Understanding the diversity of membrane lipid composition

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Abstract | Cellular membranes are formed from a chemically diverse set of lipids present in various amounts and proportions. A high lipid diversity is universal in eukaryotes and is seen from the scale of a membrane leaflet to that of a whole organism, highlighting its importance and suggesting that membrane lipids fulfil many functions. Indeed, alterations of membrane lipid homeostasis are linked to various diseases. While many of their functions remain unknown, interdisciplinary approaches have begun to reveal novel functions of lipids and their interactions. We are beginning to understand why even small changes in lipid structures and in composition can have profound effects on crucial biological functions.

Stereoisomers
Isomeric molecules that have the same molecular formula but different structures.

Membrane contact sites
Regions where two organelles are in close proximity, typically at a distance of less than 30 nm, where nonvesicular exchange of lipids is proposed to occur.

Lipid mediators
Lipids with signalling functions, such as the eicosanoids, which are derived from arachidonic acid released from the membrane and serve as ligands for their receptors.

Lipid droplets
Organelles where the excess of triglycerides, cholesteryl esters and acylceramides is stored.

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The lipid bilayer provides a functional barrier between subcellular compartments and between the cell and its environment. The requirements for barrier functions are not enough to explain the enormous degree of structural diversity of lipids (FIG. 1), which ranges from subtle differences (for example, position of a double bond in the acyl chain) to major ones (for example, different backbones). The numerous genetic diseases associated with mutations in enzymes involved in lipid metabolism, remodelling and modification (collectively referred to as lipid-related enzymes) and in lipid and lipid precursor transporters[1,2] demonstrate the multiple functions of lipids in physiology (see Supplementary information S1(table)). Lipids have several major functions in cells, including as membrane structural components[3], energy and heat sources[4], signalling molecules[5], protein recruitment platforms[6] and substrates for post-translational protein–lipid modification[7]. One can imagine that signalling lipids have diverse structures to mediate specific ligand–receptor interactions; however, the reasons for diversity in membrane structural lipids are less obvious. Understanding the reasons for this diversity is a fundamental challenge in biology.

We can define two types of diversity in lipids. First, chemical diversity, which is the diversity of chemical structures of cellular lipids, including many stereoisomers. Second, is compositional diversity (ratio of different lipids), which is seen over various scales: between species[8–10]; between tissues and/or cells within an organism[9,11,12]; between different organelles[3,13]; and between membrane leaflets and even membrane subdomains[3,14]. Chemical diversity confers specific properties on lipids[11,13], and compositional diversity affects the collective behaviour of lipids in membranes[3,14], dependent upon lipid–lipid and lipid–protein interactions. Therefore, it is important to understand how these two types of diversity are acquired and how lipid structures and their local composition are translated into functions.

Although the mechanisms whereby lipids exert their functions have been less clear than those of proteins, the identification of many lipid-related enzymes[11,15], the extensive use of lipidomics[5,16] and combinatory approaches to analyse lipid functions[12,17–22] have enabled a better understanding of how and for what purpose structural and compositional diversities of lipids are generated. Clearly, membrane lipid composition changes dynamically[23,24] while also being under homeostatic control[25,26], and misregulation of this homeostasis causes various diseases[2]. In this Review, we discuss recent findings about how chemical and compositional diversities are generated, sensed, adapted and maintained and how different lipid structures affect biological functions. We focus on membrane lipids and discuss how a small difference in lipid structure (a double bond, one atom or a slightly longer hydrophobic chain length) can generate differences in function. Aspects such as lipid transport between membrane compartments[27], the role of membrane contact sites[28], lipid mediators released from the membrane[5,29], lipids as an energy source[4], lipid droplets[30,31] and protein lipidation[7] will not be covered here in detail, although these are also exciting areas of investigation in lipid biology.

Introduction to lipid diversity

The physicochemical properties of lipids rely on their chemical structures. Therefore, it is crucial to first understand the extent of the chemical diversity of lipids and how membranes differ in lipid composition before addressing the biological consequences of lipid diversity.

Variety of building blocks and their combinations leads to chemical diversity. The major membrane lipids are classified into glycerophospholipids (GPLs; FIG. 1a), sphingolipids (FIG. 1b) and sterols (mainly cholesterol in mammals; FIG. 1c)[3]. Combinations of the various building blocks shape the chemical diversity of GPLs and sphingolipids. Fatty acids (FIG. 1d) found in GPLs and sphingolipids vary in chain length, double bond number, double bond position and hydroxylation. The chemical diversity of GPLs arises from the combinations of the two fatty acids, the linkage at the *sn*-1 position and the head group (FIG. 1a). The *sn*-1 fatty acid tends to be saturated (without double bonds) or monounsaturated (one double bond), whereas the *sn*-2 fatty acid is more often monounsaturated or polyunsaturated (multiple double bonds)[9]. Two examples of GPLs with less heterogeneity are phosphatidylinositol (PtdIns), which in most tissues incorporates predominantly stearoyl and arachidonoyl-acyl chains (18:0 and 20:4; of note, in the XX:Y nomenclature of fatty acids, XX denotes the number of carbons in the chain, whereas Y indicates the level of chain desaturation (the number of double bonds)) and phosphatidylserine (PtdSer), which tends to have stearic acid at the *sn*-1 position[9,32]. Sphingolipid chemical diversity arises from the variety in the length and type of the sphingoid base, *N*-acyl chain and head group (FIG. 1b). The *N*-acyl chains of sphingolipids tend to be more saturated and can be longer than the acyl chains of GPLs[11]. Therefore, GPLs tend to be unsaturated and sphingolipids saturated; however, many exceptions to this pattern can be found. Another degree of diversity is obtained from head group phosphorylation of PtdIns or from the complexity of oligosaccharides found in glycosphingolipids (GSLs). These various head groups act as 'codes' (FIG. 1e) mediating various biological functions, mostly through specifying lipid–protein interactions[33,34]. Sterols, oxysterols and their derivative bile acids are also diverse in their structures and functions[35]. Further details about phosphoinositides[33], complex GSL[34] and sterols[35] can be found elsewhere.

Compositional diversity of membranes. Compositional diversity is well exemplified by fatty acids found in GPLs[9,12,32] (BOX 1) or the *N*-acyl chains of sphingolipids[11,36–38], which differ largely between tissues and are required for specific tissue functions. At the subcellular scale, the endoplasmic reticulum (ER) contains low cholesterol and more unsaturated GPLs, whereas cholesterol and sphingolipids are abundant in the plasma membrane[3,13]. Extreme examples of differences between organelles are the organelle-specific lipids such as cardiolipin in mitochondria[39] or the lysobisphosphatidic acid (LBPA; also known as bis(monoacylglycero)phosphate) in late endosomes[40,41]. To mention a few examples of suborganellar compositional diversity, the composition of basolateral and apical plasma membranes of polarized cells is different[42] and plasma membrane nanodomains are enriched in cholesterol, sphingolipids and probably PtdSer[14,43]. In addition, membrane leaflets are asymmetric; for example, PtdSer is almost exclusively found in the cytoplasmic leaflet of the plasma membrane (except in special cases such as apoptosis or platelet activation)[44,45].

Generating lipid diversity

FIGURE 2 illustrates the enzymatic pathways involved in membrane lipid metabolism, with information on enzyme localizations (based on gene ontology[46]; full list in Supplementary information S2 (table)). Molecular identification of these enzymes advanced dramatically in recent decades, and now at least one gene is attributed to almost all steps, with a few exceptions (such as some steps in plasmalogen and LBPA synthesis).

Characteristics of the metabolic map of lipids. Three characteristics of this metabolic map may explain the generation of lipid chemical and compositional diversities, including promiscuity, preference and redundancy. Lipid-metabolizing enzymes are often promiscuous, utilizing a broad range of more or less similar substrates[15,47]. This promiscuity diversifies lipid structures, because fewer enzymes combine a wider range of building blocks (for example, few enzymes are sufficient to generate GPLs and sphingolipids with various acyl-chain combinations). In parallel, despite their promiscuity, enzymes have individual substrate preference, generating different ratios of the different products. Because many steps of lipid metabolism utilize redundant enzymes with different preferences, diverse lipid compositions are generated depending on the expression levels of enzymes. Large differences in enzyme expression patterns are seen between tissues (provided in Supplementary information S3 (table)), contributing to tissue compositional diversity. However, as reviewed for ceramide synthases[48], tissue-specific transcriptional regulation has only been partially studied. Projects such as the ENCODE Project (Encyclopaedia of DNA Elements)[49] provide enormous data sets about transcription regulatory elements, the analysis of which might provide mechanisms of tissue-specific enzyme expression patterns (Supplementary information S4 (figure)).

Because mammals cannot synthesize polyunsaturated fatty acids (PUFAs) (with the exception of mead acid[50]), dietary supply is required and affects cellular lipid composition (FIG. 2a). Fatty acids are first incorporated in GPLs during synthesis of the common precursor, phosphatidic acid (PtdA; FIG. 2b). Once synthesized, GPLs undergo dynamic modification of their acyl chains, called fatty acid remodelling (FIG. 2c; details in REFS 9,15). The diversity in the composition of phosphatidylcholine (PtdCho)[12,18] and the homogeneity of PtdIns[51,52] across tissues is generated by the complex relationship of various enzyme steps, which illustrate well the three characteristics of lipid metabolism described above (see also BOX 1). Ceramides, the precursors of complex sphingolipids, are synthesized by redundant ceramide synthases that transfer acyl chains to sphingoid bases with various preferences, contributing to compositional diversity of sphingolipids[11,36] (FIG. 2d).

