Review

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# The ins and outs of endoplasmic reticulum-controlled lipid biosynthesis

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## Abstract

**Endoplasmic reticulum (ER)-localized enzymes synthesize the vast majority of cellular lipids. The ER therefore has a major influence on cellular lipid biomass and balances the production of different lipid categories, classes, and species. Signals from outside and inside the cell are directed to ER-localized enzymes, and lipid enzyme activities are defined by the integration of internal, homeostatic, and external information. This allows ER-localized lipid synthesis to provide the cell with membrane lipids for growth, proliferation, and differentiation-based changes in morphology and structure, and to maintain membrane homeostasis across the cell. ER enzymes also respond to physiological signals to drive carbohydrates and nutritionally derived lipids into energy-storing triglycerides. In this review, we highlight some key regulatory mechanisms that control ER-localized enzyme activities in animal cells. We also discuss how they act in concert to maintain cellular lipid homeostasis, as well as how their dysregulation contributes to human disease.**

**Keywords** CCTα; *de novo* lipid synthesis; lipin; mTOR; SREBP
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See the Glossary for abbreviations used in this article.

## Introduction

The ER produces the membrane building-block lipids such as phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn), as well as less abundant membrane lipids such as phosphatidylinositol (PtdIns) and basic sphingolipid structures. The ER also houses the enzymes that synthesize cholesterol, as well as triacylglycerides (TAG) for energy storage (Box 1). These ER-synthesized lipids are distributed to other cellular organelles via the secretory pathway and/or ER contact sites, while TAG is transferred to lipid droplet organelles that bud from the ER membrane. While some organelles also have lipids that are absent from the ER (like cardiolipin in mitochondria), nearly all of these are derived from enzymatic modification of ER-synthesized lipids, and thus, the ER is the central regulator of lipid levels across the cell.

Since most lipids are embedded within cell membranes or lipid droplet storage depots, there are few classic feedback inhibition loops controlling lipid synthesis (where end products directly inhibit the synthetic enzyme). Instead, complex regulatory mechanisms have evolved to modify the activity of ER-localized enzymes. Here, we review these mechanisms, focusing on animal cells that differ in important aspects from yeast where pathways and control points have been described in several comprehensive reviews [1–3]. Some regulatory mechanisms act slowly via altered gene transcription, while others can rapidly modify lipid levels via post-translational modification of enzymes. In addition, depending on the enzyme target, regulatory mechanisms can broadly affect flux into or through a synthetic pathway or specifically affect levels of an individual type of lipid. These regulatory mechanisms act in concert to maintain cellular lipid homeostasis. In addition, they are also central to how the cell modifies lipid synthesis in response to physiological and developmental stimuli, and their dysregulation is implicated in some important pathologies.

## The organization of lipid synthesis within the ER network

The majority of lipid synthesis enzymes are transmembrane proteins (Box 1; Fig 1A), and classic biochemical fractionations established that most are in the ER (microsomal) membranes. However, this does not explain where enzymes are spatially located within the architecture of an animal cell ER. The ER is the largest organelle and can account for more than 50% of total cell membrane in some cell types [11]. It forms a continuous network extending throughout the cell so that other organelles and cytosol are at most 1–2 μm away from a piece of ER (Fig 1B). The organization of the ER is only now being defined in many cell types [12], but overall there is a conserved subdivision into three domains based on ultrastructural appearance: "rough ER", "smooth ER", and the double-membrane "nuclear envelope" that surrounds the nucleus. There are also functionally defined ER domains such as organelle contact sites, ER exit sites of secretory pathway vesicle budding, and sites where lipid droplets bud (Fig 1B) [13].

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**Glossary**

| | |
|---|---|
| **AGPAT** | 1-Acylglycerol-3-phosphate O-acyltransferase |
| **ATF6** | Activating transcription factor 6 |
| **CCT** | CTP:phosphocholine cytidylyltransferase |
| **CDP** | Cytidine diphosphate |
| **CDS** | PtdA cytidylyltransferase |
| **CEPT** | Choline/ethanolamine phosphotransferase |
| **CerS** | Ceramide synthase |
| **Cho** | Choline |
| **CK** | Choline kinase |
| **CTDNEP** | C-terminal domain nuclear envelope phosphatase |
| **DAG** | Diacylglycerol |
| **DGAT** | Diacylglycerol O-acyltransferase |
| **eIF-2α** | α-Subunit of eukaryotic initiation factor 2 |
| **ELOVL** | Elongation of very long fatty acid protein |
| **EPT** | Ethanolamine phosphotransferase |
| **ER** | Endoplasmic reticulum |
| **G-3-P** | Glycerol 3-phosphate |
| **GL** | Glycerolipid |
| **GPAT** | Glyceraldehyde-3-phosphate O-acyltransferase |
| **GPL** | Glycerophospholipid |
| **HMG-CoA** | 3-Hydroxy-3-methylglutaryl-CoA |
| **HMGCR** | 3-Hydroxy-3-methylglutaryl-CoA reductase |
| **INM** | Inner nuclear membrane |
| **Insig** | Insulin-induced gene |
| **IRE** | Inositol-requiring enzyme |
| **LBR** | Lamin B receptor |
| **LCB** | Long-chain base |
| **LPA** | Lysophosphatidic acid |
| **Lyso-GPL** | Lyso-glycerophospholipid |
| **MAM** | Mitochondria-associated membrane |
| **mTOR** | Mechanistic target of rapamycin |
| **NEP1R1** | Nuclear envelope phosphatase 1-regulatory subunit 1 |
| **NLS** | Nuclear localization signal |
| **NPC** | Nuclear pore complex |
| **ONM** | Outer nuclear membrane |
| **PAP** | Phosphatidate phosphatase |
| **PEMT** | Phosphatidylethanolamine N-methyltransferase |
| **PERK** | Double-stranded RNA-activated protein kinase-like ER kinase |
| **Pi** | Orthophosphate |
| **PIS** | Phosphatidylinositol synthase |
| **PPARα** | Peroxisome proliferator-activated receptor alpha |
| **PSS** | Phosphatidylserine synthase |
| **PtdA** | Phosphatidate |
| **PtdCho** | Phosphatidylcholine |
| **PtdEtn** | Phosphatidylethanolamine |
| **PtdGly** | Phosphatidylglycerol |
| **PtdIns** | Phosphatidylinositol |
| **PtdSer** | Phosphatidylserine |
| **SAM** | S-adenosylmethionine |
| **SCAP** | SREBP cleavage-activating protein |
| **SPT** | Serine palmitoyltransferase |
| **SREBP** | Sterol response element-binding protein |
| **SRE** | Sterol response element |
| **TAG** | Triacylglycerol |
| **TSC1/2** | Tuberous sclerosis proteins 1 and *2* |
| **UPR** | Unfolded protein response |
| **XBP1** | X-Box-binding protein 1 |

Information on where lipid enzymes are localized in the ER is still surprisingly limited and/or comes from tagged enzymes that may not fully recapitulate the localization of native enzymes. Lipid synthesis was traditionally considered as a "smooth ER" function. However, there is little evidence that lipid enzymes are excluded from ribosome containing regions, and transmembrane ER proteins, such as those mediating lipid synthesis, typically diffuse through the continuous membrane system unless specifically anchored in a particular domain. On the other hand, there are data supporting the concept that some lipid enzymes concentrate in functionally defined ER regions and that this may be dynamically controlled. One example is that enzymes of the pathway leading to TAG (GPAT, AGPAT, lipin, and DGAT enzymes; Fig 1A) relocalize from the bulk ER to growing lipid droplets under conditions where TAG synthesis is stimulated [14] (Fig 1B (I)). This clustering may promote TAG production by shuttling lipid intermediates through the pathway and preventing their metabolism by enzymes in the GPL pathways that lead to membrane lipids (Fig 1A), as well as promoting the efficient transfer of TAG into the growing lipid droplet.

