CHAPTER SIX

Phospholipids and the Unfolded Protein Response

Ilias Gkikas and Nektarios Tavernarakis

CONTENTS

6.1 INTRODUCTION

Eukaryotic membranes, encircling cells and organelles, consist of proteins and lipid molecules such as phospholipids and sterols. The dynamic interplay between these constituents underpins membrane integrity and fluidity, and ultimately cellular protein homeostasis, or proteostasis. The endoplasmic reticulum (ER) and mitochondria possess a unique composition of proteins and lipids which allows maintenance of organelle structure and function. The ER is a highly regulated organelle forming large and dynamic membranous networks. This complex membrane architecture is essential for more than one-third of cellular protein synthesis, folding and secretion (Pendin et al., 2011). In addition, most abundant phospholipids such as glycerophospholipids (GPLs) including phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) are predominantly synthesized in the ER (Ridgway, 2016).

Similarly, mitochondria are double-membrane organelles forming tubular networks with diverse size, number and position within cells (Kuhlbrandt, 2015). Beyond their crucial role in energy production, mitochondria are also involved in the synthesis of specialized phospholipids. While ER-synthesized phospholipids can

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be transported to mitochondria, a string of enzymatic activities within mitochondria is required for phosphatidylglycerol (PG), cardiolipin (CL) and PE synthesis (Horvath and Daum, 2013).

Interestingly, alternation of phospholipid composition within membranes can cause ER and mitochondrial proteotoxic stress (Halbleib et al., 2017; Ho et al., 2018). To maintain proteostasis, the load of newly synthesized and the degradation of unwanted proteins should be kept in balance. From yeast to mammals, compartmentalized protein quality control mechanisms, such as the unfolded protein response (UPR) and autophagy, assure the integrity of these processes (Senft and Ronai, 2015). In this chapter, we discuss recent findings on UPR activation by proteotoxic and phospholipid bilayer stress. Emphasis will also be placed on the role of UPR signaling in phospholipid biosynthesis (hereafter we will refer to both the ER and mitochondrial UPRs as UPR unless otherwise noted).

6.2 REGULATION OF ENDOPLASMIC RETICULUM AND MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE

6.2.1 The UPR^{ER} Signaling

The ER proteostasis is confronted with diverse insults often leading to lipid disequilibrium, accumulation of unfolded proteins, depletion of Ca^{2+} and glycosylation defects among others, which in turn activate the UPR^{ER} (Hetz and Papa, 2018; Ho et al., 2018; Metcalf et al., 2020). The UPR^{ER} signal is emanated from three discrete ER transmembrane proteins, namely the inositol-requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6), and double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK). While IRE1 is well conserved across species, PERK and ATF6 are only present in metazoans (Hetz and Papa, 2018). A member of the HSP70 chaperone family of proteins, the binding immunoglobin protein (BiP), determines their activity. Beyond its classical ER chaperone role, BiP binds to ER luminal domain (LD) of IRE1, PERK and ATF6. Accumulation of unfolded proteins results in BiP release from IRE1, PERK and ATF6 LDs and subsequently activates UPR^{ER}. Several models have been proposed to rationalize the transient binding of either misfolded proteins or ER stress sensors to BiP (Kopp et al., 2019). Nevertheless, the exact mechanism underpinning the inverse correlation between its role as an ER chaperone and as a UPR^{ER} activator is not fully understood.

6.2.1.1 Sensing by IRE1

Structurally and functionally conserved, IRE1 is the only UPR^{ER} transducer expressed from lower to higher eukaryotic cells. Unlike yeast, the mammalian IRE1 has two isoforms, IRE1α and IRE1β, exhibiting tissue-specific expression. Through their cytosolic regions, both exert Ser/Thr kinase and endoribonuclease activities, and sense ER stress through their N-terminal ER LDs. Under physiological conditions, IRE1α remains monomeric and inactive. Mammalian IRE1α is activated upon either BiP uncoupling or direct binding to unfolded proteins. The latter is accompanied by IRE1α oligomerization and/or dimerization, and trans-autophosphorylation (Hetz and Papa, 2018). Activated IRE1α catalyzes the unconventional splicing of X-box binding protein 1 (XBP1) mRNA more effectively than IRE1β (Imagawa et al., 2008). Concurrently, the spliced and functional isoform of XBP1 triggers the UPR^{ER} response by orchestrating the transcription of genes involved in protein folding and quality control (Uemura et al., 2009). Apart from XBP1 splicing, activities of both IRE1α and IRE1β endonucleases have been involved in the degradation of several ER-targeted and cytosolic mRNAs via a process known as IRE1-dependent decay (RIDD). Under physiological conditions, RIDD sequence-specific cleavage is essential for sustaining the normal load of newly synthesized proteins targeting the ER. Interestingly, cleavage sites recognized by IRE1α and IRE1β appear to be similar for both XBP1 and RIDD substrates. Notably, substrate recognition is a highly selective process. As such, activation of IRE1α favors XBP1 mRNA splicing to preserve ER homeostasis, whereas IRE1β preferentially recognizes RIDD substrates to induce cell death signaling upon irremediable ER stress (Maurel et al., 2014).