Lipid synthesis is compartmentalized over multiple organelles, contributing in part to the localization of lipids, such as the enrichment of sphingolipids in the plasma membrane[29]. Compartments exist even at the suborganellar scale, as shown by the enrichment of many lipid-related enzymes in ER–mitochondria membrane contact sites[53]. A novel ER subdomain containing

Protein lipidation
A post-translational modification of proteins encompassing a covalent attachment of a lipid.

Sphingoid base
The structural backbone of sphingolipids, which also acts as one of the hydrophobic chains.

Cardiolipin
A mitochondria-specific (in mammals) glycerophospholipid with four acyl chains, the malfunction of which is involved in Barth syndrome.

Membrane nanodomains
Lateral heterogeneities in membranes, often very small and dynamic, where lipids are postulated to have important roles affecting membrane properties and function.

Plasmalogen
Glycerophospholipids with a vinyl-ether bond at the *sn*-1 position, which depend upon peroxisomes for their synthesis.

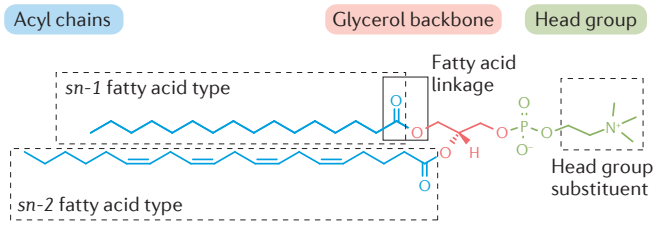
Promiscuity
In terms of enzymology, the ability of an enzyme to utilize a broad range of substrates.

Redundancy
A situation where different molecules (for example, enzymes) have (at least partially) overlapping functions.

ENCODE Project
(Encyclopaedia of DNA Elements). An international collaboration with the objective of comprehensively elucidating functional elements in the human genome.

Mead acid
A polyunsaturated fatty acid (20:3 n-9) that can be synthesized endogenously in mammals, which is produced under polyunsaturated fatty acid insufficiency.

a GPL diversity



GPL	Head group substituent
Phosphatidic acid	–
PtdCho	Choline
PtdEtn	Ethanolamine
PtdSer	Serine
PtdIns	Inositol
PtdGro	Glycerol
Cardiolipin	PtdGro
LBPA	LPA
PtdGlc	Glucose

Fatty acid linkage

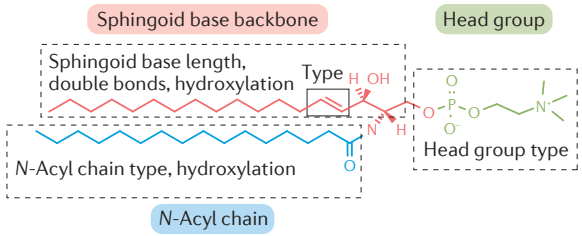
Ester (acyl)

Ether GPLs

Ether (alkyl)

Vinyl-ether (alkenyl)

b Sphingolipid diversity



Sphingoid base type

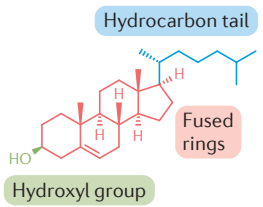
DHS

SPH

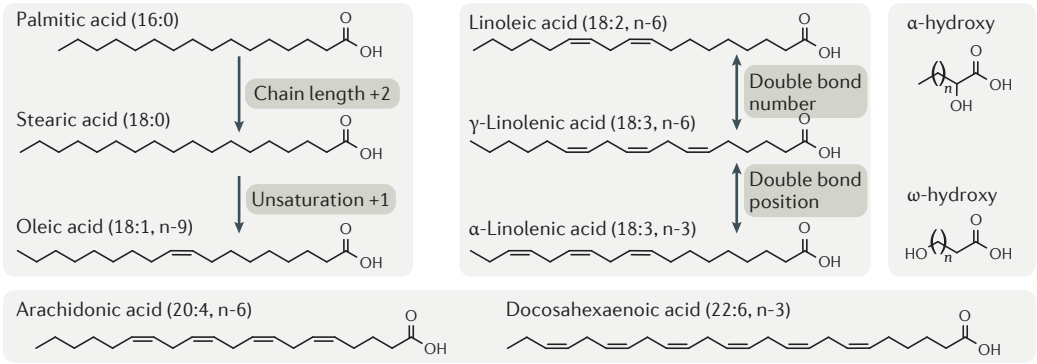
PHS

Sphingolipid	Head group
Cer	Hydroxyl
Sphingomyelin	Phosphocholine
CerPE	Phosphoethanolamine
GlcCer	Glucose
GalCer	Galactose
Complex GSLs	Oligosaccharides
C1P	Phosphate

c Cholesterol

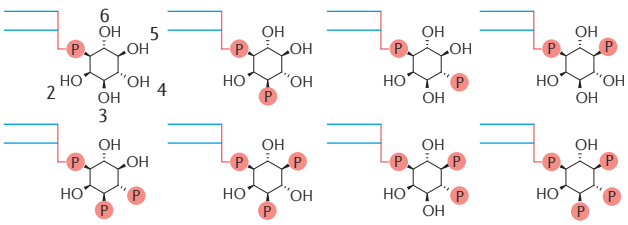


d Fatty acid diversity



e Head group "code"

Phosphoinositides



Glycosphingolipids

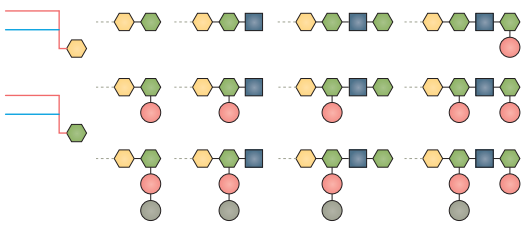


Figure 1 | **Chemical diversity of membrane lipids in mammals.** **a** | Glycerophospholipids (GPLs) have a glycerol backbone with fatty acids at the *sn*-1 and *sn*-2 positions. The head group consists of a phosphate and an alcohol, which defines the GPL name. Ether-GPLs are GPLs with ether or vinyl-ether linkage at the *sn*-1 position. Boxed parts of the GPL structure represent building blocks that confer diversity (same for part **b**). Cardiolipin and lysobisphosphatidic acid (LBPA) have acyl chains in their glycerol head group substituent; thus, they cannot be accommodated by the illustrated structure. **b** | Sphingolipids consist of a sphingoid base (which is simultaneously the backbone and a hydrophobic tail), an *N*-acyl chain and a head group. Hydroxylation and unsaturation define the sphingoid base type, whereas the head group defines the sphingolipid name. **c** | The major mammalian sterol, cholesterol. **d** | Fatty acids differ in chain length, level of unsaturation and the position of double bonds, illustrated as (XX:Y, n-Z), where XX, Y and Z are carbon number, double bond number and the position of the first double bond from the omega end, respectively. Fatty acids can be hydroxylated. **e** | Head group phosphorylations at various positions of phosphatidylinositol (PtdIns) generate the phosphoinositides. Complex glycosphingolipids (GSLs) have diverse oligosaccharides as head groups. The different shapes and colours of the designed GSL structures are only to note that their structures are composed of various building blocks (mainly sugars) in different constellations. C1P, ceramide 1-phosphate; Cer, ceramide; CerPE, ceramide phosphoethanolamine; DHS, sphinganine; GalCer, galactosylceramide; GlcCer, glucosylceramide; LPA, lysophosphatidic acid; PHS, 4-hydroxy-sphinganine; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGlc, phosphatidylglucoside; PtdGro, phosphatidylglycerol; PtdSer, phosphatidylserine; SPH, sphingosine.

