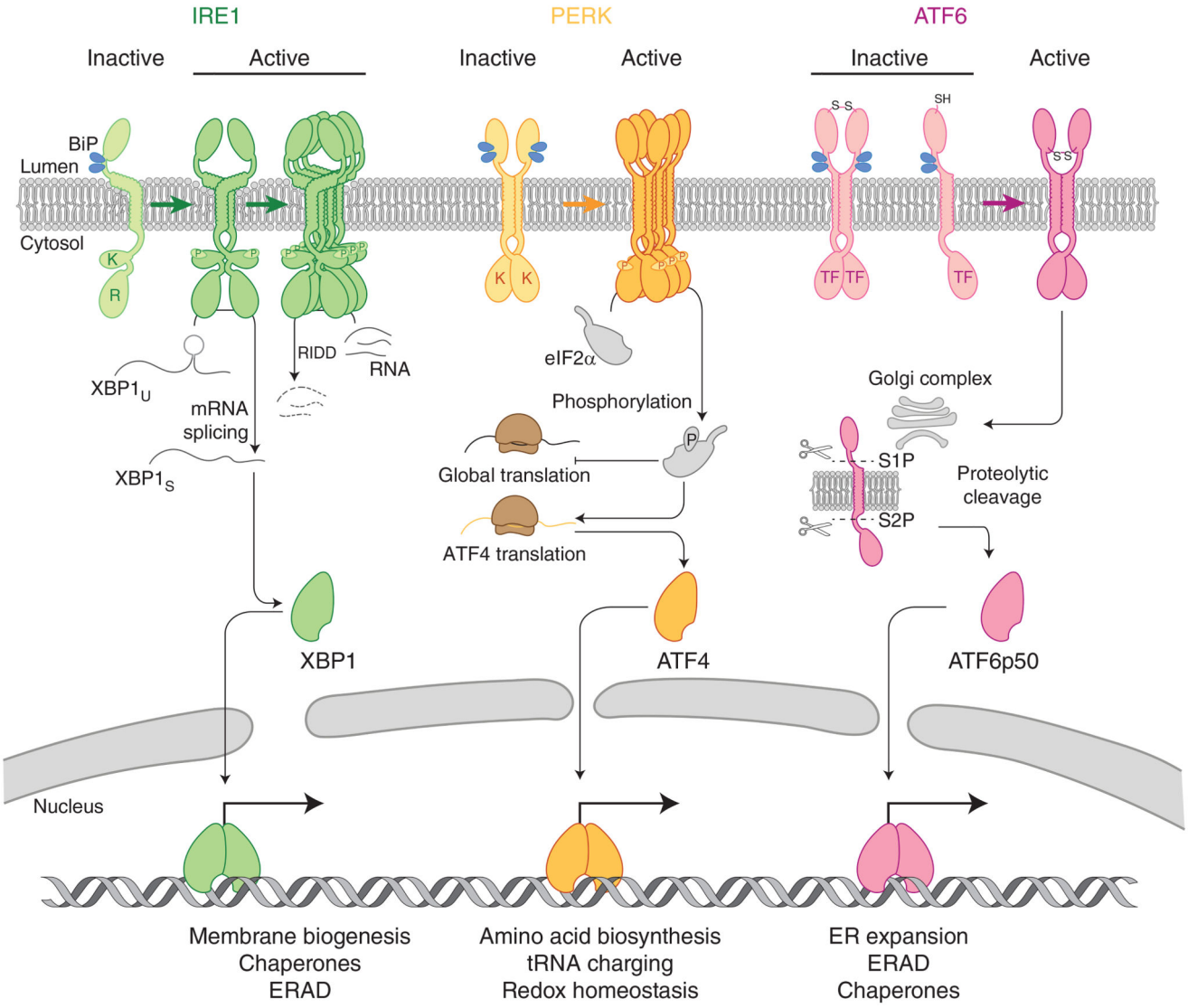

Figure 2.

The three branches of the unfolded protein response (UPR) in mammalian cells. The UPR in mammalian cells has three branches, relying on the stress sensors IRE1, PERK, and ATF6. Each branch regulates the expression of specific UPR target genes by activating branch-specific transcription factors. IRE1 interacts with the endoplasmic reticulum (ER) chaperone BiP and forms monomers and dimers in resting cells. ER stress stabilizes IRE1 dimers and higher oligomers, thereby activating the cytosolic kinase “K” and RNase “R” domains. The activated RNase domain removes an intron from XBP1 mRNA and initiates unconventional splicing that yields spliced XBP1 mRNA (XBP1S). Translation of the matured mRNA yields the XBP1 transcription factor. Like IRE1, PERK is activated by oligomerization. Dimerization and the formation of higher order oligomers activate the cytosolic kinase domain. Phosphorylation of the eukaryotic initiation factor 2α (eIF2α) decreases global transcription via the integrated stress response, but selectively promotes the production of the transcription factor ATF4. ATF6 is a membrane-tethered basic leucine-

zipper transcription factor. During ER stress, a specific disulfide-linked ATF6 dimer of the redox-sensitive protein is transported to the Golgi complex, where it is proteolytically cleaved bysite-1 and site-2 proteases (S1P, S2P), releasing the soluble p50 transcription factor (ATF6p50). The ER chaperone BiP (blue) interacts with all three UPR transducers in the unstressed ER.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