6.2.1.2 Sensing by PERK

Under normal conditions, PERK exists in its monomeric inactive form bound by BiP. In response to ER stress, BiP is released while

PERK LD and kinase domains facilitate its oligomerization. Concomitantly, autophosphorylation of its kinase domain results in activation of PERK-mediated UPR^{ER} signaling followed by cytosolic protein synthesis inhibition. Crystalized PERK in dimeric and tetrameric forms has been also reported to trigger UPR^{ER} signaling (Carrara et al., 2015). Whether and how dimer-tetramer transition correlates with a more or less active state of PERK remains elusive. Furthermore, direct binding of misfolded proteins to PERK-LD is indispensable for PERK activation (Wang et al., 2018). Intriguingly, a by-product of choline metabolism, trimethylamine N-oxide (TMAO), has been also reported to bind exclusively PERK-LD and thus induce UPR^{ER} (Chen et al., 2019). Once activated, stimulation of PERK kinase domain phosphorylates eukaryotic translation initiation factor 2 alpha (eIF2α) at Ser51 and inactivates it, eventually attenuating global translation (Harding et al., 1999). While limited, the phosphorylated eIF2α favors the translation of several mRNAs possessing upstream open reading frames (uORFs) in their 5′-untranslated region (5′-UTR) (Lee et al., 2009). Among them, activated transcription factor 4 (ATF4) has a central pro-survival and pro-death activity depending on the origin, severity and duration of ER stress. Upon activation, ATF4 controls the expression of genes involved in protein homeostasis, autophagy, apoptosis, amino acid and redox metabolism (Carrara et al., 2015; Han et al., 2013). Particularly, ATF4 exerts its pro-apoptotic role by controlling the expression of C/EBP homology protein (CHOP) and growth-arrest and DNA-damage-induced transcript 34 (GADD34). Expression of GADD34 restores protein synthesis by promoting the dephosphorylation of eIF2a (Hetz and Papa, 2018).

6.2.1.3 Sensing by ATF6

From the two isoforms found in mammals, ATF6α has been studied to a greater extent than ATF6β. ER-located ATF6α exists as a mixed population of monomers, dimers and oligomers, linked by disulfide bridges formed between ATF6α-LD cysteine residues (Nadanaka et al., 2007). In unstressed cells, BiP binding to ATF6α-LD cancels its engagement with coat protein complex II (COPII) transport vesicles at the ER–Golgi interface. In response to superfluous or misfolded proteins, BiP is released from ATF6α-LD which in turn promotes ATF6α ER-to-Golgi translocation via COPII vesicles (Sato et al., 2011). Activation of ATF6α relies on Golgi translocation of the ATF6α precursor, therein it is processed by site-1 and site-2 proteases (S1P and S2P), that sequentially remove the LD and the transmembrane anchor. Subsequently, the liberated N-terminal fragment of 50 kDa (p50ATF6α) can enter the nucleus and activate UPR^{ER} downstream transcriptional targets (Ye et al., 2000). Recently, it has been shown that ER-resident thrombospondin 4 (Thbs4) binding to ATF6α-LD is sufficient for COPII-mediated transport to the Golgi apparatus. As such, nuclear entry of p50ATF6a fragment and transactivation of its UPR^{ER} target genes allows restoration of ER proteostasis (Lynch et al., 2012). Interestingly, upon ER stress, oxidoreductase activity of protein disulfide isomerase A5 (PDIA5) promotes accessibility of ATF6α into COPII vesicles in a BiP-independent manner (Higa et al., 2014). More recently, an additional ER oxidoreductase, ERp18, has been associated with ATF6α in response to ER stress. In this context, moderate ATF6α activation through improved cleavage by S1P/S2P and controlled ER-to-Golgi transport by ERp18 has been documented (Oka et al., 2019). Despite the effort, how BiP binding and release occur and how other ATF6 interacting partners ultimately mediate ATF6 response are not fully understood.

6.2.2 The UPR^{mt} Signaling

When excess misfolded and/or unfolded proteins accumulate within mitochondria, the UPR^{mt}-mediated transcriptional response emerges. Since its discovery in mammals, only few sensing and transducing molecules have been uncovered. Unequivocally, UPR^{mt} activation occurs through a mitochondria-to-nucleus signaling, initiated upon failure of mitochondrial tricarboxylic acid cycle (TCA) activity, electron transport chain (ETC) activity, fatty acid beta-oxidation (FAO), translation, protein import system etc., leading to proteotoxic stress (Nargund et al., 2012; Rolland et al., 2019). In turn, specific transcription factors allow adaptive transcriptional changes and restore collapsed mitochondrial proteostasis. Alongside chaperones and

proteases, the UPR^{mt}-mediated transcriptional response favors glycolytic gene expression while it dampens ETC target genes (Nargund et al., 2015). The latter suggests that alternative energy sources are triggered to reduce the pressure on ETC function. Moreover, adequate evidence of how mitochondrial misfolded or unfolded proteins are recognized and how signaling is transduced across the mitochondrial membrane is missing.