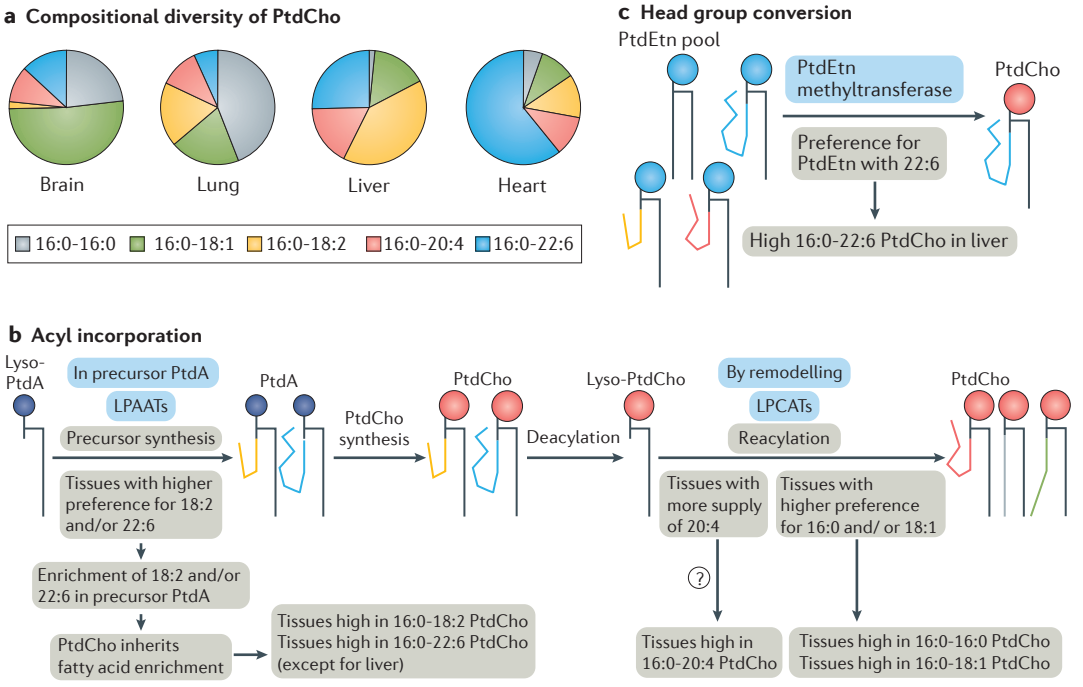
Omega end
In fatty acid nomenclature, the end of a fatty acid that has a methyl group. The other end with a carboxyl group is called the alpha end.

enzymes for PtdIns synthesis[54] and remodelling[55] (and many other lipid-related enzymes[56]) was recently identified, which is involved in ER dynamics[57], local synthesis of phosphoinositides[55], their replenishment in other pools upon stimulation[54] and autophagy[56]. Discoveries of novel subdomains warrant the need to establish more precise localization data of known lipid-related enzymes in order to understand the consequences of localized lipid synthesis for lipid compositional diversity and downstream biological functions.

Diversity of lipids is modulated by metabolic bias. Strong substrate preference of lipid-related enzymes and lipid transporters leads to biased metabolism of certain lipids, depending on the supply of the preferred substrate and the expression and regulation of these enzymes and transporters (for example, favouring certain isoforms over others)[58]. If this bias occurs at a step where metabolites diverge (for example, downstream of PtdA; see FIG. 2b,c), only a subset of substrates that have a certain signature (for example, a certain composition of acyl chains) will feed efficiently into the subsequent enzymatic reactions, thereby fuelling selected metabolic pathways. This metabolic bias implies that the specific composition of lipid species in a class of lipids, and not necessarily only the total amount of lipids in that class, might drastically affect the generation of downstream metabolites. An example of such

Box 1 | **Regulation of glycerophospholipid acyl-chain composition**

Fatty acids at the *sn*-2 position of phosphatidylcholine (PtdCho) differ largely between tissues (ratios of five PtdCho species in mouse tissues[12] are shown in figure, part **a**). Acyl-chain incorporation occurs at two different steps[15]: during *de novo* synthesis of the precursor phosphatidic acid (PtdA) from lysophosphatidic acid (lyso-PtdA) by lysophosphatidic acid acyltransferases (LPAATs) and during fatty acid remodelling, catalysed by lysophosphatidylcholine acyltransferases (LPCATs; see figure, part **b**). Because enzymes that catalyse these reactions are expressed as redundant isoforms that have different substrate preferences[15] and are characterized by tissue-specific transcriptional regulation (and in consequence variable tissue distribution; see Supplementary information S4 (figure)), fatty acid incorporation at each step differs between tissues. It was shown that both steps affect tissue distribution of fatty acids in PtdCho, with different contributions depending on the fatty acid at the *sn*-2 position[12]. Although remodelling by LPCATs is important to enrich palmitic (C16:0), oleic (C18:1) and arachidonic acids (C20:4)[12,18], the substrate preference of LPAATs regulates the tissue distribution of linoleic acid (C18:2) and docosahexaenoic acid (DHA; C22:6)[12,91,92]. Palmitic and oleic acid enrichment in specific tissues is caused by LPCAT substrate preference, whereas arachidonic acid cannot be similarly explained. One might speculate that the arachidonate supply differs between tissues. In the liver, DHA is enriched in PtdCho through the biased conversion from DHA–phosphatidylethanolamine (PtdEtn) by PtdEtn methyltransferase[61] (figure, part **c**). Therefore, the acyl-chain composition of PtdCho (and possibly other glycerophospholipids) is regulated by a complex contribution of substrate supply, enzyme preference during precursor synthesis, acyl-chain remodelling and head group conversion. Apparently, there is no single PtdCho species, levels of which would depend on only one of these contributions. Although the description here ignores factors such as diet or degradation, it seems to explain the major contributors to PtdCho compositional diversity. Phosphatidylinositol (PtdIns) is remodelled at both the *sn*-1 and *sn*-2 positions, but only single enzymes per position were identified to date, in contrast to PtdCho[51,52]. The absence of redundancy could explain the homogeneity of PtdIns composition, in addition to the metabolic bias effect during the PtdIns cycle (in text).



a bias is seen in the PtdIns cycle, which is a PtdIns head group remodelling sequence (FIG. 2c), with all the intermediates containing mostly 18:0 and 20:4 acyl chains, similar to PtdIns[9,58]. Multiple enzymes of this cycle are selective for 18:0–20:4 acyl-chain-containing species[59], and many of the intermediates of this cycle have signalling functions that require these acyl chains. For example, 18:0–20:4 diacylglycerol (DAG) induces calcium responses, whereas other species do not[60]. Therefore, the 18:0–20:4 signature of PtdIns seen in most tissues is beneficial for preventing intermediates from exiting the PtdIns cycle into other metabolic pathways.

The concept of metabolic bias suggests that enzyme malfunctions can generate metabolites that are not optimal substrates for subsequent enzymes or pathways, leading to phenotypes caused by insufficiency or overaccumulation of downstream lipids. Phosphatidylethanolamine (PtdEtn) methyltransferase, which converts PtdEtn into PtdCho, regulates incorporation of docosahexaenoic acid (DHA; 22:6 n-3) into PtdCho through metabolic bias[61] (BOX 1). Despite its liver-specific expression, loss of this enzyme affects brain lipid composition and function[62], likely because the brain takes up DHA from the blood only when esterified to lyso-PtdCho[63,64], which may originate from the liver. Metabolic bias might be present in various pathways. Differences in *N*-acyl chains between sphingolipid classes[65] suggest that biosynthetic enzymes for each complex sphingolipid have preferences for different ceramides, where the same concept of metabolic bias might apply. Indeed, RNAi knockdown of different ceramide synthases to change the ratio between different ceramide species led to various quantitative changes in downstream sphingolipids[65], suggesting that *N*-acyl chain diversity in ceramides partially regulates downstream sphingolipid levels through metabolic bias.

Membrane lipids in health and disease

The importance of lipid composition is clear from the number of genetic diseases that are related to lipids (provided in Supplementary information S1 (table)). Most of these diseases result from mutations in specialized pathways, rather than the key steps of GPL synthesis, as the consequences of mutations in the latter are too severe. Identifying enzymes involved in lipid metabolism uncovers novel disease mechanisms, as witnessed by the identification of a link between sphingolipid degradation and the genetic disease Sjögren–Larsson syndrome[66].

Genetic diseases related to ether lipid synthesis (mutations in *GNPAT*, *FAR1* or *AGPS*) and sphingolipid synthesis (mutations in *SPTLC1* or *SPTLC2*) show the importance of small differences in lipid chemical structures. Ether lipids differ only in the *sn*-1 fatty acid linkage, yet their deficiency leads to severe diseases[67,68]. Serine palmitoyltransferase 1 (SPTLC1) and SPTLC2 catalyse the first step for sphingolipid synthesis, and mutations that make them less selective for the main substrate serine (in favour of glycine or alanine incorporation) cause hereditary sensory and autonomic neuropathy[69]. The use of alanine instead of serine leads to the production of toxic 1-deoxysphingolipids, which differ only by the lack of one oxygen atom and therefore cannot be degraded or converted to complex sphingolipids[70]. The exact mechanisms of how these lipid changes lead to disease remain unclear.

In addition to single-trait heritable diseases, correlations between lipidome changes and disease are often seen[71] (BOX 2). Ceramides are increased in diseases such as type 2 diabetes, cancer, Alzheimer disease and cystic fibrosis[11,72]. Some studies revealed that a higher level of DHA in plasma-isolated PtdCho leads to lower risk of dementia[73]. Changes in PtdCho acyl chains are also found in many cancers[74]. It will be crucial to understand whether these lipidome changes are causal, and if so, by which molecular mechanism (BOX 2). From the symptoms of genetic diseases and the phenotypes of knockout animals, we identify new lipid functions, but not necessarily the exact mechanism. For lipids for which the known function relates to their physical properties, explaining phenotypes can be fairly straightforward. Dipalmitoyl-PtdCho (16:0–16:0) is the major component of pulmonary surfactant, which reduces surface tension of the airway epithelium to assist breathing[12]. As expected, reduced dipalmitoyl-PtdCho leads to partial neonatal lethality[75] and susceptibility to lung injury[12]. In skin, ω-*O*-acylceramides are extremely important for maintaining barrier functions[38]. Defects of ω-*O*-acylceramide synthesis cause the loss of skin barrier functions in mice[76–78] and the skin disease autosomal recessive congenital ichthyosis in humans[79].