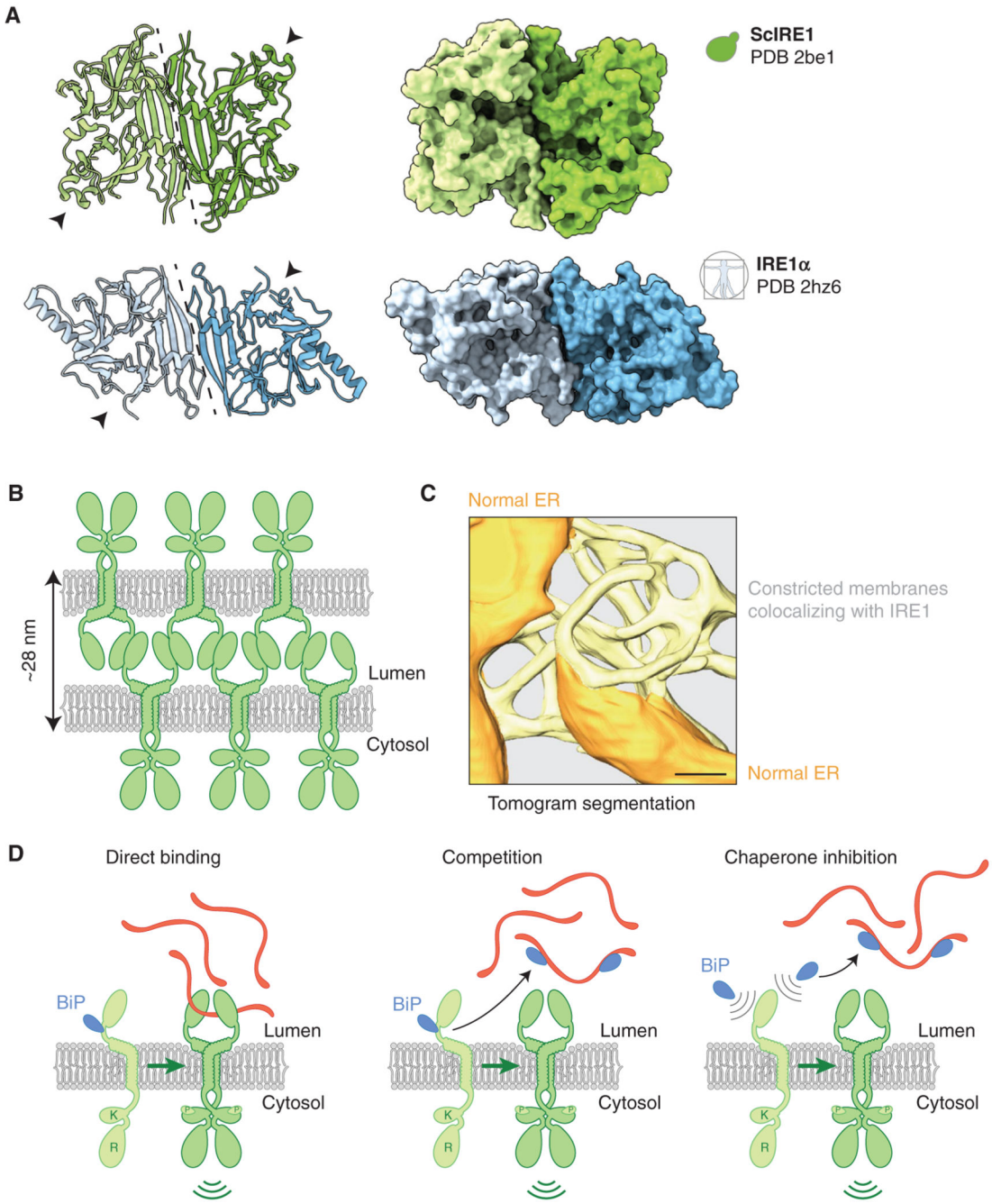

Figure 3. Sensing proteotoxic stress by IRE1.

(*A*) Crystal structures of the lumenal domains of yeast (green) and mammalian (blue) IRE1, as determined by Credle et al. (2005) and Zhou et al. (2006), respectively. Cartoon models (*left*) and surface models (*right*) are depicted. The interfaces for dimerization (dashed line) and oligomerization (arrowheads) are indicated in the cartoons. (*B*) Oligomerization of IRE1α can shape the endoplasmic reticulum (ER) into constricted tubes. The ER–luminal domains of dimeric IRE1α serves as a proteinaceous zipper that juxtaposes two ER membranes. (*C*) Segmented tomograms showing normal ER (bright orange) and constricted

ER membranes that colocalize with IRE1. Scale bar, 100 nm. (Tomogram is adapted, with permission, from Tran et al. 2021.) (*D*) Three models of proteotoxic stress sensing by IRE1, as explained in the text. The ER chaperone BiP and accumulated unfolded proteins are depicted in blue and red, respectively.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