The mammalian bZIP transcription factor ATF5 and its *Caenorhabditis elegans* homolog ATFS-1 are the master regulators of UPR^{mt} signaling. In unstressed cells, ATFS-1 is targeted predominantly to mitochondrial matrix, where degradation of ATFS-1 is facilitated by AAA^{+}-protease LON. As reported recently, the mitochondrial targeting sequence (MTS) of ATFS-1 is able to sense changes in mitochondrial membrane potential and thus initiate UPR^{mt} signaling (Rolland et al., 2019). In response to mitochondrial stress, mitigated import of ATFS-1 precursor into mitochondria favors its nuclear translocation. Nuclear accumulation of ATFS-1 triggers the expression of mitochondrial chaperone- and protease-encoding genes. Subsequently, nuclear ATFS-1 mitigates the expression of ETC transcripts to a sustainable level of bioenergetics, where less but still efficient ETC complexes are formed (Nargund et al., 2012). Interestingly, paraquat-induced (ETC inhibitor) expression of mitochondrial chaperones such as mtHSP70 and HSP10, and the protease LONP1, requires ATF5 activity in mammalian cells (Fiorese et al., 2016). It has been reported that, contrary to wild type, oligomycin- (inhibitor of ATP synthase) or doxycycline (mitochondrial translation inhibitor)-treated ATF5 knockout mice failed to activate UPR^{mt} as a protective mechanism against cardiac ischemia-reperfusion injury (Wang et al., 2019).

6.3 PHOSPHOLIPID BIOSYNTHESIS IN THE ENDOPLASMIC RETICULUM AND MITOCHONDRIA

Phospholipids are amphipathic molecules possessing a polar phosphate group (hydrophilic heads) and two non-polar long fatty acid (FA) chains (hydrophobic tails). Two FA chains can be attached either to glycerol or a sphingosine backbone, forming GPLs and/or sphingophospholipids (SPLs), respectively. Here, we review GPL biosynthesis (referred to as phospholipid from now on) derived from a glycerol backbone. Infrequently found in cells, PA is the metabolic precursor of all phospholipids composed of a glycerol backbone, two FA chains and a phosphate group. Glycerol contains two hydroxyl groups esterified by FAs while esterification or not of the third glycerol hydroxyl group (sn3 position) with a phosphate defines PA or diacylglycerol (DAG) formations (Blanco and Blanco, 2017). Given that phospholipids are amphipathic molecules, their spontaneous self-assembly forms a continuous bilayer (Ridgway, 2016). Therefore, membrane formation of all mammalian cells relies primarily on the proper synthesis and distribution of these phospholipid classes (Figure 6.1).

6.3.1 Phosphatidylcholine

Mostly synthesized in the liver, PC appears to be the major phospholipid in biliary lipids, secreted lipoproteins and lipid droplet membranes (Van der Veen et al., 2017). PC synthesis requires the activity of several ER- and cytosolic-resident enzymes of the cytidine diphosphate (CDP) choline pathway, alternatively named as the Kennedy pathway. Choline is the main substrate for PC synthesis. Cytosolic choline kinase (CK) enzymatic activity promptly phosphorylates choline to phosphocholine which in turn is catalyzed by CTP:phosphocholine cytidyltransferase (CCT; encoded by *Pcyt1*), to form CDP-choline. Next, the ER-embedded 1,2-DAG choline phosphotransferase (CPT) catalyzes the formation of PC by attaching a phosphocholine moiety from CDP-choline to the third hydroxyl group of DAG (Ridgway, 2016). Alternatively, PE can be used as a substrate for PC synthesis. In this context, the ER- and mitochondrial-associated ER membrane (MAM)-embedded PE N-methyltransferase (PEMT) catalyzes the sequential methylation of PE to PC (Vance, 2014).

6.3.2 Phosphatidylethanolamine

PE is the second most abundant phospholipid in mammalian cells. Interestingly, coordinated *de novo* synthesis of PE in both ER and mitochondria has been reported. Of note, partial inhibition of mitochondrial PE synthesis activates the