However, in most cases how aberrant lipid composition leads to disease and phenotypes is unclear. Human patients and knockout mice with abnormal acyl chains in PtdIns develop brain malfunctions[52,80], the mechanisms of which remain unknown. Similarly, knockout mice of different ceramide synthases have distinct phenotypes[36], which suggests that sphingolipid functions are affected by their *N*-acyl chains, but how this difference emerges is still mysterious[11]. These problems are partly due to the inherent difficulties of studying lipids, for which highly multidisciplinary approaches are needed, but also because disrupting metabolic pathways, such as lipid biosynthesis, can lead both to loss of the expected product and to accumulation of substrates and other unexpected products (likely through the mechanisms involving metabolic bias discussed above).

Functional implications

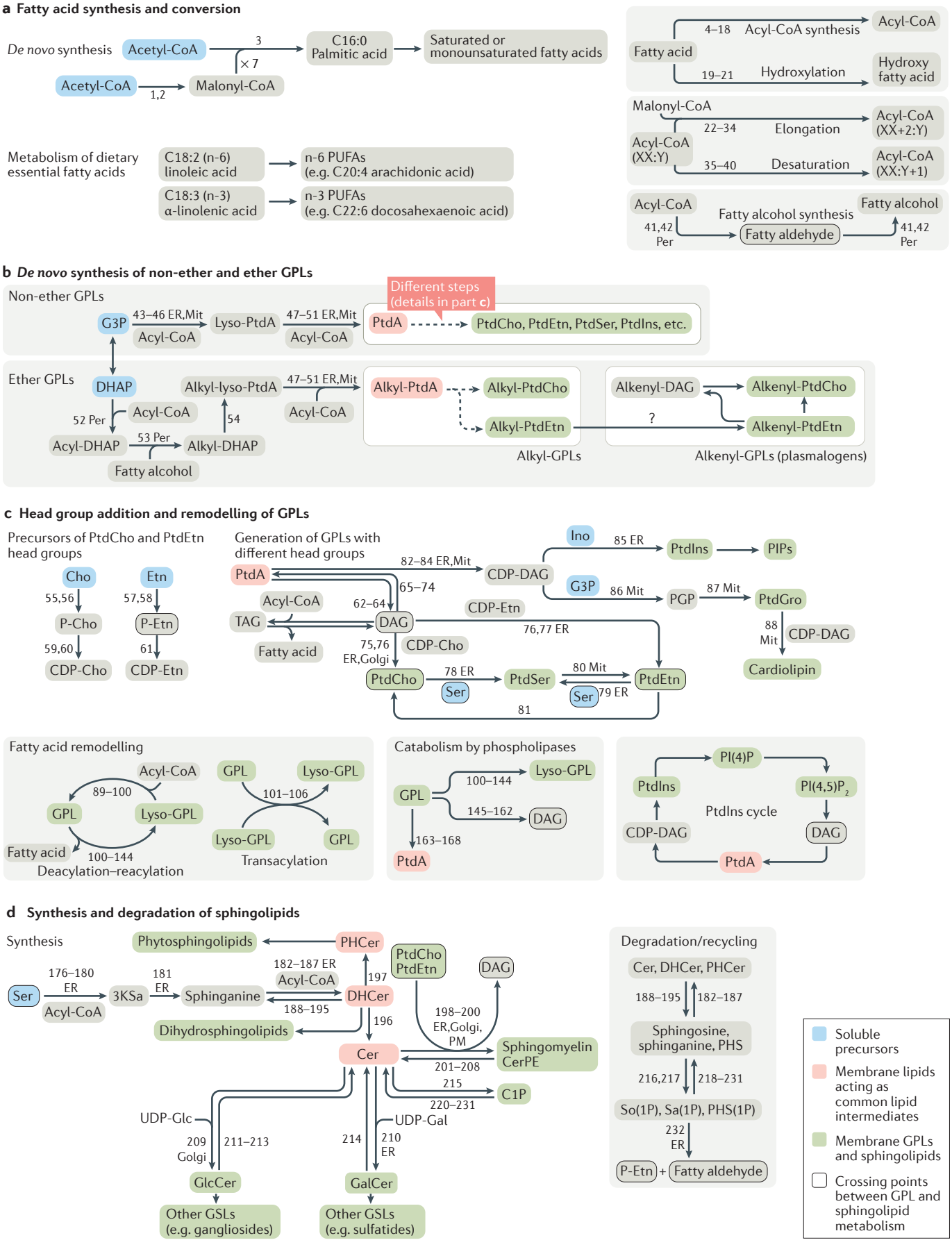
As a comprehensive understanding of how lipids affect biological functions is lacking, we need to draw conclusions from a limited number of examples. Changes in membrane lipid composition will inevitably result in changes in membrane properties. Lipids can also affect the activity of both integral membrane proteins and non-membrane proteins that recognize specific lipid species and function as effectors in other processes.

Modulation of membrane physicochemical properties by lipids. One way that lipids execute their functions is through modulating membrane physicochemical properties.

Sjögren–Larsson syndrome
A genetic disease with skin and neurological problems, caused by mutations in a fatty aldehyde dehydrogenase involved in sphingolipid degradation.

Hereditary sensory and autonomic neuropathy
A genetic disease affecting the nervous system characterized by a loss of pain sensation, among other symptoms.

ω-*O*-acylceramides
Ceramides with another fatty acid *O*-esterified at the ω-end of the *N*-acyl chain.



SNARE
A group of proteins involved in membrane fusion.

Osteoclast
Multinucleated cell type generated by cell fusion that has a role in bone resorption.

Liquid-ordered domains
A state of membrane lipids where hydrophobic chains are ordered but lateral diffusion is still high.

Sertoli cells
Testicular cells that assist spermatogenesis.

Ferroptosis
A non-apoptotic cell death triggered by overaccumulation of peroxidized lipids.

The relative size of the head group and hydrophobic tails of lipids affects the shape of the lipid and the spontaneous curvature of the membrane[81] (FIG. 3a). The negative spontaneous curvature of PtdEtn leads to bilayer-disrupting properties, which might promote processes that involve the generation of non-bilayer membrane intermediates, such as fusion. Indeed, the importance of conical lipids has been shown *in vitro* for SNARE-mediated fusion[82] and in cells for osteoclast fusion[83]. Local deacylation–reacylation to interconvert lyso-PtdA and PtdA, which largely differ in spontaneous curvature, regulates Golgi membrane fission[84]. Therefore, lipid composition might be regulated to assist membrane deformation through spontaneous curvature.

Lipids with long and saturated fatty acids (for example, sphingolipids) make membranes thicker and less fluid owing to the tight packing of their hydrophobic tails and stronger lipid–lipid interactions[3,14]. Unsaturated lipids do the opposite because acyl-chain kinks prevent tight packing. In artificial lipid membranes, saturated lipids, unsaturated lipids and cholesterol separate into phases depending on their ratio, generating regions with high lipid packing (liquid-ordered domains) and less lipid packing (liquid-disordered domains)[3] (FIG. 3b). This local phase separation is the underlying principle behind the highly debated lipid raft hypothesis, postulating lateral heterogeneities in the plasma membrane that show high lipid order and the ability to concentrate 'raftophilic' proteins[14,85]. Recent results suggest that proteins (for example, B cell receptors or cytoskeletal components) initiate the formation of membrane heterogeneities on the nanoscale level and that lipids have roles in the stabilization of these nanodomains and their expansion[43,86]. Because these protein-initiated nanodomains differ in composition depending on the initiating protein, local, specific lipid–protein and lipid–lipid interactions might better explain plasma membrane heterogeneity than lipid-initiated phase separation (detailed discussion in Supplementary information S5 (box)).

As examples of lipid–lipid interactions (FIG. 3c), cholesterol and sphingolipids interact through hydrogen-bonding (in addition to the interaction caused by saturated hydrophobic chains), which might stabilize nanodomains[14]. Strong genetic interactions between sterol and sphingolipid metabolism are seen in yeast, which adapt to mutations in sterol metabolism-related genes by changing the sphingolipid composition[87]. This finding suggests that sterols and sphingolipids often act as functional pairs, likely owing to their interactions. Lipids also interact across the bilayer. Actin-initiated aggregation of PtdSer in the cytosolic leaflet of the plasma membrane causes clustering of glycosylphosphatidylinositol anchors in the other leaflet[43]. This interaction requires cholesterol, and lipids on both leaflets should contain at least one long and saturated acyl chain, providing a novel view of nanodomain formation, perhaps different from the previously described phase separation. This finding, together with another study showing the importance of long acyl chains for lateral interaction between PtdSer and cholesterol[88], might explain why stearic acid (C18:0) is enriched in PtdSer[9,32]. Thus, lipid–lipid interactions help nanodomain formation, but they might also be involved in other membrane-associated processes through their collective effect on membrane properties.