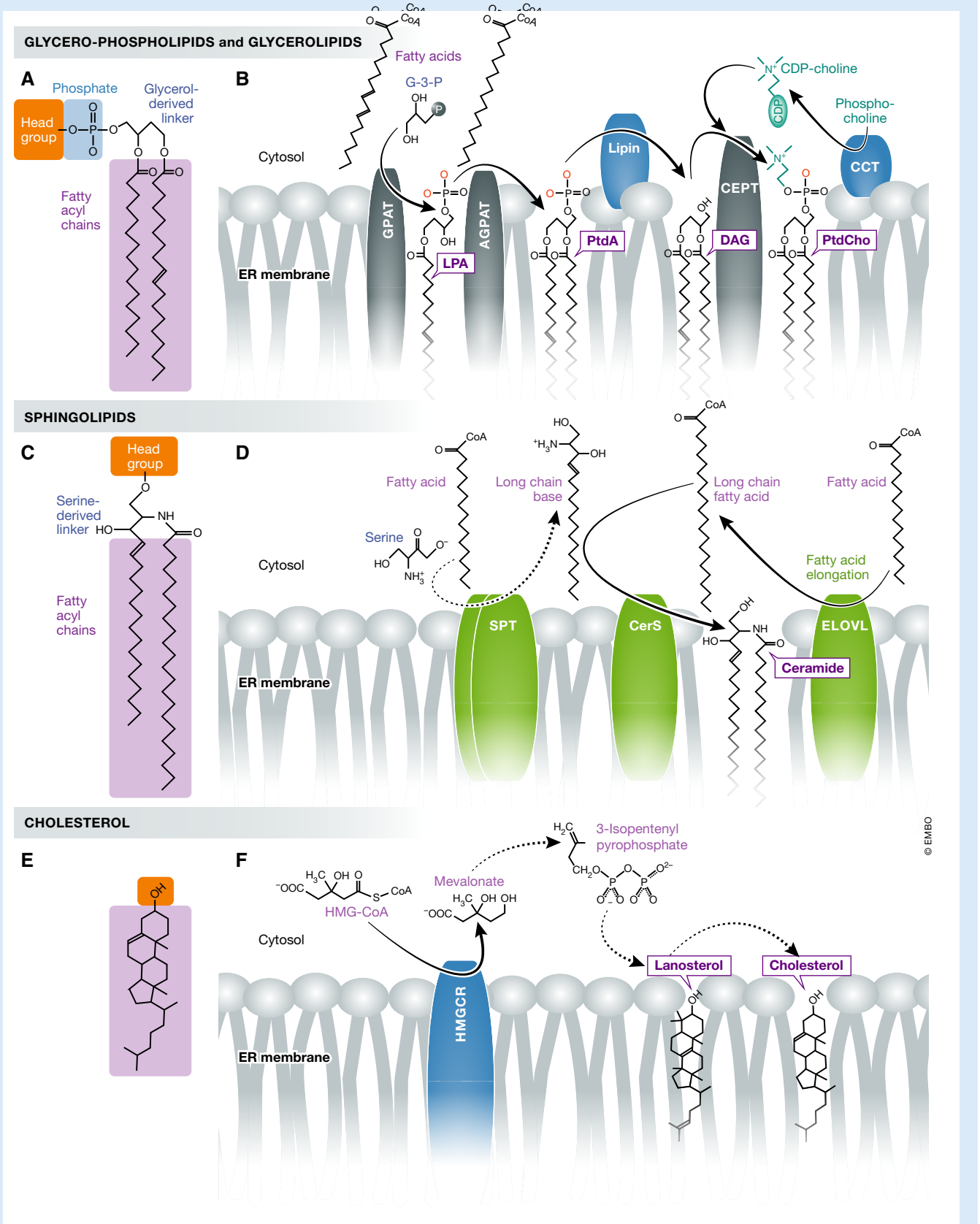
There is also evidence that some lipid synthesis reactions concentrate at ER–organelle contact sites. These specialized ER domains can traffic lipids out of the ER via lipid transfer proteins (vesicle-independent transfer), for example, allowing newly synthesized PtdCho to arrive at the plasma membrane 10 times faster than the 20–30 min newly synthesized proteins take via the secretory pathway (vesicle-dependent transfer) [15,16]. In yeast, contact sites are indeed enriched in lipid-synthesizing capacity [17]. Further, the PSS1 and PSS2 enzymes are particularly enriched in mitochondria-associated membranes of animal cells (Fig 1B (II)), as is the PEMT enzyme that methylates PtdEtn into PtdCho in some cell types. The DGAT2 enzyme that produces TAG has also been reported to concentrate in mitochondria-associated membranes under certain conditions [18,19].

This arrangement where lipids are synthesized close to their target destinations may facilitate efficient lipid transfer and allow feedback from target organelles to the activity of ER-localized lipid-synthesizing enzymes. Another example is that the PtdIns synthase enzyme (PIS; Fig 1A) concentrates in a rapidly moving ER-derived subcompartment that potentially contacts a variety of organelles to supply PtdIns to different membranes [20]. However, whether the bulk of *de novo* lipid synthesis takes place in specialized ER domains is less clear. Immunolabeling or expression of tagged lipid synthetic enzymes typically finds these broadly distributed, particularly under basal conditions [20–24]. In addition, new techniques such as click-chemistry labeling show newly synthesized PtdCho appearing across the ER system without signs of contact site "hot spots" [25], although with the caveat that these experiments require several minutes of exposure to labeled choline, during which time newly synthesized lipids may diffuse in the bilayer and thus preclude precise detection of where they are synthesized.

Surprisingly, there is considerable evidence pointing to the inner nuclear membrane as a site where some lipid synthetic reactions are concentrated. Lipin and CCTα are key soluble GPL/GL enzymes (Fig 1A). They both contain nuclear localization signals (NLS), traffic between the cytosol and nucleus through nuclear pore complexes (NPC) (Fig 1B (III)), and both strongly concentrate in the nucleus under specific conditions [26–28]. These two enzymes also translocate onto membranes to exert their activity. From the cytosol, they access the membranes of the main ER. However, they are restricted to the nucleoplasmic face of the inner nuclear membrane once in the nucleus (Fig 1B (III)). Transmembrane lipid enzymes can also access the inner nuclear

**Box 1: Membrane lipid structures and synthesis**



The majority of animal cell lipid biomass comes from members of the structurally related glycerophospholipid (GPL) and glycerolipid (GL) categories. Phosphatidic acid (PtdA) is the simplest GPL and contains only a phosphate group as a hydrophilic moiety (Box 1 Fig, panel A; blue), while PtdCho, PtdEtn, phosphatidylserine (PtdSer), and PtdIns are GPLs with choline, ethanolamine, serine, and inositol headgroups, respectively (Box 1 Fig, panel A; orange). GLs differ from GPLs in that they lack the phosphate linker. Important GLs are diacylglycerol (DAG) that has a hydroxyl group attached to the glycerol linker, and TAG that has a third fatty acyl chain.

GPL/GL synthesis begins with the transmembrane GPAT (glyceraldehyde-3-phosphate O-acyltransferase) and AGPAT (1-acylglycerol-3-phosphate O-acyltransferase; also known as LPAAT) enzymes sequentially combining soluble precursors into PtdA (Box 1 Fig, panel B; note that this figure does not depict enzyme membrane topology). The majority of this *de novo*-synthesized PtdA is then dephosphorylated into DAG by the lipin PtdA-phosphatase (Box 1 Fig, panel B), including in the pathway leading to the abundant membrane lipids of PtdCho and PtdEtn (Fig 1A). PtdCho is generated when CDP–choline is combined with DAG, and is catalyzed by the dual specificity CEPT (choline/ethanolamine phosphotransferase) that is responsible for most *de novo* PtdCho and PtdEtn production in animal cells. The rate of PtdCho synthesis is typically limited by CDP–choline levels, which is generated by the CCTα (CTP:phosphocholine cytidylyltransferase) enzyme that reversibly interacts with the ER membrane (Box 1 Fig, panel B). There are also enzymes catalyzing the reverse reactions, including phospholipases that remove headgroups and return complex lipids to the simpler DAG or PtdA structures. Furthermore, lipids in the ER membrane are also targeted by phospholipases ($PLA_2$ type) and acyltransferases that remove and replace fatty acyl chains, respectively (called the Lands cycle) [4]. The overall diversity of lipid acyl chains derives from both *de novo* synthesis via PtdA (the Kennedy pathway) and this Lands cycle "remodeling", with the relative contribution of each varying between cell types [5].

The sphingolipid category includes ceramide, sphingomyelin, and ganglioside lipids. Some membranes, including the plasma membrane, are particularly enriched in sphingolipids where their inclusion reduces fluidity and deformability while increasing thickness [6,7]. Sphingolipids are built around a serine backbone, with the different sphingolipid classes differentiated by their headgroup moieties (Box 1 Fig, panel C). Examples are choline on sphingomyelin, a hydroxyl group on ceramide, and complex sugar residues on gangliosides.

The ER is central for sphingolipid synthesis because the rate-limiting step of producing a "long-chain base" (LCB; also known as a sphingosine) is performed by ER transmembrane enzymes (serine palmitoyltransferase (SPT) in yeast, or SPTLC1-3 in mammals) [8,9] (Box 1 Fig, panel D). Ceramide synthase (CerS) enzymes that add a second fatty acyl chain are also ER enzymes [6], and the ER houses the fatty acid elongase (ELOVL) enzymes that produce the longer and more saturated fatty acyl chains that characterize sphingolipids (Box 1 Fig, panel D). Once the basic sphingolipid structures are synthesized by the ER, they are exported and further processed into other classes, such as sphingomyelin.