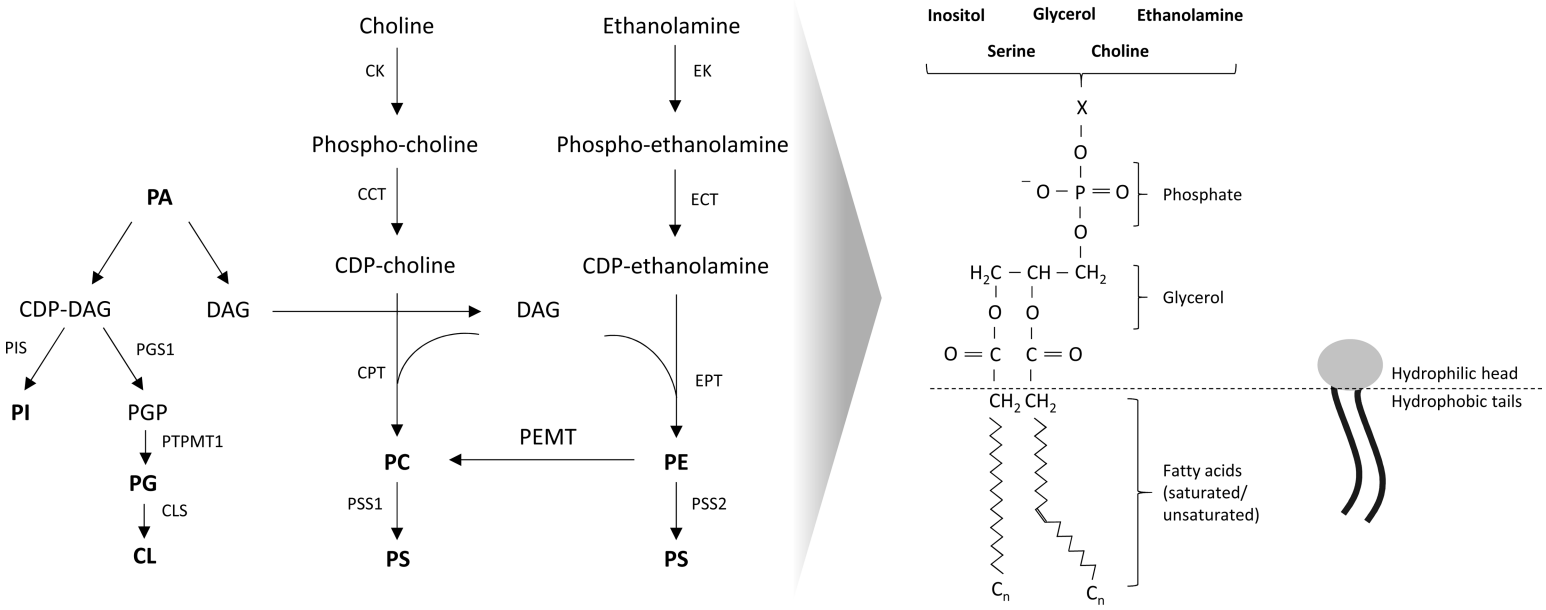

Figure 6.1 **Overview of phospholipid biosynthesis and structure in mammalian cells.** The enzymatic activities required for phospholipid biosynthesis are discussed in the text. CK: Choline kinase, CCT: CTP:phosphocholine cytidyltransferase, CPT: 1,2-DAG choline phosphotransferase, EK: ethanolamine kinase, ECT: CTP:phosphoethanolamine cytidylyltransferase, EPT: 1,2-DAG ethanolamine phosphotransferase, PSS1: PS synthase 1, PSS2: PS synthase 2, PIS: PI synthase, PGS1: PGP synthase, PTPMT1: PTP localized to the mitochondrion 1, CLS: cardiolipin synthase, C_n: number of carbons.

CDP-ethanolamine pathway to restore imbalance of cellular PE levels (Steenbergen et al., 2005). ER-synthesized PE relies on the CDP-ethanolamine pathway. Initially, the cytosolic ethanolamine kinase (EK) catalyzes the phosphorylation of ethanolamine to phosphoethanolamine. Next, the rate-limiting CTP:phosphoethanolamine cytidylyltransferase (ECT; encoded by *Pcyt2*) converts phosphoethanolamine to CDP-ethanolamine. The last step involves the ER-localized enzymatic activity of 1,2-DAG ethanolamine phosphotransferase (EPT) which catalyzes the transfer of the phosphoethanolamine moiety of CDP-ethanolamine to the free hydroxyl group of DAG, to form PE (Gibellini and Smith, 2010). Mitochondrial synthesis of PE requires the proper trafficking of its precursor PS from MAMs of the ER to the inner mitochondrial membrane (IMM). Therein, PS decarboxylase (PSD) converts PS to PE, which is either assimilated into mitochondria or exported to the ER (Patel and Witt, 2017).

6.3.3 Phosphatidylserine

Contrary to high quantities of PC and PE, PS represents a minor phospholipid in mammalian cells. Its synthesis occurs in the MAMs and requires both PS synthase 1 (PSS1) and PS synthase 2 (PSS2). Based on intracellular calcium levels, PSS1 and PSS2 catalyze the replacement of either choline or ethanolamine polar heads from PC and PE, respectively, with L-serine (Vance, 2008). In yeast, a single PSS enzyme exists which catalyzes PS formation, in the expense of CDP-DAG and L-serine (Vance and Tasseva, 2013). Interestingly, PSS1 possesses distinct but complementary activity, since lack of PSS2 exhibits relatively normal amounts of PS and PE attributed to PSS1 activity (Arikketh et al., 2008).