PUFAs decrease membrane bending rigidity[89,90] (FIG. 3d). Through this property, DHA in GPLs was shown to promote rapid endocytosis[20]. Mice with reduced membrane DHA also display male infertility and visual dysfunctions[91,92]. The former can be explained by the role of DHA in membrane shaping during sperm formation. During spermatogenesis, Sertoli cells contact spermatids and remove their excess cytosol by endocytosis through narrow, highly curved tubes called tubulobulbar complexes, the formation of which is likely compromised under DHA insufficiency owing to impaired membrane bending[91]. In hepatocytes and enterocytes, arachidonic acid (20:4 n-6) in membrane GPLs facilitates transport of triglycerides into the ER lumen, likely by generating a curved membrane that clusters triglycerides between leaflets[18] (others explain the phenotype by membrane fluidity[93]), thereby preventing cytosolic triglyceride overaccumulation. Thus, PUFA levels in GPLs are regulated to assist membrane deformation (FIG. 3d). All these examples show the importance of lipid composition for maintaining the physical properties (for example, spontaneous curvature and bending stiffness) of membranes.

Different lipids also have different susceptibility to modifications. For example, PUFAs are highly prone to oxidation. Excessive oxidation of lipids leads to an atypical type of cell death called ferroptosis, which is a

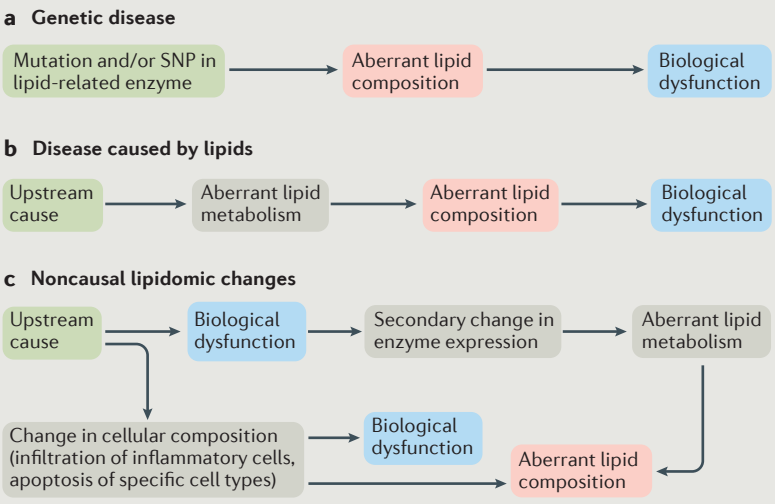
◀ Figure 2 | **Metabolism of membrane lipids in mammals.** Metabolic pathways for synthesis, conversion and degradation of fatty acids, GPLs and sphingolipids. Cofactors are omitted for simplicity. Numbers are attributed to different enzymes listed in Supplementary information S2 (table). **a** | Fatty acid synthesis and conversion. Endogenous synthesis generates saturated or monounsaturated fatty acids. PUFAs come from the diet. Elongation, desaturation, partial β-oxidation (not depicted) and hydroxylation modify fatty acids, generating their vast diversity[157]. **b** | Synthesis of GPLs with various *sn*-1 fatty acid linkage. Biosynthetic pathways downstream of alkyl-PtdA and alkenyl-DAG, generating ether GPL species, are the same as their ester counterparts. **c** | Synthesis of GPL head group precursors (for PtdCho and/or PtdEtn) and GPL remodelling. GPLs are subjected to fatty acid remodelling (different transacylases also exist[9]). Phospholipases cleave GPLs at different positions, producing lyso-GPLs, DAG or PtdA. PtdIns engages in a cycle of head group conversion. **d** | Sphingolipid synthesis and degradation depicted for Cer and Cer-derived complex sphingolipids. Of note, synthesis of complex phytosphingolipids and dihydrosphingolipids uses the same pathways as conversion of Cer into complex sphingolipids (not shown). Synthesis of most of the sphingomyelin and GlcCer begins in the Golgi compartment. 3KSa, 3-ketosphinganine; C1P, ceramide 1-phosphate; Cer, ceramide; CerPE, ceramide phosphoethanolamine; Cho, choline; DAG, diacylglycerol; DHAP, dihydroxyacetonephosphate; DHCer, dihydroceramide; ER, endoplasmic reticulum; Etn, ethanolamine; G3P, glycerol 3-phosphate; Gal, galactose; Glc, glucose; GlcCer, glucosylceramide; GPL, glycerophospholipid; GSL, glycosphingolipids; Ino, inositol; Mit, mitochondria; P-, phospho-; PGP, phosphatidylglycerol phosphate; PHCer, phytoceramide; PHS(1P), 4-hydroxy-sphinganine (1-phosphate); PI(4)P, PtdIns 4-phosphate; PI(4,5)P_2, PtdIns 4,5-bisphosphate; PIPs, phosphoinositides; $PLA_1/A_2/B/C/D$, phospholipase $A_1/A_2/B/C/D$; PtdA, phosphatidic acid; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PUFAs, polyunsaturated fatty acids; Sa(1P), sphinganine (also known as dihydrosphingosine) (1-phosphate); So(1P), sphingosine (1-phosphate); TAG, triacylglycerol.

Nuclear receptors
A family of proteins that serve as transcriptional regulators in the nucleus upon ligand binding.

potential target for cancer treatment[94]. Not surprisingly, multiple enzymes regulating membrane PUFA levels were implicated in ferroptosis[95]. PUFAs in GPLs are also oxidized enzymatically (for example, during platelet activation)[96]. Despite having strong effects on membrane physical properties[97], the biological relevance of GPLs with oxygen-modified acyl chains remains largely uninvestigated.

Box 2 | **Lipid composition and disease**

Changes in lipid composition are found in numerous diseases, such as cancer[74] or type 2 diabetes[72], but it is often unclear whether these changes are causal for the disease. For genetic diseases of lipid metabolic enzymes (Supplementary information S1 (table)), the causality is clear (see figure, part **a**). In other cases, lipid composition might be a therapeutic target only if aberrant lipid metabolism caused by some environmental or genetic factors (not necessarily connected to the metabolism of the altered lipid species) contributes to disease progression (figure, part **b**). However, in many cases, the lipidomic changes might be a symptom of the disease (figure, part **c**). Nonetheless, if the changes are specific for the disease, they serve as good biomarkers. Overall, for therapeutic approaches, lipidomic changes associated with diseases should be carefully interpreted and evaluated for causality. These therapeutic approaches can lead to potential treatments oriented to reverse the aberration of lipid composition. As an example, preventing ceramide overaccumulation in major depression animal models is beneficial for alleviating symptoms of depression in animal models, suggesting a major contribution of ceramide to the disease[154]; another example is the inhibition of glucosylceramide synthesis, which reduced hepatocellular carcinoma driven by overactive TOR complex 2 (REF. 71) (example where lipid composition changes are downstream from other pathways; see figure, part **b**). Ceramide accumulation often correlates with cellular stress[29], but ceramide species overaccumulation is unlikely to mediate all stress responses. For example, accumulation of toxic dicarboxylic fatty acids induces liver failure and is associated with increased ceramide levels. However, it is likely that treatments that reduce ceramides will only provide partial benefit for liver dysfunction, owing to other processes upstream of ceramide accumulation[155] (example for the scenario shown in figure, part **c**). To establish a strategy for the treatment of diseases caused by lipid composition, it is critical to understand the underlying molecular mechanisms. If the effect of lipid composition on membrane physical properties is causal, interventions to correct lipid composition would probably be required, either by interfering with metabolism or by delivery of lipids. In cases where proteins mediate the effect of aberrant lipid composition, protein inhibitors or activators might also be used. For example, in the case of blood–brain barrier dysfunction caused by docosahexaenoic acid insufficiency, depletion of a single protein, caveolin 1, rescued the disease in mice[156]. Therefore, when interpreting the relationship between lipid composition and diseases, not only the causality but also the mechanism linking composition to disease should be correctly understood to establish therapeutic strategies. SNP, single-nucleotide polymorphism.



Impact of lipid composition on protein function. Except for cases where lipid and membrane physicochemical properties have direct biological functions, most lipid functions are probably mediated through their ability to modulate proteins. Many lipids recruit proteins with lipid-binding domains to specific membrane compartments or membrane subdomains[6] (FIG. 4a). These domains specifically recognize lipids (for example, on the basis of their charge). The best understood lipids that engage in protein recruitment are the phosphoinositides. These lipids are dynamically regulated by phosphorylation and dephosphorylation, which allows them to recruit proteins in a concerted manner, as reviewed elsewhere[33]. PtdA, DAG and PtdSer are also known to recruit proteins[98]. The importance of PtdSer was shown for actin-initiated nanodomain formation[43]. PtdSer also affects other processes through protein binding, such as intracellular trafficking[99]. A systematic study showed that many protein–lipid interactions involve cooperative binding with multiple lipids, thereby increasing target specificity[100]. Another type of lipid–protein interaction involves lipid-binding pockets, which extract lipids from the membrane and incorporate them (FIG. 4a). Some nuclear receptors bind specific lipids with a defined chemical structure and act as transcription factors[101]. For example, PtdCho acts as a ligand for peroxisome proliferator-activated receptor-α (PPARA) or liver receptor homologue 1 (LRH1; also known as NR5A2) (although PtdCho is not the only ligand), which recognize only PtdCho that have specific acyl chains[102,103]. Lipid transfer proteins[27] also use lipid-binding pockets to regulate lipid localization and downstream functions. Interestingly, some lipid transfer proteins transport lipids by counterexchange, where two different lipids are transferred in the opposite direction between membranes[104]. This counterexchange mechanims implies that the aberrant composition of a lipid can affect the localization of the other counterexchange partner lipid, which is seen when the phosphatidylinositide phosphatase SAC1 (SACM1L) is lacking, causing aberrant distribution of PtdSer in response to increased PtdIns 4-phosphate[105]. Therefore, lipid levels are maintained not only to preserve their own functions but also the functions of other lipids that are counterexchanged.