Sterols are the third major membrane lipid (Box 1 Fig, panel E) and can account for 30% or more of total membrane mass. Sterols are highly insoluble and pack between longer lipids to affect membrane flexibility and fluidity [10]. Cholesterol is the sole sterol of mammalian membranes and is synthesized by a series of enzymes, many of which are ER membrane proteins including HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase) that is typically rate-limiting for cholesterol production (Box 1 Fig, panel F). Cholesterol synthesis is not strongly conserved. Many animals, including the model organisms of *C. elegans* and *Drosophila melanogaster*, lack the cholesterol synthesis pathway, acquire sterols through their diet, and have membranes that contain relatively little sterol compared to many mammalian cells.

membrane by diffusing in the ER membranes (via the NPC-associated membrane) and will concentrate specifically in this nuclear-associated ER membrane if they are retained by a binding interaction [29]. It is likely that enzymes of TAG synthesis access the inner nuclear membrane given that lipid droplets bud into the nucleus under some circumstances [30]. The lamin B receptor (LBR) sterol reductase specifically concentrates in the inner nuclear membrane and is required in the pathway from lanosterol to cholesterol (Box 1 Fig, panel F) [31]. Furthermore, some exogenously expressed tagged AGPAT enzymes also appear to concentrate in nuclear membranes compared to their levels in the bulk ER [32,33].

It is difficult to understand why the inner nuclear membrane would be important for lipid synthesis. One possible explanation comes from lower eukaryotes where there is evidence for a role of nuclear lipid synthesis in expanding nuclear size during the cell cycle [34,35]. An alternative hypothesis is that this is a site where specific lipid enzymes and their reactions are isolated away from the bulk ER, perhaps to localize changes in lipid levels and avoid that they broadly influence ER membrane composition. Another possibility is that enzymes active on the inner nuclear membrane couple lipid synthesis with gene transcription regulation. Support for this comes from the fact that lipin has a dual function as the PtdA-phosphatase of *de novo* lipid synthesis and, through a different domain, as a coactivator of PPAR (peroxisome proliferator-activated receptor) transcription factors [36]. There is also strong competition in yeast between the use of the methyl donor SAM (S-adenosylmethionine) for lipid methylation versus histone methylation [37]. This may also be relevant in mammalian cell types where PtdEtn methylation into PtdCho occurs, although this does not necessarily need enzymes that localize in the nuclear membranes since SAM is soluble. Whatever the reason, it appears that enzyme targeting to the inner nuclear membrane is regulated and several lipid biosynthesis reactions occur on either the bulk ER or nuclear membranes depending on (currently poorly defined) differences in cell physiology.

## The composition of ER membranes as a central feedback regulator of lipid synthesis

The ER membranes contain high levels of PtdCho and PtdEtn and relatively little of the sphingolipids and cholesterol that promote thicker membranes, lipid order, and tighter lipid packing (Fig 2A) [38–40]. This is a unique composition and means that the ER membranes are relatively thin and primarily exist in a liquid disordered phase, which is synonymous with looser packing and greater disorder [41,42]. Here, we describe four mechanisms by which the unique lipid composition of the ER membrane controls the activity of enzymes in GPL/GL and cholesterol synthesis (note that the regulation of sphingolipid production remains less understood [7,43]). It appears that these are core feedback mechanisms underlying how the cell maintains ER membrane homeostasis and, since other organelles draw on ER lipids, they can also couple the broader needs of the cell to ER-localized lipid-synthesizing enzymes. Furthermore, as we discuss later, the same mechanisms are repeatedly involved with how a cell reprograms lipid synthesis in response to internal or externally derived signals.

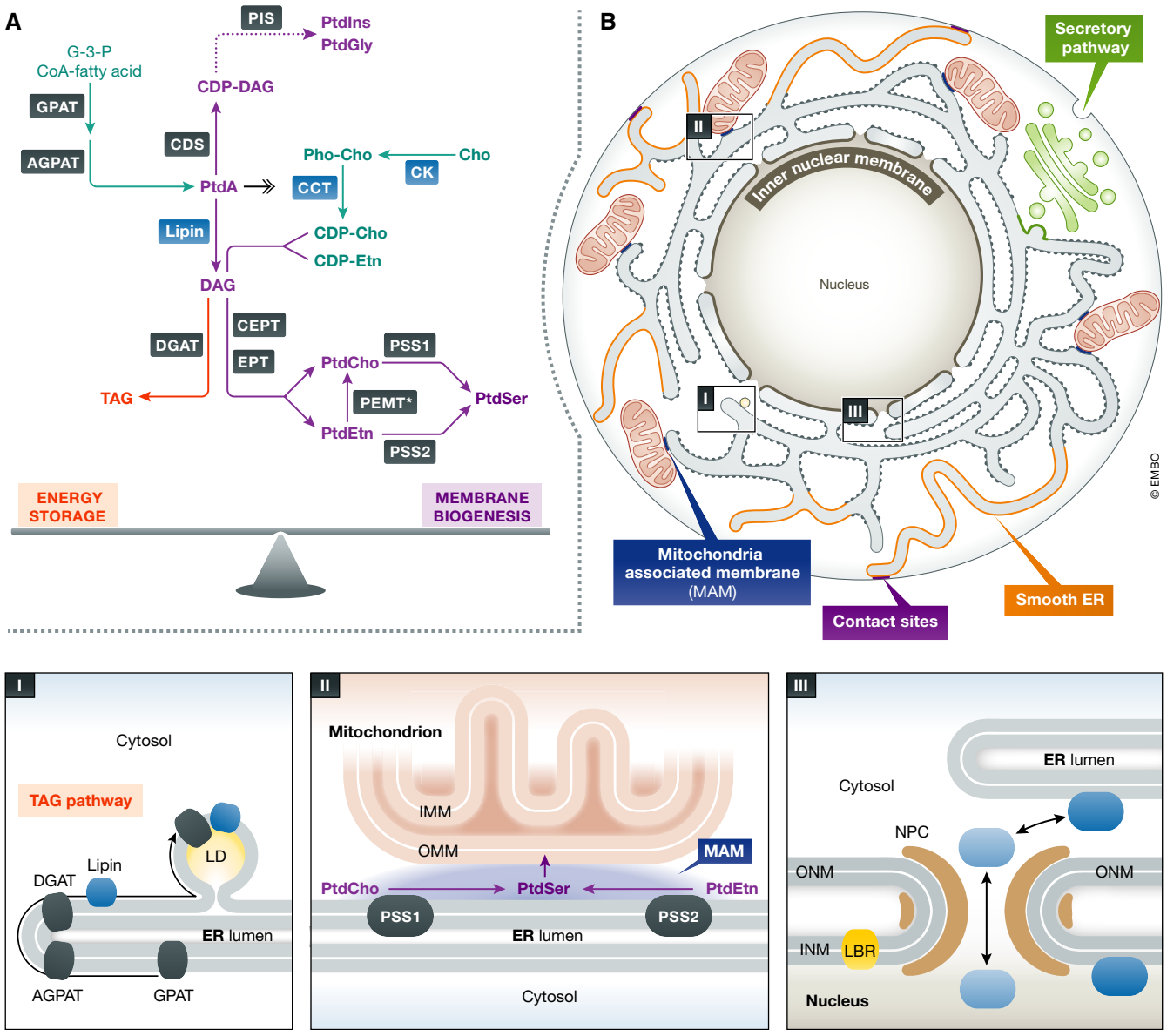



**Figure 1. The organization of ER-localized GPL/GL synthesis.**
(A) The GPL/GL enzyme network where membrane lipids are in purple, storage lipids are in orange, and water-soluble intermediates are in blue. Only key nodes are shown. The enzymes shown in gray are transmembrane ER proteins, while blue indicates a protein that reversibly associates with the ER membrane. Double arrow highlights the feedback loop where PtdA activates CDP–choline synthesis, which is a key control point for PtdCho production. Dotted lines indicate multiple reactions, and "*" indicates that PEMT has a restricted expression pattern in animals and its physiological importance is largely reported for hepatocytes. (B) Lipid synthesis organization within the ER system. Schematic of ER organization in a simple animal cell. There are structurally and/or functionally distinct domains, such as the inner and outer nuclear membrane (INM and ONM), and specialized contact sites between the ER and most organelles (not all shown). The localization of lipid enzymes within the ER system is still poorly defined, but some new concepts are emerging including the following: (I) Enzymes of the TAG pathway can relocalize from a broad distribution to concentrate at sites of growing lipid droplets. (II) Enzymes of PtdSer synthesis (PSS1 and PSS2) concentrate at mitochondria-associated membranes that are also the sites where newly synthesized PtdSer is transferred to mitochondria. (III) The lipin and CCTα enzymes that reversibly bind membranes also carry NLS and shuttle between the cytosol and nucleus through nuclear pore complexes. This affects whether these enzymes interact with the main-ER membrane or inner nuclear membrane. The LBR sterol reductase is also a well-characterized INM resident. AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; CEPT, choline/ethanolamine phosphotransferase; CK, choline kinase; CDP, cytidine diphosphate; Cho, choline; CDS, PtdA cytidylyltransferase; EPT, ethanolamine phosphotransferase; G-3-P, glycerol 3-phosphate; PtdGly, phosphatidylglycerol; PSS, phosphatidylserine synthase; MAM, mitochondria-associated membrane.