6.3.4 Phosphatidylinositol

In eukaryotes, PI is found in relatively small amounts compared to other phospholipids. While DAG derived from PA dephosphorylation is the intermediate for PC and PE synthesis, conversion of PA to CDP-DAG provides the major precursor for PI synthesis (Yang et al., 2018). Particularly, the mammalian CDP-DAG synthases 1 and 2 (CDS1 and CDS2) as well as translocator assembly and maintenance homolog protein 41 (TAMM41) catalyze the formation of CDP-DAG from PA and CTP, in the ER and mitochondria, respectively (Jennings and Epand, 2020). Despite this, mitochondrial CDS1 and CDS2 activity cannot be excluded. Subsequently, utilizing inositol and CDP-DAG as substrates, ER-embedded PI synthase (PIS) catalyzes the formation of PI (Blunsom and Cockcroft, 2020).

6.3.5 Phosphatidylglycerol

PG is also found in small quantities, accounting for approximately 1% of total mammalian phospholipid content. Of note, PG is significantly enriched in pulmonary surfactant comprising approximately 15% of phospholipid mass, and it prevents alveolar collapse. Although sharing a similar biosynthetic route with PI, PG is exclusively synthesized in mitochondria. From substrate incorporation to final PG synthesis, most of the associated enzymes are well conserved between yeast and mammals. Once PA is transported into IMM, it serves as a substrate for TAMM41 CDS activity required for CDP-DAG formation (Blunsom and Cockcroft, 2020). Next, a condensation reaction of CDP-DAG and glycerol-3-phosphate through PGP synthase 1 (PGS1) results in phosphatidylglycerol-phosphate (PGP) formation. Subsequently, PTP localized to the mitochondrion 1 (PTPMT1) phosphatase activity dephosphorylates PGP to PG (Ridgway, 2016).

6.3.6 Cardiolipin

In mammals, synthesized CL is predominantly assimilated into either the IMM or the OMM (outer mitochondrial membrane), where it accounts for approximately the 20% and 3% of total phospholipid mass, respectively. Contrary to other phospholipids, CL possesses a unique molecular structure which is constituted of two phosphate and three glycerol moieties as well as four acyl chains (Dudek, 2017). In mammalian and yeast cells, CL synthesis is amenable to a common biosynthetic pathway utilizing mitochondrial PA, CDP-DAG and PG precursors. When PG is synthesized, it rapidly reacts with CDP-DAG to form premature CL through the activity of CL synthase (CLS). Finally, premature to mature remodeling of CL requires the transacylase activity of taffazin (TAZ) (Dudek, 2017).

6.4 PHOSPHOLIPIDS AND UNFOLDED PROTEIN RESPONSE SIGNALING

Contrary to the prevailing notion that UPR is activated upon sensing of unfolded protein accumulation, aberrant phospholipid composition has also been shown to trigger UPR signaling. For instance, alterations in acyl chain length and/or degree of saturation of various phospholipid classes result in phospholipid bilayer stress, which in turn activates UPR. Perturbation of phospholipid homeostasis has been associated with various metabolic disorders including obesity and diabetes, among others. Conversely, in response to ER or mitochondrial proteotoxic stress, UPR activation has been shown to control phospholipid abundance. Here, our current understanding of phospholipids and UPR mutual regulation will be discussed.

6.4.1 Unfolded Protein Response as a Sensor of Phospholipid Bilayer Stress

Phospholipid biosynthesis in mammalian and yeast cells is primarily compartmentalized in the ER and mitochondria (Ridgway, 2016). In addition, locally synthesized phospholipids are convoyed to a designated cellular membrane in time of need. Once they have reached their destination, phospholipids are organized and self-assembled into bilayers, or used as a substrate for the synthesis of other phospholipid classes. Regardless of where they are synthesized or transported, their bilayer heterogeneity determines membrane fluidity and provides a versatile environment for integral membrane proteins to be embedded, folded, modified and recruited (Harayama and Riezman, 2018). Specifically, membrane fluidity relies on the acyl chain composition of various phospholipid classes which is unique among cell types and subcellular organelles (Ridgway, 2016). Moreover, the presence of saturated and unsaturated acyl chains in various phospholipid classes renders membranes as non-fluidic and fluidic, respectively (Harayama and Riezman, 2018; Manni et al., 2018). Importantly, the acyl chain ordering and length affects linearly the thickness of the PC bilayer in the presence of sterols. Together, acyl chain length and degree of saturation regulate PC bilayer thickness and engender proper self-assembly of membrane-bound proteins (Anbazhagan and Schneider, 2010). In addition, membrane protein conformation and function necessitate the proper matching of the hydrophobic core of membranes with the hydrophobic span of transmembrane helix. Similarly, to ER-synthesized phospholipids, acyl chain remodeling of CL affects mitochondrial membrane properties, and the function of the embedded ETC proteins (Pennington et al., 2019). Although membrane-bound proteins account for approximately 30% of the human proteome, the interplay between phospholipid bilayers and incorporated proteins is still not fully understood. Interestingly, it has been shown that phospholipid bilayer stress, as a result of aberrant phospholipid composition and remodeling, mitigates the harnessing of membrane properties while it promotes ER stress and proteostasis collapse (Shyu et al., 2019). Thus, it is not surprising that changes in the phospholipid composition in the ER and mitochondrial membranes can have profound effects on the activity of UPR transducers. Expectedly, disruption of phospholipid and protein homeostasis has also been associated with numerous pathologies including cancer, obesity, type II diabetes, liver and heart failure (Wang and Tontonoz, 2019).