Several classes of proteins interact with the membrane by sensing membrane properties[106] (FIG. 4b). The BAR-domain-containing proteins[107] are well-characterized curvature-sensing proteins. Lipid composition, which affects both the deformability and the intrinsic curvature of the membrane, may thus affect the recruitment of BAR domains. It was recently shown that upon cholesterol depletion, increased density of PtdSer in the plasma membrane induces curvature owing to repulsive forces between the charged head groups[108]. This curvature then facilitates recruitment of the BAR-domain-containing protein endophilin, showing that lipid composition indeed influences recruitment of BAR domains. Another important membrane property is called a 'packing defect', meaning the degree of exposure of membrane hydrophobic regions to the aqueous environment[13] (FIG. 4b). Such defects are caused by both membrane bending and

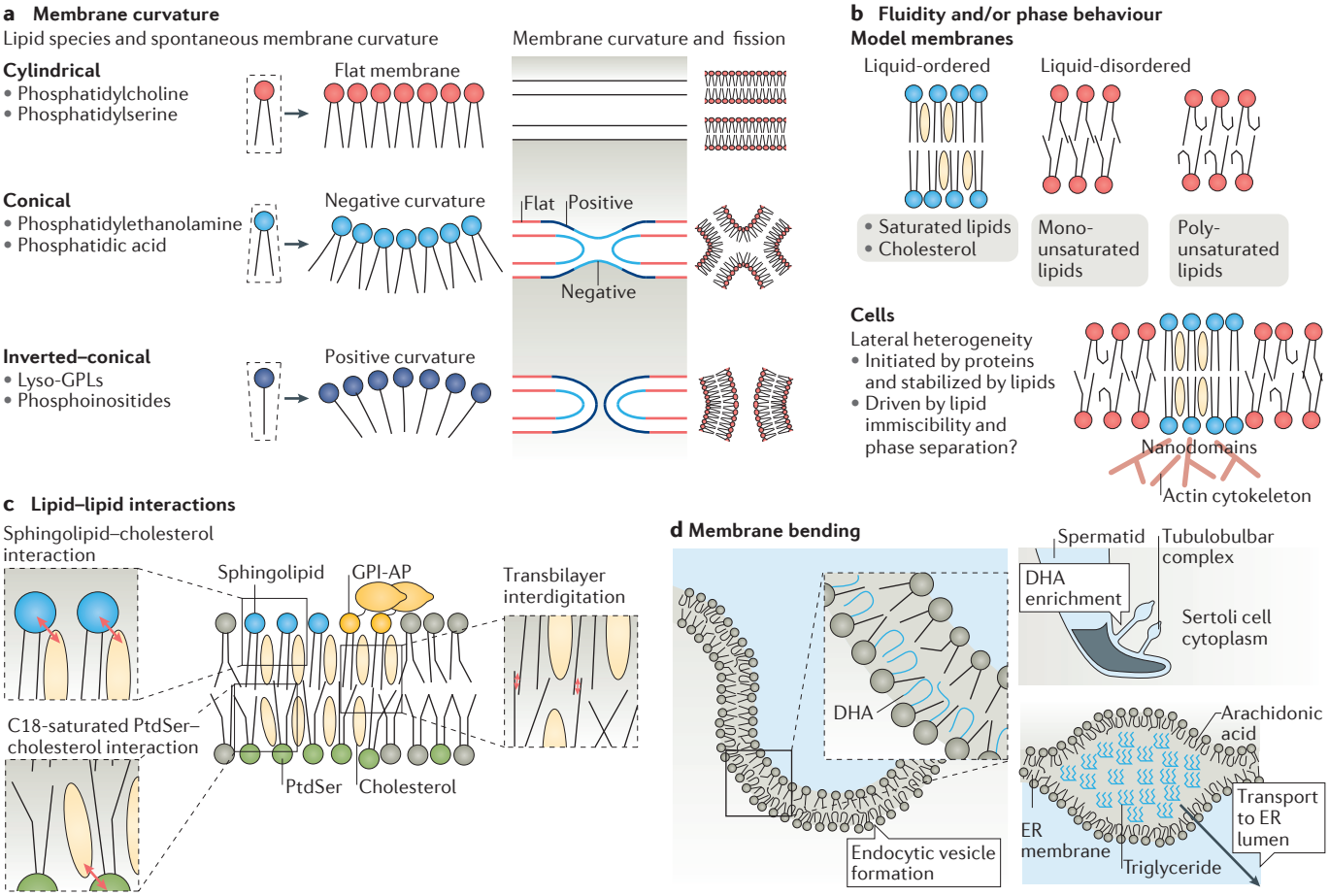


Figure 3 | **Lipids regulate biological processes through membrane properties. a** | The size balance between the head group and hydrophobic tails affects membrane spontaneous curvature. During membrane remodelling processes such as fusion and fission (which are initiated by proteins), non-bilayer intermediates and dynamic changes in curvature occur (illustrated by colours) where such spontaneous curvature might be important for membrane remodelling. **b** | Unsaturation in acyl chains increases membrane fluidity; thus, the level of unsaturation of lipids in the membrane might affect its organization. Saturated lipids and cholesterol generate liquid-ordered phases, and unsaturated lipids generate liquid-disordered phases, at least *in vitro*. Gel phases generated by pure saturated lipids are not depicted. Lateral heterogeneities in membrane fluidity generating distinct nanodomains are likely to exist in cells, although the mechanisms generating them are under debate. Recent results suggest that protein-initiated nucleation of these domains occurs first, followed by their stabilization through protein–lipid and lipid–lipid interactions (see also Supplementary information S5 (box)). **c** | Lipid structures affect lipid–lipid interactions occurring laterally or across leaflets, which affects physical properties and lateral heterogeneity in membranes. The chain length of phosphatidylserine (PtdSer) is proposed to affect its lateral interaction with cholesterol and transbilayer interdigitations (for example, with glycosylphosphatidylinositol-anchored proteins (GPI-APs)). **d** | Polyunsaturated fatty acids in glycerophospholipids (GPLs) reduce membrane bending rigidity, which might help cellular processes where membrane deformation occurs, such as endocytosis (left panel). Highly bent membranes facilitated by polyunsaturated GPLs also occur under various other circumstances. For example, during spermatogenesis, the excess cytosol of spermatids is removed by surrounding Sertoli cells through the tubulobulbar complexes, which have highly curved tubular membranes (top right panel). High curvature is also proposed to be generated in the endoplasmic reticulum (ER) of enterocytes and hepatocytes to promote local triglyceride accumulation between leaflets (bottom right panel). This pool is an efficient substrate for the transport into the ER lumen for the generation of intestinal or hepatic lipoproteins, and deficiency of curvature-promoting arachidonic acid was shown to lead to cytosolic triglyceride accumulation. DHA, docosahexaenoic acid.

lipid composition[106]. Small head groups and fatty acid unsaturation promote packing defects. Polyunsaturated GPLs generate shallower defects than monounsaturated GPLs[20]. Proteins with amphipathic helices recognize and bind to packing defects, and different amphipathic helices discriminate defects of variable depth[106]. In other words, these proteins sense lipid composition and discriminate between different membranes through packing defects. This mechanism is used to recognize and filter intracellular vesicles coming from different sources, enabling selective entry of ER-derived vesicles in the *cis*-Golgi[109].

Lipid composition affects transmembrane protein localization and conformation (FIG. 4c). A handful of proteins (listed elsewhere[110–112]), such as ion channels, are known to be influenced by lipids, but it is quite possible that this is a more general phenomenon, as membrane proteins and lipids have evolved together. PUFA-containing GPLs enhance touch sensation in *Caenorhabditis elegans*, suggesting that membrane composition and the resulting membrane properties affect mechanosensitive channels[22]. In addition, surrounding lipids can exert lateral pressure on the protein (for details

BAR domain
A protein domain that has the ability to bind and/or to induce a specific curvature in membranes.