*Membrane composition and PtdCho synthesis*

PtdCho is the most abundant lipid of mammalian membranes, and its synthesis is central to membrane biogenesis. The CCTα enzyme (human gene: *PCYT1A*) is typically rate-limiting for PtdCho production in animal cells (Fig 1A). CCTα cycles between a soluble and membrane-bound state (or onto the single lipid leaflet surrounding lipid droplets [44]) and is subject to auto-inhibition mediated by a carboxy-terminal region termed the M-domain [44,45]. When CCTα is soluble, the M-domain inhibits the catalytic domain and prevents CDP–choline synthesis. However, this inhibition is relieved when the

M-domain folds into an amphipathic helix and absorbs onto a lipid surface (Fig 2C). Thus, CCTα membrane binding represents an on–off switch regulating catalytic activity and therefore PtdCho production.

Whether domains such as the M-domain interact with membranes to fold into a helix depends on the biophysical properties of available membrane surfaces. One critical factor is that membrane binding is typically promoted by looser lipid packing and voids between lipid headgroups (Fig 2B). Such packing voids are more frequent when membranes contain a higher percentage of conical lipids compared with cylindrical lipids (Fig 2B) [38,44,46].

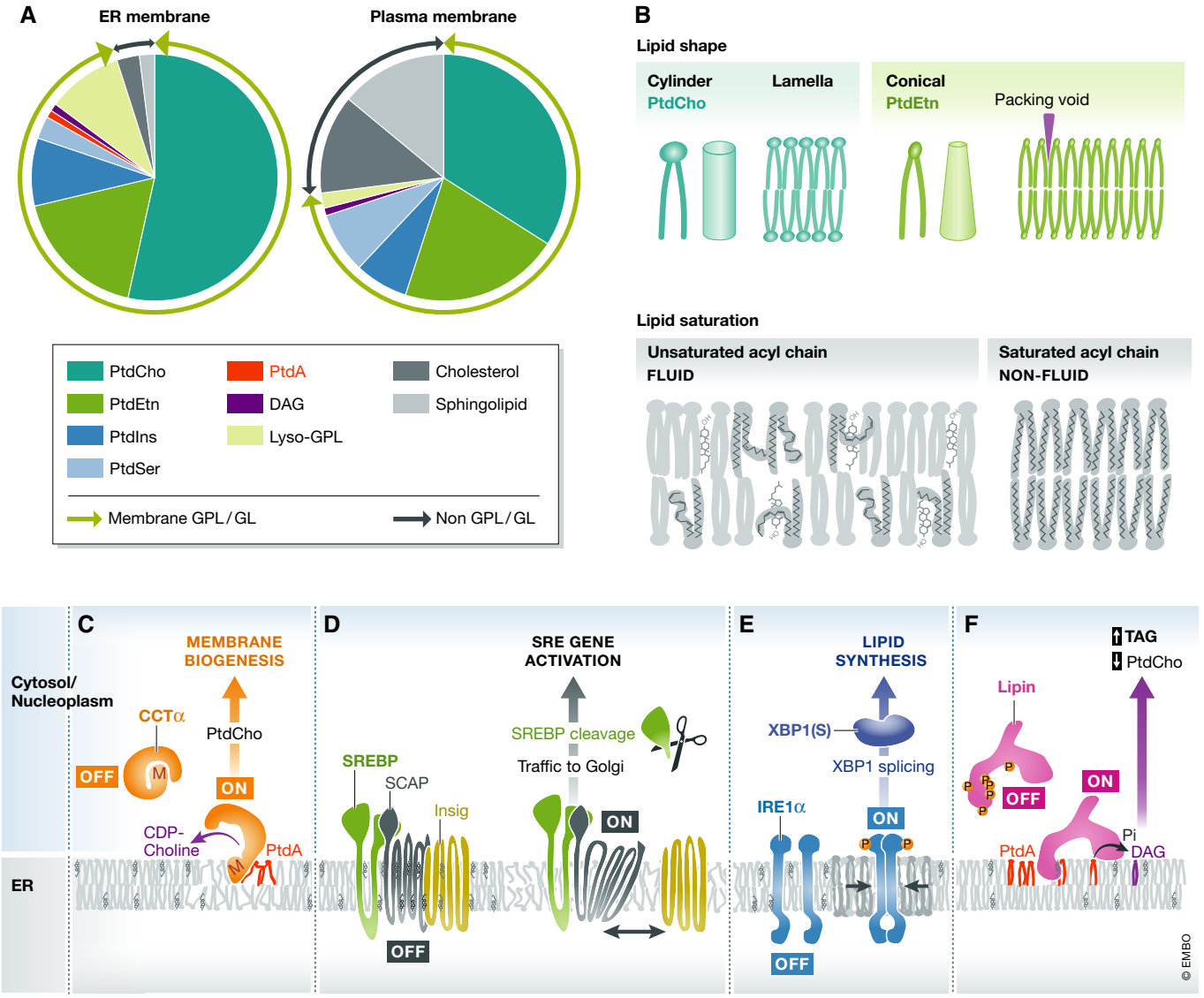



**Figure 2. Mechanisms of ER membrane homeostasis.**
(A) Hepatocyte membrane compositions. The ER membranes are enriched in GPL and contain little sphingolipid or cholesterol. PtdA is also detected in the ER membrane, but not the plasma membrane. PtdIns includes phosphorylated and non-phosphorylated species. These data are modified from [39] and are in line with several other studies, although not all lipid species were assessed in each report [120,133,134]. (B) Intrinsic properties of lipids effect membrane properties. PtdCho is a cylindrical lipid where the headgroup and acyl chains occupy a similar lateral area, while PtdEtn is conical because it has a smaller headgroup. PtdCho spontaneously assembles into bilayers and stabilizes bilayers *in vivo*, while PtdEtn promotes voids between lipid headgroups within a bilayer and forms curved "hexagonal" structures in isolation [135]. Note that the looser packing associated with PtdEtn also exposes small lipid headgroups, such as that of PtdA, for protein interactions [136]. Acyl chain saturation also affects membrane properties such as thickness and lipid packing density [137,138]. (C) CCTα is activated by membrane absorption of the M-domain that is regulated by lipid packing and PtdA. Activated CCTα produces the CDP–choline that is typically rate-limiting for PtdCho production and therefore membrane biogenesis. (D) Immature, inactive SREBP is a transmembrane ER protein. It is activated when altered membrane environment causes SCAP to change conformation and dissociate from Insig. This alters SREBP/SCAP trafficking (not shown) and allows protease-dependent release of the mature SREBP transcription factor from the membrane. This then can enter the nucleus and activate gene transcription via binding to SRE, including genes of lipid synthesis. (E) The IRE1/XBP1(S) branch of the UPR is activated by increased lipid saturation and packing density in the ER membrane. This causes IRE1 dimerization, self-phosphorylation of cytosolic residues, and activation of IRE1 RNA splicing activity that removes an intron from the XBP1 mRNA. The XBP1 (S) protein is then translated and acts as a transcription factor. (F) Lipin PtdA-phosphatase activity requires that it integrates into a membrane. This is positively regulated by PtdA and negatively regulated by lipin phosphorylation state. Lipin activity often promotes TAG production while reducing PtdCho. Pi, orthophosphate.

While this can be a relatively non-specific activation mechanism, in the case of the ER it tunes CCTα activity to PtdCho levels since these are the main cylindrical, bilayer-stabilizing lipids of mammalian membranes. Thus, CCTα membrane binding and activity increase when PtdCho levels drop relative to the conically shaped PtdEtn. Conversely, a high concentration of PtdCho reduces CCTα membrane binding and activity.

Amphipathic helix integration into a membrane is also influenced by the surface charge of a membrane, via interactions between the protein and charged lipid headgroups. Typically, this involves negatively charged lipids that further stabilize the membrane–protein binding interaction. While phosphorylated PtdIns species are centrally important for how such proteins associate with most membranes, these lipids are rare in the ER where instead PtdA often promotes protein–membrane binding, including that of CCTα [47] (Fig 2C). This inherently matches CDP–choline production to the amount of PtdA being produced at the beginning of the GPL/GL network (Fig 1A). Interestingly, there are also several other mechanisms by which PtdA sensitivity further tunes CCTα activity to detect changes in ER membrane composition. One is that the small charged headgroup of PtdA is typically more accessible for protein binding interactions when membranes contain more conical lipids (e.g., PtdEtn) than cylindrical PtdCho (Fig 2B). This means again that the membrane binding and activity of CCTα increase when PtdCho levels drop. In addition, PtdA exists in mono- and divalent forms, and proteins typically interact more strongly with the greater charge of divalent PtdA. In turn, several factors regulate the relative abundance of mono- and divalent PtdA that intriguingly includes that the primary amine headgroup of PtdEtn promotes divalent PtdA through hydrogen bonding interactions [48]. Thus, although unproven *in vivo*, a membrane lipid imbalance with high PtdEtn levels relative to PtdCho can potentially increase CCTα activity via elevating divalently charged PtdA, and thus counteract the imbalance.