Toward this direction, muscle cells from mice deficient of Lipin1 (a phosphatidate phosphatase enzyme catalyzing the dephosphorylation of phosphatidate to DAG) exhibit increased phospholipid biosynthesis which coincides with high levels of several phospholipid substrates. In addition to phospholipids, the levels of triglycerides (TAGs) and their DAGs precursors are also augmented. This accumulation of divergent lipid classes has been ascribed to higher expression of various lipogenic genes controlled by the sterol regulatory element-binding protein 1c/2 (SREBP1c/2) when Lipin1 is depleted. Ultimately, sarcoplasmic reticulum stress, resulting from dysregulation of lipid composition, is accompanied by active XBP1 and ATF6 forms (Rashid et al., 2019). Moreover, obese mice with high liver PC/PE ratio display irregular ER Ca^{2+} signaling and UPR^{ER} activation (Fu et al., 2011). Likewise, increased phospholipid acyl chain saturation in mouse liver due to lysophosphosphatidylcholine acyltranferase 3 (Lpcat3) deficiency is accompanied by heightened UPR^{ER} signaling (Rong et al., 2013). In addition, stearoyl-CoA desaturase

1 (SCD1)-deficient mice exhibit more saturated fatty acids (SFAs) and less monounsaturated fatty acids (MUFAs) of phospholipids followed by activation of CHOP, GRP78 and increased of spliced XBP1 transcripts (Ariyama et al., 2010). Since SCD1 catalyzes the biosynthesis of MUFAs (e.g. palmitate) from SFAs (e.g. oleate), when palmitate is supplemented, activation of UPR^{ER} is expected. In agreement, incubation of rat insulinoma cell line (INS1-β cells) with palmitate initiates UPR^{ER} signaling, which is reversed upon oleate supplementation (Sommerweiss et al., 2013). It is noteworthy that in INS1 β-cells, palmitate-induced ER stress is also accompanied by altered phospholipid composition (Moffitt et al., 2005). Furthermore, exposing hepatic cells to palmitate leads to ER membrane integrity collapse and accumulation of saturated phospholipids, followed by increased CHOP expression. These adverse conditions could be reversed upon oleate supplementation (Leamy et al., 2014). As evidenced in yeast and mammalian cells, IRE1 and PERK1 responsiveness in increased acyl chain saturation, resulting by phospholipid perturbation, has also been reported. Of note, IRE1 and PERK LDs, required for sensing unfolded proteins, are dispensable for UPR^{ER} activation in response to phospholipid bilayer stress. As expected, direct sensing of bilayer stress predominantly relies on their transmembrane domains (Ho et al., 2020; Volmer et al., 2013). Despite this, whether phospholipid bilayer stress and misfolded proteins act in parallel or not, for UPR^{ER} signaling to be activated, is far from understood. Further mechanistic insight into IRE1 oligomerization and activation showed that an amphipathic helix region within IRE1 transmembrane helix controls its responsiveness to both phospholipid bilayer and proteotoxic stress (Halbleib et al., 2017).

Interestingly, yeast cells lacking ubiquitin-like (UBX)-domain-containing protein 2 (UBX2) display more saturated than unsaturated phospholipid acyl chains resulting in UPR^{ER} activation. It is noteworthy that while total phospholipid saturation is increased, high discrepancies between cone-shaped PE and cylinder-shaped PC saturation have been reported (Surma et al., 2013). Most likely, high levels of saturated PE compensate for low levels of saturated PC to sustain FA composition at an optimal range, which in turn favors bilayer-forming propensity (Basu Ball et al., 2018). In addition, knockout of acetyl-CoA synthase Fat1 in yeast increases abundance of very long-chain fatty acids (VLCFAs) with compensatory activation of UPR^{ER}. Specifically, lipidomic analysis of Fat1-deficient cells showed accumulation of PC species including FA chains with 32 and 34 carbons. Importantly, the ratio of di-unsaturated/monounsaturated PC species appeared higher in Fat1 mutants compared to wild type. Both increased acyl chain length and saturation of PC reflect membrane disruption and are necessary for UPR^{ER} activation (Micoogullari et al., 2020).