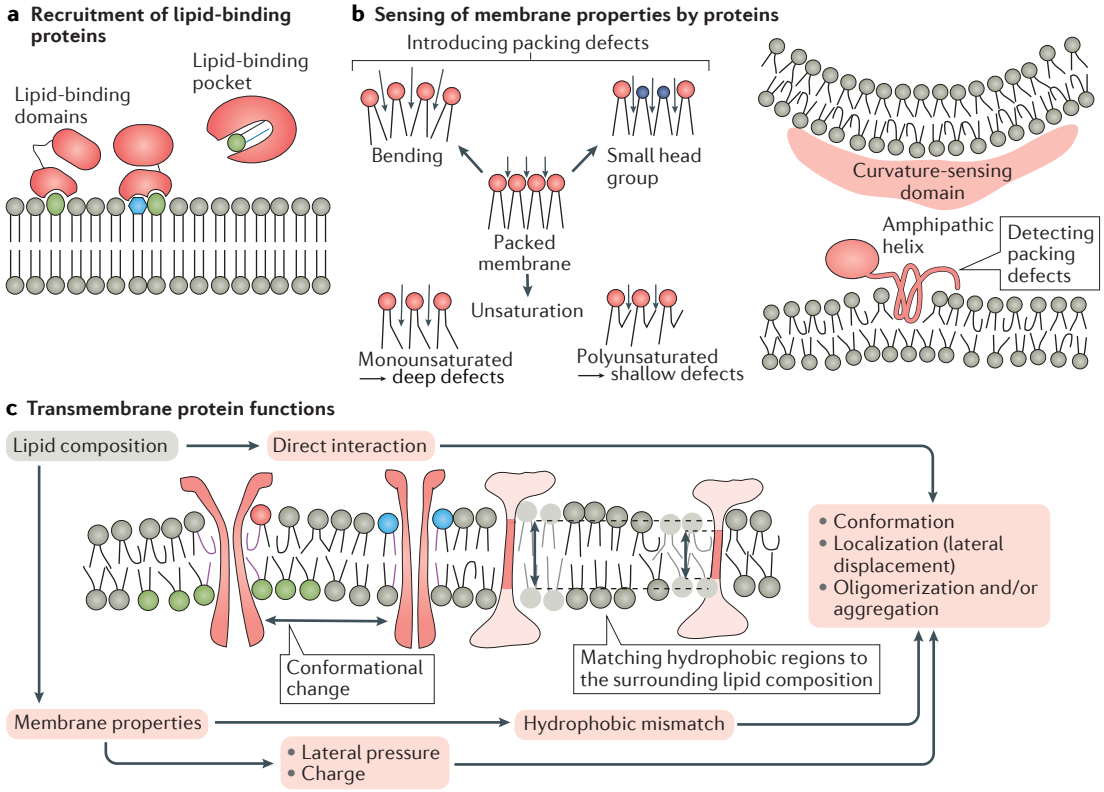


Fig. 4 | **Lipids regulate protein-mediated biological processes. a** | Lipids with specific structures recruit lipid-binding proteins or can be ligands for proteins, such as nuclear receptors, thereby regulating protein activity. **b** | Membrane curvature, head group size and unsaturation affect the degree of lipid packing and can cause packing defects (exposition of hydrophobic regions to the aqueous environment). Several proteins are recruited through recognition of membrane properties such as curvature and packing defects. Proteins with amphipathic helices bind to packing defects of different depth, depending on the bulkiness of the amino acid side chains. **c** | The conformation, distribution and oligomerization states of membrane proteins are affected by the surrounding lipid composition. These transitions could occur through conformational changes caused by direct lipid–protein binding (for example, as a nonannular lipid), or indirectly through membrane properties. A mismatch between the length of the transmembrane segment and membrane thickness causes various effects, such as lateral displacement of the protein, protein aggregation, tilting of the transmembrane segment or adjustment of transmembrane segment length by deformation (hydrophobic mismatch can also induce adjustment of membrane thickness by lipid deformation). The lateral pressure of the membrane (influenced by lipid head groups and hydrophobic chains) and the lipid head group electrical charge apply various forces on membrane proteins, affecting their functions. The lateral heterogeneities discussed in Supplementary information S5 (box) can also be regarded as a way in which lipid composition affects membrane protein functions.

about lateral pressure, see REFS 81,112), which is likely to affect protein conformation. One striking example of the importance of specific transmembrane protein–lipid interaction is the regulation of the protein p24 by sphingomyelin[17]. This protein regulates the budding of vesicles from the Golgi apparatus, and its transmembrane domain interacts with sphingomyelin that contains a C18 *N*-acyl chain but not with other species. This specific interaction is important for p24 dimerization and Golgi protein transport. Transmembrane protein segments also interact with their surrounding lipids[113] (called annular lipids or nonannular lipids depending on the mode of interaction[110,112]), with various specificities and affinities. During nanodomain initiation by a transmembrane protein (see discussion above), it is likely that such initial lipid–protein interactions will establish a larger interaction network with other lipids, thereby promoting nanodomain formation. In more general terms, the effect of membrane composition on membrane thickness might affect lateral protein distribution, as proteins strive to match the length of their transmembrane domains with local membrane thickness. Lack of fit results in hydrophobic mismatch with an energy penalty that will cause lateral displacement of the transmembrane domain, adjustment of local membrane thickness or tilting of the transmembrane domain for a better hydrophobic match[14,112,114]. Hydrophobic mismatch affects the lateral distribution of SNARE proteins (for example, where a single amino acid difference in transmembrane domain length is sufficient to sort closely homologous proteins separately[115]). A comprehensive analysis of proteins localized differently along the secretory pathway suggested that transmembrane domains have an appropriate match with lipid composition in different compartments, which might also help the sorting of proteins in the secretory pathway[116].

Annular lipids
Lipids that stick to the surface of membrane protein transmembrane regions with fairly weak interactions, being in rapid exchange with the bulk of membrane lipids.

Nonannular lipids
Lipids that bind strongly to, or are buried in, membrane protein transmembrane regions.

Thus, lipid composition might affect transmembrane protein recruitment, conformation, activity and localization through specific lipid–protein interactions or through their effects on membrane properties.

Studying protein–lipid interactions. It is possible to analyse the effect of lipid composition on functions of a selected protein — for example, by reconstitution of proteins in liposomes or expression in hosts with altered lipid composition. An elegant study used *C. elegans* as a host to express human transient receptor potential cation channel subfamily V member 4 (TRPV4) and analysed its function in genetically modified worms with various lipid alterations[21]. This study revealed the importance of membrane PUFAs, especially those carrying an epoxide group, for the function of this channel involved in blood pressure regulation. This study sheds light on the poorly studied, oxygen-modified class of membrane GPLs. Structural analyses also have the potential to reveal how lipids affect protein functions[112]. A recent structural study used X-ray solvent contrast modulation to visualize the interplay between lipids of the bilayer and a transmembrane protein, revealing the importance of phospholipids in the dynamic structural rearrangements of a calcium pump[117]. However, except for a few examples, it is often difficult to define how protein-bound lipids affect protein conformation. Emerging methodologies use ion mobility and/or native mass spectrometry to correlate the lipid-binding state and folding stability or oligomerization of the protein of interest[118,119]. These experiments showed that specific lipids can help to stabilize protein structures and protein–protein interfaces. Despite being technically challenging, these examples show that the effect of lipid composition on a protein of interest can be analysed. However, it remains difficult to understand the effect of lipid composition when the affected proteins are not known (for example, to explain a lipid-related disease).

Chemical biology approaches using 'bifunctional' probes enabled a proteome-wide assessment of protein–lipid interactions. In such approaches, lipid analogues are crosslinked with neighbouring proteins and isolated, followed by proteomics analyses to detect lipid–protein interactions in a high-throughput manner. This approach has successfully identified proteins that interact with lipids containing various fatty acids[120,121], cholesterol[122] and sphingolipids[123]. The list of these interacting proteins likely includes those in which function is affected by lipid composition or specific protein–lipid interactions. However, it is labour-intensive to refine the candidate list of proteins to dissect these that are regulated by the interaction with lipids. In addition, because many membrane proteins will be missing from these types of high-throughput studies, many challenges remain in understanding the roles of lipid–protein interactions and their contribution to disease. Methods that enable proteome-wide identification of protein conformation changes by lipid composition would be of great usefulness, and their development warrants further attention.

Sensing lipids to maintain homeostasis

Lipid composition is highly dynamic because it depends upon many factors, including diet, circadian rhythms[124,125] and cell cycle[23]. Thus, lipid and cellular homeostasis have to be coordinated with these processes by sensing the lipid composition. To maintain homeostasis, cells sense lipid composition with various strategies (by detecting lipid levels or the consequent membrane physical properties) and use various feedback mechanisms such as transcription regulation or phosphorylation to adjust lipid levels and maintain compositional homeostasis (FIG. 5a).

Sensing composition to maintain lipid homeostasis. Cells can sense the levels of various lipids (either directly or through precursors or by-products). An example of lipids that are sensed directly are sterols. In mammals, maintenance of cholesterol homeostasis is achieved through the transcription factor sterol regulatory element-binding protein 2 (SREBP2), which is proteolytically modified in response to sterol insufficiency and in this cleaved form moves to the nucleus to upregulate sterol biosynthetic genes[126]. In yeast, sterol sufficiency is sensed through the transcription factor sterol uptake control protein 2 (Upc2), which controls expression of sterol biosynthetic genes and is regulated by direct ergosterol (fungal sterol) binding. When ergosterol is abundant, it inhibits nuclear translocation of Upc2, which results in downregulation of sterol synthesis upon sterol sufficiency[127]. Another example of the direct effect of the sensed lipid on its own biosynthetic pathway is PtdSer synthesis. PtdSer synthase 1 is directly inhibited by its enzyme product PtdSer, thereby maintaining correct amounts of PtdSer[128]. Importantly, gain-of-function mutations in PtdSer synthase 1 that reduce this end-product inhibition cause a severe human disease called Lenz–Majewski syndrome.