Through these mechanisms, the CCTα M-domain represents an elegant means by which a water-soluble step of lipid synthesis is regulated by the end product lipid, even though this is packed within a lipid bilayer. The membrane binding of CCTα also shows how a lipid-synthesizing activity is "tuned" to the composition of the ER membranes, and thus inherently corrects against membrane disequilibrium. Furthermore, once bound into the membrane, the amphipathic helix of CCTα is exposed to the surrounding lipid environment and may undergo further conformational adaptation—such as shown for the lipid packaging sensor Mga2 and inositol-requiring enzyme (IRE1) [49,50]—to further influence CCTα activity and PtdCho production.

### *Sterol regulatory element-binding proteins (SREBPs)*

The SREBP system also allows lipid species in the ER membrane to feedback on their own biosynthesis. SREBPs are transmembrane ER proteins that were first identified as players in mammalian cholesterol synthesis. Mammals have three *SREBP* gene products: SREBP-1A, SREBP-1C, and SREBP-2 (Fig 2D). All three have a cytosolic N-terminal domain that is a transcription factor of the basic helix-loop-helix leucine zipper family. This domain activates gene transcription when it binds to sterol response elements (SREs) in promoters. However, the domain is held inactive and unable to bind DNA while attached to the transmembrane C-terminus of SREBP. The proteases responsible for SREBP cleavage are localized in the Golgi, while under basal conditions SREBP is retained in the ER via interactions with SCAP (SREBP cleavage-activating protein) and the ER-resident protein, Insig (insulin-induced gene) (Fig 2D) [13]. The system is sensitive to the membrane lipid environment as it influences SCAP conformation [51,52]. In the classic cholesterol feedback model, low cholesterol in the ER membrane causes SCAP to alter its conformation and release from Insig. SREBP/SCAP then escape the ER in secretory pathway vesicles, arrive in the Golgi, and undergo proteolytic processing that releases the active N-terminus of SREBP into the cytosol from where it can traffic to the nucleus and access SREs (Fig 2D) [53]. The importance of this mechanism for cholesterol synthesis is supported by experiments showing that forced expression of the mature isolated N-terminal domain of mammalian SREBP-2 causes a 75-fold increase in the levels of *HMGCR* mRNA that encodes the rate-limiting enzyme of cholesterol synthesis. This led to a ~30-fold increase in cholesterol synthesis [54]. Additionally, ER membranes contain particularly low amounts of cholesterol (Fig 2A). This means that even small molar changes in cholesterol levels represent a relatively large percentage change, and thus can trigger SCAP/Insig dissociation [55]. This mechanism of SREBP activation is therefore another example where ER membrane composition is a central regulator of cellular lipid synthesis.

Although it was first described in cholesterol regulation, the SREBP system is not specific to cholesterol synthesis; 1,003 different genes show statistically significant expression changes in the liver of mice with forced expression of the N-terminus of SREBP-1A, while 505 are affected by SREBP-2 [56]. Not all genes are directly affected, however, and the overall picture from many studies of SREBP-1 and SREBP-2 signaling is that SREBP-2 has the classic, originally described role in cholesterol synthesis, while SREBP-1 isoforms predominantly upregulate genes of fatty acid and GPL/GL metabolism, particularly TAG synthesis. This includes that SREBP-1 isoforms elevate the expression of fatty acid synthase, fatty acid elongase, and GPAT enzymes to upregulate flux into the GPL/GL network. The general importance of SREBP signaling is also clear when considering non-mammalian organisms. *Caenorhabditis elegans* and *Drosophila* do not synthesize cholesterol, but still have SREBP signaling. In these organisms, SREBP activation specifically affects GPL/GL synthesis. Indeed, *C. elegans* SBP-1 is activated by low PtdCho, as well as by abnormal ratios of PtdA and DAG [57,58], while *Drosophila* SREBP is activated by low PtdEtn [59]. In both organisms, SREBP is an essential positive regulator of membrane lipid biogenesis [59–61]. Thus, although SREBP was named for its role in mammalian cholesterol homeostasis, this may be a relatively recent evolutionary adaptation of a general membrane regulator. The next steps will be to better understand the disturbances in membrane composition that activate the different SREBPs. In addition, as we explain below, it is important to fully catalog the molecular mechanisms of activation given that SREBP-1, in particular, contributes to cellular programs such as cell growth and TAG lipogenesis, and has been implicated in the proliferative capacity of cancer cells [62].

### *The unfolded protein response (UPR); lipids as well as unfolded proteins*

The UPR maintains ER protein homeostasis in response to the accumulation of misfolded proteins in the ER lumen [63–68]. Higher eukaryotes have three UPR branches mediated by different

transmembrane sensors: (i) IRE1 which signals through XBP1 (Fig 2E), (ii) ATF6, and (iii) PERK that acts via elF-2α. All three branches are activated by misfolded proteins in the ER lumen and classically signal to slow translation, upregulate chaperone expression, and/or induce apoptosis [69]. Additionally, as we describe here, they also strongly affect lipid metabolism.

A major link between the UPR, proteopathic defects, and lipid synthesis is the importance of ER volume for resolving ER dysfunction. In fact, increasing ER volume helps rescue luminal protein misfolding independent of factors such as chaperone levels [70]. In turn, increased ER volume is synonymous with increased membrane, and thus increased membrane lipid production. The IRE1/XBP1(S) branch, in particular, is a well-characterized positive regulator of membrane lipid synthesis. Forced XBP1(S) expression is sufficient to increase the size of the ER [71,72], while genetic deletion of the *Xbp1* gene reduces ER size [73]. The ER membrane expansion induced by XBP1(S) is concomitant with elevated PtdCho and PtdEtn biosynthesis [71], and increased expression of lipid synthesis genes including several of fatty acid synthesis, *LIPIN* and *AGPAT* isoforms [71,74]. The specific mediator between IRE1/XBP1(S) and PtdCho and PtdEtn synthesis has been difficult to pin down. It appears to involve increased CCTα activity but, surprisingly, given that IRE1/XBP1(S) is primarily a transcriptional mechanism, *PCYT1A*/CCTα mRNA and protein are relatively unaltered [71,74]. There is however evidence that CCTα might be activated via changes to the ER membrane environment [74], such as increased PtdA levels that elevate CCTα membrane binding.

ATF6 and PERK also affect lipid biogenesis. Some of their reported effects may be indirect given that, at least in some conditions, ATF6 promotes IRE1 and XBP1 expression alongside increasing membrane production [75,76]. On the other hand, forced ATF6 signaling can drive *de novo* membrane lipid synthesis to the same extent as XBP1(S), and independent of XBP1(S) [77]. In addition, ATF6 signaling (rather than XBP1(S)) underlies the lipid synthesis and ER proliferation that occurs when the ER is overloaded with membrane proteins [78]. The lipid enzymes targeted by ATF6 also differ from those of XBP1(S), further supporting that this UPR branch has its own (currently less defined) role in promoting membrane lipid production.

The UPR has been primarily considered as a protein homeostatic mechanism. However, there are now substantial data challenging the concept that UPR control of lipid biosynthesis is a corollary to its role in protein homeostasis. Indeed, not only does the UPR control cellular lipid levels, but UPR sensors are activated by abnormal ER membrane composition including abnormal lipid saturation and altered sterols [79–82]. Furthermore, it is clear that this indeed occurs because UPR sensors are directly influenced by their surrounding membrane, and not just by the presence of luminal unfolded proteins. Excess saturated lipids activate IRE1 or PERK even when these sensors lack the luminal regions that detect misfolded proteins [82]. Similarly, mutant yeast IRE1 that cannot detect misfolded proteins nevertheless mediates UPR activation in cells with abnormal lipid metabolism [83]. Further, IRE1 in *C. elegans* is activated by disturbed lipid metabolism even when this fails to induce protein misfolding, and in a manner that is insensitive to chemical chaperones that rescue protein misfolding [79].