Attenuation of PC synthesis by knocking down ptm-2 in worms also induces UPR^{ER} signaling. Intriguingly, tunicamycin-treated (N-glycosylation inhibitor) and PTM-2 deficient worms have differential expression of UPR-related transcripts (Koh et al., 2018). Moreover, worms deficient of the mediator complex subunit 15 (MDT-15) display compromised unsaturated over saturated phospholipids ratio and reduced membrane fluidity, leading to UPR^{ER} activation, independent of proteotoxic stress. Despite the ER membrane disequilibrium and UPR^{ER} activation, it is surprising that MDT-15 deficient worms do not display altered CL abundance and FA composition and fail to activate the UPR^{mt} (Hou et al., 2014). Nevertheless, worms with reduced CL or PE levels upon inhibition of the CRLS-1 or PSD-1, respectively, have been shown to engender activation of UPR^{mt} (Rolland et al., 2019). Notably, worms deficient of the S-adenosyl methionine synthase 1 (SAMS-1) also exhibit compromised PC synthesis and increased UPR^{ER} signaling (Ehmke et al., 2014; Ho et al., 2020). Collectively, both the UPR^{ER} and UPR^{mt} act as sensors of imbalanced phospholipid biosynthesis and remodeling which, in turn, allow specialized transcriptional responses.

6.4.2 UPR as a Regulator of Phospholipid Abundance

Perturbation of phospholipids comes with a unique UPR transcriptional outcome, yet this is not a unidirectional interaction. Activation of UPR has been also shown to promote membrane biogenesis, as it regulates lipid metabolism, including phospholipids biosynthesis. Among UPR^{ER} transducers, the well-conserved IRE1 has been primarily identified in yeast cells

to mediate inositol synthesis, which serves as a substrate for PI biosynthesis (Nikawa and Yamashita, 1992). Further characterization of the UPR^{ER} transcriptional response in yeast identified various genes involved in phospholipid biosynthesis to be activated upon tunicamycin or dithiothreitol (DTT) treatment (a strong disulfide-reducing agent) (Travers et al., 2000). Through UPR^{ER} signaling, increased expression of OPI3 and INO1, involved in PC and PI synthesis, respectively, has also been documented. This mutual activation of UPR^{ER} signaling and phospholipid biosynthesis is necessary for proper membrane expansion upon ER stress (Schuck et al., 2009).

Following XBP1 activation in mammals, the enzymatic activity of several constituents of the CDP-choline pathway is also increased. Specifically, it has been suggested that increased PC synthesis contributes to the ER membrane biogenesis (Sriburi et al., 2004). Similarly, palmitate-exposed murine macrophages exhibit increased phospholipid content and enlarged ER, in an XBP1-dependent manner (Kim et al., 2015). Notably, chemically induced IRE1, ATF4 and PERK in the mouse lung result in elevated expression of lipogenic proteins such as SDC1, SREBP1c and FA synthase. The latter is evidenced by increased TAG and phospholipid contents, and a higher degree of PC saturation. When SCD1 is inhibited, defective UPR^{ER} activation with increased expression of profibrotic factors comes with compromised phospholipid composition. On the contrary, elevated activity of SREBP1c accompanied by increased neutral- and phospholipid abundance alleviates both ER stress and expression of pro-fibrotic factors in lung epithelial cells (Romero et al., 2018).

Similarly, UPR^{mt} activation is accompanied by changes in mitochondrial phospholipid content. Previous studies have shown that perturbation of mitochondrial proteostasis activates a mitochondrial-to-cytosolic stress response (MCSR) which functions in concert with the UPR^{mt} and the cytosolic heat shock response (HSR). Interestingly, worms deficient of ATFS-1 failed to activate MCSR suggesting that UPR^{mt} is indispensable for MCSR activation. In addition, it has been shown that MCSR and UPR^{mt} activation is accompanied by increased levels of various phospholipid classes including mitochondrial PG which is a precursor of CL. Importantly, attenuation of CL synthesis in CRSL-1 deficient worms failed to activate MCRS (Kim et al., 2016). These findings further support the notion that phospholipid biosynthesis and UPR signaling are not mutually exclusive (Figure 6.2).

6.5 CONCLUSIONS

Compartmentalized in the ER and mitochondria, phospholipid biosynthesis and remodeling play an essential role in protein synthesis and secretion. When phospholipid or protein homeostasis collapses, the UPR signaling facilitates a specialized transcriptional response. Beyond its role in sensing phospholipid bilayer and proteotoxic stress, UPR signaling has also been shown to control phospholipid abundance in response to ER or mitochondrial stress. A vicious cycle linking phospholipid biosynthesis and UPR signaling therefore exists, which is responsive to both phospholipid bilayer and proteotoxic stress. Recent findings on the impact of bilayer stress and unfolded proteins on UPR signaling come with several questions regarding their mutual regulation. Notably, whether bilayer stress-mediated UPR activation relies on unfolded protein accumulation or not remains enigmatic. Moreover, whether the existence of distinct transcriptional outcomes upon UPR activation can provide a cross-protection response remains uncertain. In addition, how the composition and ratio of saturated versus unsaturated phospholipids regulate the UPR signaling is unclear. Since new biosynthetic enzymes and proteostatic factors have been identified, our understanding of their contribution to cellular homeostasis will continue to improve. Dissecting the molecular mechanisms underpinning co-regulation of phospholipids and UPR will open the path to a new era of resolving various severe metabolic disorders.