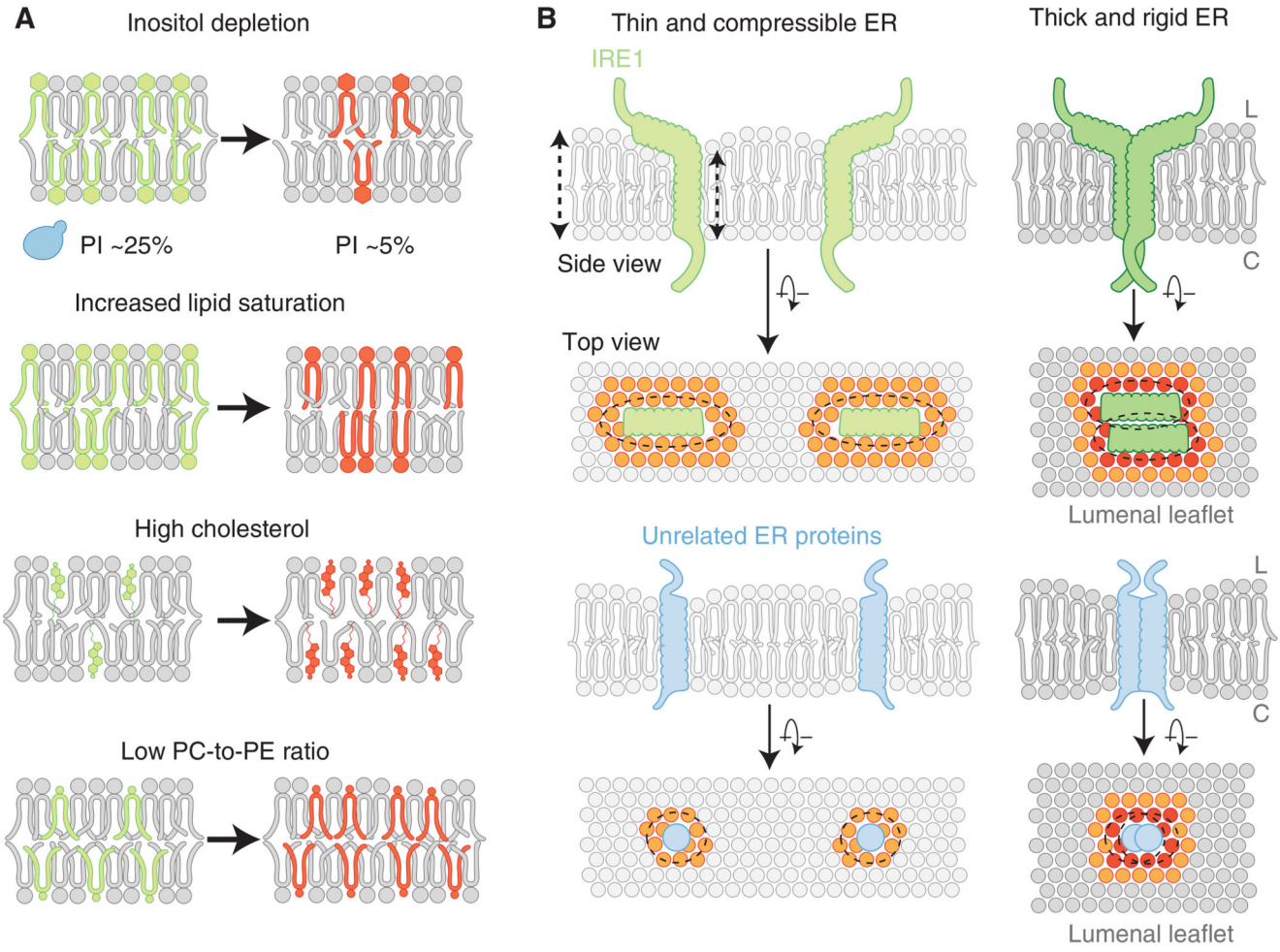

Figure 4. Sensing of lipid bilayer stress.

(*A*) A diverse set of lipid metabolic perturbations triggers the unfolded protein response (UPR) by a membrane-based mechanism. Inositol depletion, increased abundances of tightly-packing, saturated lipids, increased levels of cholesterol and reduced PC-to-PE ratios cause lipid bilayer stress. (*B*) ScIRE1 (green) uses a hydrophobic mismatch-based mechanism for sensing ER membrane compressibility. With its short transmembrane helix and an adjacent amphipathic helix, ScIRE1 induces a local membrane thinning and establishes an ellipsoid membrane footprint (orange and red color). Increased membrane thickness and reduced compressibility provide a driving force for ScIRE1 oligomerization. Upon dimerization, the total area of the membrane distortion is minimized, and large parts of the membrane footprints coalesce where the transmembrane helices “meet.” Compared to unrelated, single-pass membrane proteins (blue), ScIRE1 gains more free energy upon dimerization because a much larger distorted membrane region coalesces. The cytosolic side of the ER membrane is labeled C, the luminal side L.