Yeast studies also provided further insight into the molecular basis of how UPR proteins sense membrane composition [49]. Just as for unfolded proteins, IRE1 activation in response to abnormal ER membrane composition occurs via the formation of higher order IRE1 oligomers that splice an mRNA (XBP1 or Hac1 in yeast) to allow translation of a full-length soluble transcription factor (XBP1(S)/HAC1) that mediates many downstream events of the UPR (Fig 2E). Uniquely, however, lipid-driven UPR activation requires an amphipathic helix in IRE1 that is embedded in the luminal face of the ER membrane and lies adjacent to the transmembrane section of the sensor. This juxtamembrane amphipathic helix is also evolutionarily conserved in animal IRE1 proteins, as well as in the PERK sensor. Molecular dynamic simulations point to this helix driving IRE1 clustering if ER membrane thickness and/or lipid packing density increase; notably, the ER membrane is particularly thin and loosely packed under normal conditions. This means that IRE1 activation is sensitized to imbalances in the levels of different GPL classes, as well as GPL saturation, given how these both impact membrane packing (Fig 2B). Additionally, it likely means that the UPR is sensitized to changes in the normally low levels of ER membrane sphingolipids and cholesterol given that these also promote thicker and more densely packed membranes.

It is therefore now clear that the UPR detects and solves defects in the lipid composition of ER membranes, just as it does detect proteomic defects within the ER lumen. Additionally, the two different insults can act additively since they drive IRE1 dimerization through distinct molecular mechanisms—thus establishing a new model where abnormal ER membrane composition increases the likelihood that unfolded proteins in the ER lumen activate the UPR, and vice versa [49].

#### *Lipin PtdA-phosphatase activity at the center of GPL/GL biosynthesis*

PtdA is the basic lipid structure from which other GPL/GL derive. It lies at the branch point between the pathways that produce PtdIns versus PtdCho/PtdEtn. PtdA conversion to DAG further leads to TAG production (Fig 1A). PtdA is also a signaling lipid that is detected by many peripheral membrane proteins, and, for example, helps to activate CCTα to drive membrane production, as described above. Consequently, enzymes that control PtdA levels exert multiple layers of control over the GPL/GL synthesis network.

In eukaryotes from yeast to plants to animals, the lipin PtdA-phosphatases influence ER-localized PtdA levels [84] (note that a distinct set of lipid phosphatases (LPP enzymes) hydrolyze PtdA in other organelles [85]). Lipins hydrolyze the phosphate headgroup of PtdA to produce DAG (Box 1 Fig, panel A) with high specificity for PtdA above other phosphorylated lipids [86,87]. As mentioned above, animal lipins also directly influence lipogenic transcriptional programs through their C-terminal domain that interacts with transcriptional regulators and in a PtdA-phosphatase-independent manner [88]. However, how this role integrates with lipin's role in *de novo* lipid synthesis remains poorly understood and is not further considered here.

Despite having a substrate and product in the lipid bilayer, lipins are not integral membrane proteins and must associate with membranes to access their substrate (Fig 2F). Just as for CCTα, the regulation of lipin membrane binding has evolved as a central regulator of its catalytic activity. Lipin associates with the ER membrane through an amino-terminal amphipathic helix, and this event is especially sensitive to PtdA levels [89]. The addition of PtdA

increases yeast lipin (pah-1) binding to micelles by five- to sixfold [90]. Inclusion of PtdA also strongly promotes the otherwise weak binding of lipin1 to synthetic liposomes even when these have a mix of PtdCho/PtdEtn that mimics the ER membrane composition [91]. PtdA sensing is mediated through a series of basic amino acids that are downstream of the membrane binding domain of lipin [26,91], and it will be interesting to know how these two domains interact, as well as their relationship to the C-terminal catalytic region—information that will be gained when the structure of the lipin enzyme is solved.

Lipin is heavily phosphorylated under many conditions, and this regulates its PtdA-phosphatase activity (Fig 2F). There are more than 17 distinct serine/threonine phosphorylations recorded for mammalian lipin1, 15 in lipin2, and a similar number for yeast pah-1/lipin [27,92–94]. The roles of individual phosphorylation sites have been difficult to pin down, even in yeast where these are fewer and there is more advanced understanding of lipin biochemistry. However, phosphorylation is uniformly associated with reduced PtdA-phosphatase activity, leading to an experimentally well-supported model that dephosphorylated lipin proteins are active PtdA-phosphatases, while highly phosphorylated lipin is inactive. It is also clear that phosphorylation inhibits PtdA-phosphatase activity (at least in part) because it reduces membrane binding and/or PtdA detection (Fig 2F) [91,93].

Lipin phosphorylation and dephosphorylation also control whether lipin localizes in the nucleus or cytosol (Fig 1B (III)). Indeed, all lipins carry a NLS, and there is a consistent relationship between dephosphorylated lipin, nuclear localization, increased membrane binding, and elevated PtdA-phosphatase activity. Highly phosphorylated lipin is cytosolic, not membrane-bound, and has less activity. It is difficult to understand why nuclear localization correlates with membrane binding and *more* PtdA-phosphatase activity, although we note that nuclear-localized lipins can access PtdA within the nucleoplasmic face of the inner nuclear membrane. One interesting possibility is that the inner nuclear membrane localization relates to PtdA-dependent crosstalk between lipin and CCTα. This negative feedback between lipin metabolism of PtdA and reduced CCTα activity has been demonstrated in yeast, plant, and fly. Both lipin and CCTα carry an NLS and both often concentrate in the nucleus, and thus will cycle to and from the inner nuclear membrane (Fig 1B (III)). Perhaps concentrating both enzymes to the inner nuclear membrane amplifies how efficiently lipin regulates CCTα, and data from fly indeed show lipin regulating whether or not CCT1 interacts with the inner nuclear membrane [28]. Furthermore, segregating the lipin/CCTα crosstalk may prevent altered PtdA levels from impacting the many other proteins that bind and/or are regulated by PtdA [95,96].

There are still many unknowns about how lipin is regulated. The interplay between multiple phosphorylations, sensitivity to membrane composition, and partitioning between the nucleus and cytosol have made it difficult to dissect the roles of each event. The conserved CTDNEP1/NEP1R1 phosphatase complex (Nem1 and Spo7 in yeast) is a centrally important lipin activator in yeast and animals that often specifically localizes in the inner nuclear membrane [97–99]. As we discuss below, lipin phosphorylation is also a target of insulin and mTOR signaling. In addition, torsin ATPases, which reside inside the ER lumen, were recently identified as lipin inhibitors, and are also implicated as sensors of the ER redox state [28,100,101]. Thus, lipin is positioned to integrate multiple types of information and couple these to lipid flux through the GPL/GL metabolic network.

## Adaption of lipid synthesis to environment, differentiation, and disease

There are also many circumstances where the cell adjusts the levels of lipid synthesis or flux of intermediates moving through different synthetic pathways. This includes external and internal pro-growth signals and many cell differentiation programs. Here, we describe some key examples and discuss the evidence that these programs act by modifying the same molecular mechanisms described above for maintaining membrane homeostasis (Fig 3A). In addition, it is also becoming clear that dysregulation of these same mechanisms has pathological consequences for human health.

### *Cell growth, growth factors, and mTORC1*

Cell growth is controlled by external information such as hormones and nutrients, integrated with internal states relating to cellular energy and stress. Cell growth is an essential element for many aspects of animal development and physiology, while the dysregulation of cell growth is pathogenic in the context of cancer. Cell growth inherently requires increased cellular biomass, which includes membrane and thus increased membrane lipid production. Consistently, lipid production is a target of cell growth signaling pathways under normal as well as pathological conditions.

The evolutionarily conserved mTOR serine/threonine kinase is a master integrator of growth information and coordinates the downstream processes that increase cellular biomass. This includes signaling for the increased production of lipids [102]. There are two distinct mTOR kinase-active complexes, mTORC1 and mTORC2. mTORC1 is inhibited by rapamycin, activated by pro-growth PI3K and Akt pathways, and is the complex that most often couples pro-growth signaling to lipid biogenesis [102]. Indeed, the majority of events where nutrients alter lipid production are either blocked by rapamycin and/or stimulated by genetic knockout of the TSC1/2 upstream inhibitors of mTORC1. This means that mTORC1 is central to how pro-growth signals increase the synthesis of PtdCho and PtdEtn membrane lipids [103,104].