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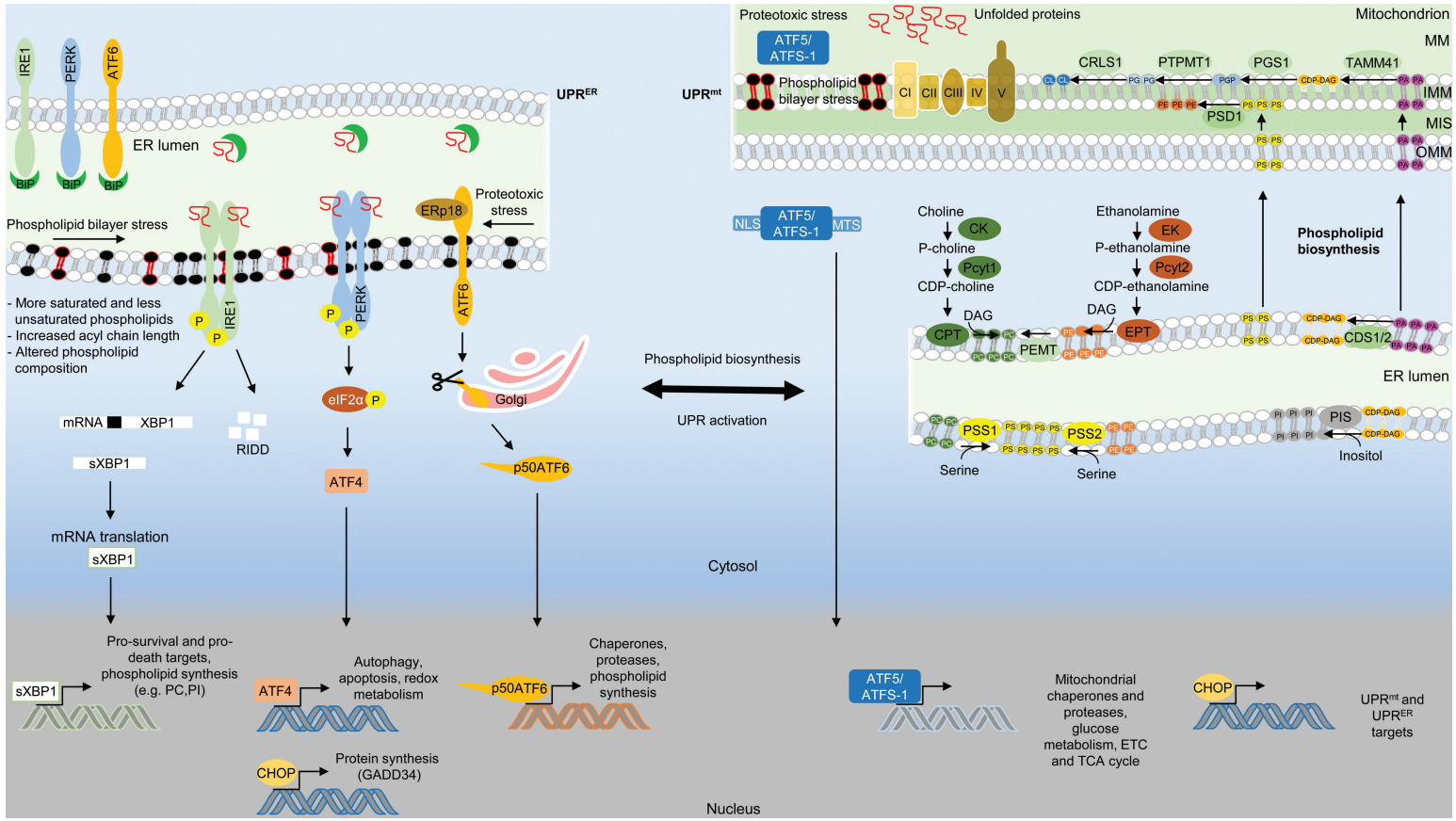

Figure 6.2 **Interplay between UPR signaling and biosynthetic pathways of phospholipids.** In mammals, sensing of ER proteotoxic and phospholipid bilayer stress relies on IRE1, PERK and ATF6 activity, which in turn triggers UPR^{ER} signaling. In *C. elegans* and mammals, sensing of mitochondria proteotoxic and phospholipid bilayer stress necessitates ATFS-1 and ATF5 activity, respectively. Intriguingly, UPR signaling proteins also regulate phospholipid abundance. While phospholipid biosynthesis is compartmentalized, trafficking of phospholipids between ER and mitochondrial also occurs. MM: Mitochondrial matrix, IMM: inner mitochondrial membrane, MIS: mitochondrial intermembrane space, OMM: outer mitochondrial membrane.

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