Pro-growth signaling/mTORC1 regulates lipid production through several mechanisms, of which SREBP-1 activation is the most consistently important (Fig 3A). Indeed, this fundamental conserved mechanism has been described driving cell growth in invertebrate and vertebrate species [103]. The mechanism of how mTORC1/pro-growth affects SREBP-1 has been most studied in liver cells, a cell type under particular scrutiny due to its central role in nutrient responses [102] and which has recently been discussed in detail [105]. In brief, coupling to SREBP-1 typically requires that mTORC1 activates its classic ribosomal S-6-kinase substrate, and impacts SREBP-1 at multiple levels including elevating SREBP-1 cleavage, increasing the half-life of cleaved mature SREBP-1, and upregulating *SREBP-1* mRNA expression at least in part because the *SREBP-1* promoter contains a SRE and thus responds to mature SREBP-1 protein [106]. How pro-growth signals promote SREBP cleavage from the membrane remains relatively poorly understood,

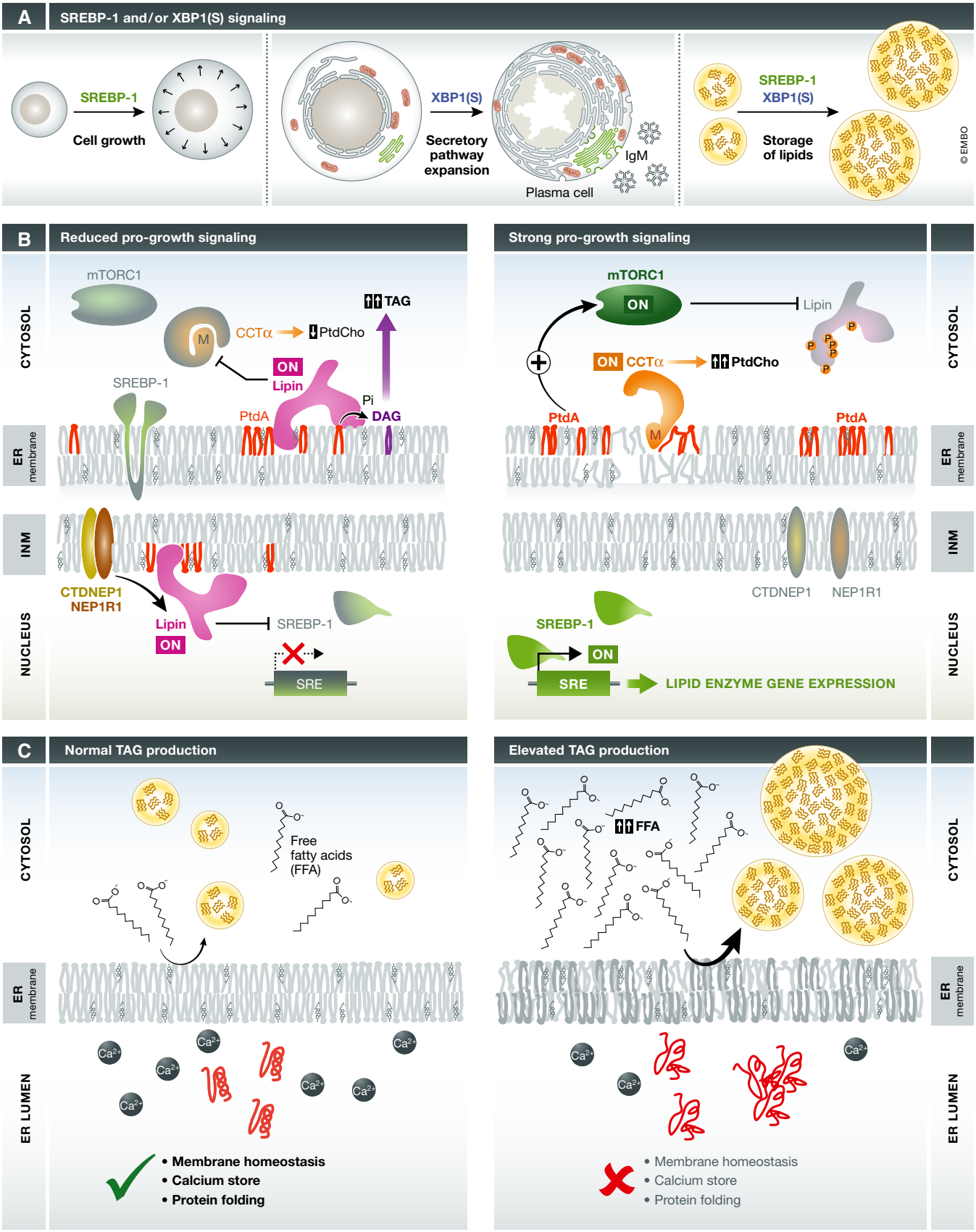



**Figure 3.**

**Figure 3. The regulation of ER-localized lipid synthesis in cell growth, differentiation, and disease.**
(A) SREBP-1 and/or XBP1(S) signaling are important for cellular adaptation of lipid synthesis to cell growth, differentiation (like secretory pathway expansion), or the environment (like TAG production in response to nutrients). The mechanisms that allow context-dependent variation in the final outcome of these pathways remain under study. (B) Growth signals, including key pathways such as insulin signaling, stimulate increased lipid synthesis associated with increased cell biomass. The activation of cell growth-associated lipid synthesis typically requires mTORC1 and couples to SREBP-1 including enhancing SREBP-1 cleavage. Pro-growth signaling also consistently causes lipin phosphorylation, which converts this enzyme from a nuclear localization to a cytosolic localization, and negatively correlates with PtdA-phosphatase activity. This in turn disinhibits CCTα by reducing PtdA metabolism to DAG. Lipin PtdA-phosphatase activity also inhibits SREBP by sequestering cleaved SREBP at the nuclear periphery. The inner nuclear membrane CTDNEP/NEP1R1 phosphatase complex maintains dephosphorylated lipin and thus counters pro-growth lipin phosphorylation [112,138]. Pi, orthophosphate. (C) Pathological impact of elevated TAG production in hepatocytes caused by excess nutrients. These cells are functionally specialized to convert carbohydrates into TAG. High-level TAG production from excess nutrients alters the ER membrane lipid saturation profile and the ratio between PtdCho and PtdEtn. This altered membrane lipid composition induces broader ER dysfunction, included impairing calcium transport and protein misfolding.

including whether/how ER membrane composition plays a role. One interesting newer discovery is that increased SCAP glycosylation decreases its binding to Insig-1, and increases SREBP traffic to the Golgi for cleavage (Fig 2D). This SCAP glycosylation is sensitive to cellular glucose levels, which, in turn, are regulated by pro-growth signals [62]. This provides a mechanism whereby SREBP cleavage is upregulated by a signaling cascade, although this particular mechanism appears to be mTORC1-independent. Overall, there is much to uncover about the coupling of pro-growth pathways to SREBP-1, including how this is adapted in different cell types, how abnormal activation of SREBP-1 helps fuel oncogenic cell growth, and whether SREBP-1 may represent a useful chemotherapy target [62,107].

Pro-growth signaling/mTORC1 also controls lipid production via lipin phosphorylation that reduces its PtdA-phosphatase activity [91,108]. Again, this is a conserved mechanism with similar events described in yeast, fly, plant, and mammalian cells. A highly conserved serine residue (position 106 in mammalian lipin1) is targeted by insulin and mTOR [93], although mTORC1 activation goes beyond this and 17 distinct mTOR-sensitive serine/threonine phosphorylations have been described in mammalian lipin1 [27]. How lipin inhibition contributes to mTOR-driven cell growth is not yet fully explained. It may relate to the feedback between PtdA and CCTα [28,47], whereby lower lipin PtdA-phosphatase activity allows PtdA levels to increase, thus activating CCTα and driving membrane GPL production (Fig 3B). In addition, PtdA is itself an mTORC1 activator via a PtdA-binding motif in the same mTORC1 domain that rapamycin binds [95,109,110], possibly meaning that lipin inhibition amplifies mTORC1 activation.

There is an additional interaction between lipin and the SREBP branch of the cell growth response. mTOR-driven lipin phosphorylation causes lipin to localize in the cytosol. Conversely, mTOR inhibition promotes a nuclear localization and thus lipin access to the inner nuclear membrane (Figs 1B (III) and 3B). When mTOR is off, the PtdA-phosphatase activity of lipin modulates nuclear morphology and sequesters cleaved SREBP at the nuclear periphery to prevent SREBP from activating gene transcription. Conversely, mTOR-dependent lipin phosphorylation overcomes this inhibitory control over SREBP (Fig 3B) [27]. The *in vivo* relevance of this inhibitory interaction between lipin and SREBP is shown by genetic knockout of an ubiquitin ligase that targets phosphorylated lipin for degradation. Its loss elevates lipin levels in parallel with reducing SREBP target gene expression and lipid synthesis in liver cells [111]. Interestingly, lipin phosphorylation driven by pro-growth signaling is reversed by the CTDNEP1 phosphatase that resides at the nuclear periphery, and pro-growth signals in yeast also control the lipin phosphorylation state via this phosphatase (Fig 3B) [112].

### *Tailoring lipid synthesis to cell-type-specific functions*

Lipid synthesis is regulated by differentiation programs concomitant with how they alter the size and morphology of cells, and/or the volume of internal organelles. In addition, organelle scaling occurs in response to environmental cues, for example, when hepatocytes expand the size of the ER to house detoxifying cytochrome P450 enzymes [113], or in professional energy-processing cells that alter TAG production according to nutritional cues (Fig 3A).

The UPR machinery, especially the IRE1/XBP1(S) branch, is the main driver of lipid synthesis coupled to changes in ER and secretory pathway volume (Fig 3A). XBP1 is required in secretory cells, such as pancreatic acinar cells and salivary gland cells, and XBP1 deletion causes lethal phenotypes in mice because these cells fail to achieve fully differentiated states that support high-level protein secretion [73]. XBP1 deletion also inhibits the ER membrane expansion that occurs when B cells differentiate into antibody-secreting plasma cells, while forced XBP1(S) expression is sufficient to induce ER expansion and differentiation of this cell type [114]. Thus, although IRE1/XBP1(S) were first described in the context of a pathological response to protein misfolding, this pathway also promotes membrane lipid synthesis during normal differentiation.

Another well-characterized scaling of lipid synthesis occurs with the synthetic pathway leading to TAG (fatty acid synthesis, GPAT, AGPAT, lipin, and DGAT enzymes; Fig 1A). It is particularly active in cell types that convert nutrients into energy stores, such as mammalian adipocytes and hepatocytes. In addition, environmental signals further refine TAG pathway activity, and there are important roles for the UPR and SREBP regulatory mechanisms in TAG production (Fig 3A). Here, again, IRE1/XBP1(S) play roles. Conditional deletion of XBP1 from mature mouse hepatocytes decreases flux into the GPL/GL network (detected by [14C]-acetate incorporation into lipids) concomitant with reduced expression of fatty acid synthesis and *Dgat2* genes. Hepatocytes that lack XBP1 are also less able to upregulate lipid synthesis in response to a high-carbohydrate diet [115]. This XBP1-mediated control over hepatic lipogenesis appears to occur via IRE1-dependent and IRE1-independent mediated mechanisms that overall remain poorly described [115]. In addition, TAG synthesis in hepatocytes is further promoted by insulin signaling acting via SREBP-1. Mouse studies have shown that hepatocytes lacking SREBP-1 release less TAG into the circulation, while forced SREBP-1 (or SREBP-2) expression leads to excess TAG deposition [116].

The mechanism(s) that allow SREBP-1 activation to elevate TAG synthesis in this context, while the same pathway promotes cell growth in other cells/situations, remain poorly defined. In addition, there are also similar questions about how the XBP1(S) pathway has differing effects in different contexts. Since both systems are transcriptional, they may target different gene sets in different cell types [117], although this is an incomplete explanation since gene expression changes usually correlate poorly with altered lipid production. It may relate to differences in whether SREBPs and XBP1(S) are activated by factors such as insulin signaling, versus activated by ER membrane composition. For example, ER membrane composition will also modify the final outcome of XBP1(S) and SREBP signaling by repressing or augmenting the activity of newly expressed lipid synthetic enzymes via mechanisms described above (Fig 2C–F). Additionally, ER membrane composition may impact on the gene targets of XBP1(S) or SREBP-1. A conceptual example of this comes from lipin. As explained above, lipin PtdA-phosphatase activity [which, in turn, is affected by ER membrane composition (Fig 2F)] controls whether cleaved SREBP is sequestered to the nuclear periphery thus preventing its binding to SREs (Fig 3B) [27].

*ER lipid synthesis in pathology*

In addition to lipid synthesis, the ER stores calcium and processes newly translated secretory pathway proteins. These core ER functions are so tightly linked that defects in one typically cascade to impact the others; for example, reduced calcium often causes protein misfolding due to impaired function of calcium-dependent chaperones, in turn activating the UPR and thus altering lipid synthesis. This means that ER dysfunction can drive altered lipid production downstream of different primary insults. In addition, there is strong evidence that abnormal lipid synthesis is itself the primary driver of ER dysfunction in some human metabolic and obesity-related pathologies [118–124]. These focus on hepatocytes that are functionally specialized to process nutrients into storage lipids, and also have large amounts of ER dedicated to secretory pathway functions.

Hepatocytes are a cell type that upregulates TAG synthesis in response to nutrients. The TAG synthetic pathway overlaps with that of membrane GPL/GL, diverging only at the final step catalyzed by DGAT (Fig 1A). Thus, when excess nutrients elevate flux into and through hepatocyte GPL/GL synthesis pathways, the network must buffer against spillover into non-TAG branches, in order to maintain membrane composition. Indeed, under normal conditions, the fatty acyl chain profile of liver ER membrane lipids is unrelated to that of dietary lipids, showing segregation of membrane GPL and TAG production. However, the saturation profile of membrane GPL is altered in obese mice toward mono-unsaturated acyl chains, suggesting that pathway buffering breaks down under conditions of excess TAG production [120]. The same study also identified that hepatic ER membranes from obese animals contain excess PtdCho compared to PtdEtn. This occurred alongside elevated expression of CCT$\alpha$, together with the PEMT enzyme (Fig 1A). The study then elegantly examined whether the abnormal ratio between PtdCho and PtdEtn had a role in the pathology of obese mice. This was done via *Pemt* knockdown, which halts PtdEtn to PtdCho interconversion and thus specifically reduces PtdCho relative to PtdEtn. This genetic manipulation not only corrected the PtdCho/PtdEtn imbalance, but also reduced ER dysfunction in the mice including restoring the function of the SERCA calcium pump that has a well-characterized sensitivity to ER membrane composition [125] (Fig 3C).

This study and others [119–124] have demonstrated that increased hepatic lipid synthesis is a primary driver of liver pathology in metabolic diseases related to excess food intake. Along these lines, genetic knockout of certain elongase and desaturase enzymes reduces obesity-related phenotypes in mice, implicating abnormal acyl chain incorporation into lipids as harmful [126]. Indeed, the interconnected nature of ER functions gives rise to a model where a vicious cycle of abnormal or excess lipid synthesis impacts the calcium and protein folding functions of the ER that, in turn, further amplify or alter lipid synthesis through membrane composition and/or UPR activation. There is also a strong correlation between hepatic ER pathology and the development of systemic metabolic defects such as hyperglycemia and insulin resistance, because of the central role of the liver in controlling circulating lipids. For example, the *Pemt* knockdown described above rescues systemic abnormalities in parallel with restoring normal hepatic ER membrane composition [120], clinical data correlate specific acyl chain TAG profiles with type 2 diabetes [127], and genetically reducing lipin degradation in mice (thereby allowing SREBP suppression) reduces high-fat-diet liver pathology and circulating insulin levels [111]. Thus, abnormal ER lipid synthesis contributes to the pathology of obesity, which explains the clinical benefit of drugs that regulate lipid enzymes such as statins (HMGCR inhibitors) as well as inhibitors of TAG production [128–131].

**Box 2: In need of answers**

(i) How are lipid synthetic enzymes spatially organized in the animal cell ER, including large and/or polarized cell types such as a neuron, and how does enzyme localization impact on synthetic pathway activity?
(ii) What regulatory mechanisms control ER-localized sphingolipid synthesis, and is there overlap with cholesterol and GPL/GL regulation?
(iii) Fatty acid elongases and desaturases also reside in the ER membranes, and there are examples from yeast where they are regulated by membrane-sensing mechanisms [50]. In animal cells, they are targeted by signaling pathways such as SREBP-1, but there is relatively little known about how ER membrane composition affects these enzymes or, in turn, the overall regulation of acyl chain diversity [126].
(iv) What underlies cell context-dependent effects of regulatory mechanisms, including how XBP1(S) or SREBP-1 activation causes different events under different conditions?
(v) The development of pharmacology that inhibits specific lipid enzymes might allow for their functional dissection (without relying on genetics). In addition, these may represent new therapies to test in lipid metabolism-associated pathologies.
(vi) The role of abnormal ER lipid metabolism in hepatic pathology is established. However, its contribution to other human pathologies, including those with ER dysfunction, remains unclear.

## Conclusions and perspectives

More than 60 years of work has identified and biochemically characterized the enzymatic networks of lipid synthesis. The field is

rapidly progressing on structural and biochemical dissection of enzyme function and regulation, as well as moving forward into the cell biology of how enzymes are spatially organized and regulated, and how synthesis adapts to developmental, physiological, and pathological circumstances. One example is the progress describing the lipid environment of the ER membrane as a central regulator of enzyme activities, including that interactions between lipid and protein are being modeled and solved at a structural level. However, it also remains experimentally difficult to characterize ER membrane lipid composition in different cell types and conditions, in part because classic density-based purification of ER membranes is itself altered by membrane lipid composition. Potentially, exploitation of click-chemistry reporters of lipid synthesis, microscopy readouts of membrane biophysics, and/or affinity-based membrane purification methods will promote our understanding of ER lipid synthesis and regulation beyond hepatocytes from where the majority of our information still derives. This is particularly important given that many human diseases feature ER dysfunction [132], and thus, there is a possibility that pathogenically altered lipid synthesis is a broadly useful therapeutic target beyond the types of liver disease where this is already established.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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