GPLs are sensed indirectly through the levels of their precursors. A key regulator of GPL homeostasis in yeast is overproducer of inositol protein 1 (Opi1)[129], which is the major transcriptional repressor of GPL biosynthetic genes. Accumulation of PtdA due to reduced production of GPLs leads to ER sequestration of Opi1, thereby promoting GPL synthesis to maintain their levels. Although mammals lack homologues of yeast Opi1, many enzymes for GPL synthesis have intrinsic properties to maintain lipid homeostasis.

CCTα (also known as CTP, phosphocholine cytidylyltransferase alpha), the rate-limiting enzyme for PtdCho synthesis, is activated when packing defects are present, reflecting PtdCho insufficiency (PtdCho has a small contribution to packing defects as compared with its precursors; see also above and refer to FIGS 3a,4b). PtdCho precursors including PtdA and DAG, both of which are lipids that promote packing defects, regulate CCTα activity, at least *in vitro*[130]. CCTα uses its amphipathic helix to bind to membranes featuring packing defects (see FIG. 4b), which promotes its activation. Thus, CCTα responds to PtdCho insufficiency by sensing PtdCho precursors and membrane properties resulting from PtdCho insufficiency.

a **Sensing lipid composition for lipid homeostasis**

Mammals Yeast

Sensing through lipid metabolite levels

Mode of sensing	Sensed aspect	Sensed lipid	Molecular mechanism	Outcome
Regulated lipid itself	Sterol insufficiency	Sterols	Cleavage, nuclear localization of SREBP2 activator	Sterol biosynthetic genes up
	Sterol sufficiency	Sterols	Cytoplasmic retention of Upc2	Sterol biosynthetic genes down
	PtdSer sufficiency	PtdSer	Feedback inhibition of PtdSer synthase	PtdSer synthesis down
By-product level	• Ceramide flux in Golgi • Sphingomyelin sufficiency	Diacylglycerol	Kinase-mediated inhibition of ceramide tranport	• Ceramide transport down • Sphingomyelin synthesis down
Precursor level	GPL insufficiency	PtdA	ER sequestration of Opi1 repressor	GPL biosynthetic genes up
	PtdCho insufficiency	Diacylglycerol and PtdA	Activation of CCTα	PtdCho synthesis up

Sensing through membrane properties

Sensed aspect	Sensed property	Molecular mechanism	Outcome
PtdCho insufficiency	Membrane packing defects	Membrane binding of the amphipathic helix of CCTα and its activation	PtdCho synthesis up
Oversaturation of lipids and unsaturated lipid balance	Lateral pressure	Cleavage, nuclear localization of Mga2	Desaturase transcription up
Sphingolipid levels	Membrane stress (membrane stretching, reduced tension)	• Relocation of Slm proteins within membrane domains • Activation of TORC2 • Orm protein phosphorylation	• Relieving inhibition of SPT • Sphingolipid synthesis up

b **Sensing cellular status through phosphatidic acid composition**

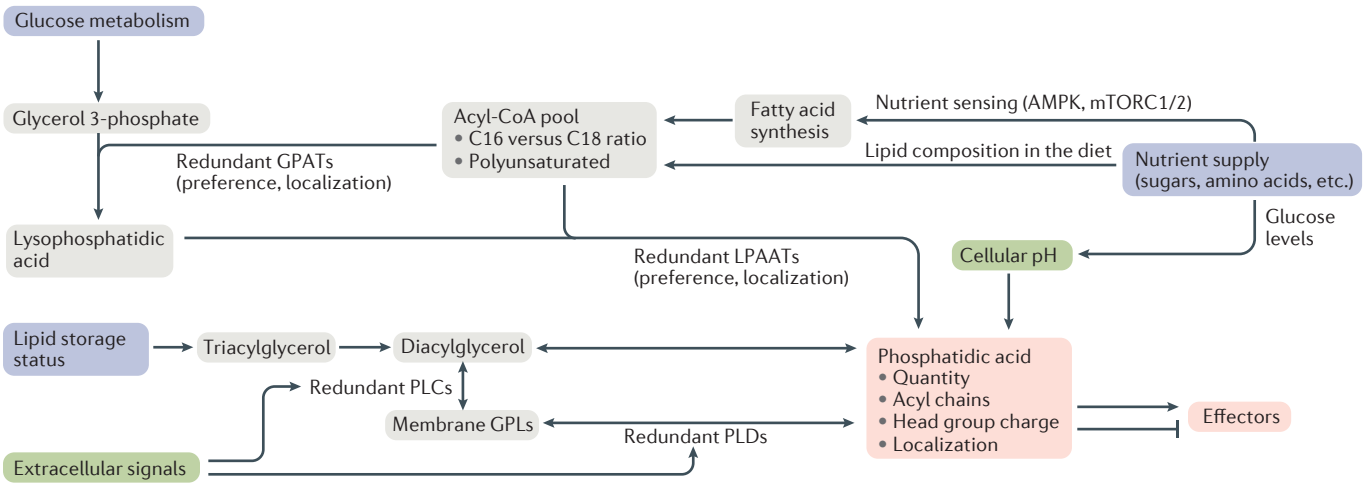


Fig. 5 | **Sensing lipid composition to maintain homeostasis. a** | Lipid composition is sensed to maintain lipid composition itself (for example, to dampen fluctuations). As is seen in the main text, maintenance of lipid composition is regulated by various mechanisms (such as regulation of expression or post-translational modifications of enzymes involved in lipid metabolism), but the mode of sensing is also diverse. Sufficiency or insufficiency of a lipid can be sensed by detecting levels of the lipid itself, the precursor of the lipid or the by-product of the reaction to synthesize the lipid. Lipid composition can also be sensed through the effects of the lipid on membrane properties. Lipid composition and membrane properties are often causally related, which makes it difficult to determine the precise property that controls recognition. Of note, proteins that sense lipid composition utilize the same mechanisms as those depicted in FIG. 4 to detect lipids and determine their composition. **b** | Lipid composition is also an indicator of cellular metabolic status. Various effectors might sense lipid composition to coordinate different cellular processes appropriately, as exemplified here by the role of phosphatidic acid sensing. Phosphatidic acid (PtdA) levels, acyl-chain composition, charge and localization are affected by multiple factors, such as lipid availability, glucose metabolism, nutrient sensing and cellular pH. In turn, this might regulate the localization and activity of various effectors (for example, mTOR complexes (mTORC1/2) in mammals and overproducer of inositol protein 1 (Opi1) in yeasts). Therefore, phosphatidic acid diversity might confer various signals to coordinate cellular metabolic status with downstream functions. PtdA is an important lipid to survey because it is at the crossroads of different lipid metabolic pathways, located between glycerophospholipids (GPLs) and triacylglycerol metabolism (see FIG. 2). AMPK, AMP-activated protein kinase; CCTα, phosphocholine cytidylyltransferase alpha (also known as CTP); ER, endoplasmic reticulum; GPAT, glycerol 3-phosphate acyltransferase; LPAAT, lysophosphatidic acid acyltransferase; PLC, phospholipase C; PLD, phospholipase D; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; SREBP2, sterol regulatory element-binding protein 2; SPT, serine palmitoyltransferase; TORC2, Tor complex 2; Upc2, sterol uptake control protein 2.

Membrane properties other than packing defects can also be sensed to maintain lipid composition. The yeast transcription factor Mga2 is activated by proteolytic cleavage when membrane lipids are too saturated and translocates to the nucleus, resulting in the upregulation of acyl-CoA desaturase 1 (Ole1)[131]. Mga2 activation was recently described in detail, where lipid composition affects the rotation of the transmembrane helix depending on specific amino acids. In mammals, membrane saturation induces proto-oncogene c-Src (SRC) clustering on endosomal membranes, which mediates stress responses with a potential implication in insulin resistance[132]. In addition, ER membrane saturation leads to the unfolded protein response (UPR)[133,134]. Although the importance of the UPR transducer IRE1α (also known as ERN1) for this response was suggested[134], it remains unclear how this protein senses membrane properties. IRE1α response is strikingly insensitive to mutations in transmembrane residues[135], suggesting that mechanisms similar to yeast Mga2 are unlikely. Cells not only adapt to membrane lipid saturation but also respond to elevated PUFAs by increasing saturated PtdCho[136], suggesting the presence of unidentified sensors for the degree of polyunsaturation.

Orm family proteins are important negative regulators of sphingolipid synthesis, which form a conserved complex and inhibit serine palmitoyltransferase, the first and rate-limiting enzyme in sphingolipid production[137]. In yeast, reduced sphingolipid levels cause membrane stress. This membrane stress is sensed by PtdIns 4,5-bisphosphate-binding proteins Slm1 and Slm2 and transmitted to Tor complex 2 (TORC2)[25], which then relieves Orm inhibition through a protein phosphorylation cascade[26]. In mammals, three Orm homologues mediate feedback regulation of sphingolipid synthesis upon increased ceramide levels[138] through their protein–protein interactions[139], but this regulation is complex owing to their redundancy and stoichiometry[140,141]. In addition to the inhibition of synthesis, when sphingomyelin accumulates in Golgi membranes owing to increased ceramide flux, ceramide transport is inhibited to prevent overproduction of complex sphingolipids[142]. Interestingly, sphingomyelin accumulation is sensed through the by-product of sphingomyelin synthesis, DAG (for molecular details, see REF. 142). This circuit might enable correct balance of complex sphingolipid and ceramide levels in addition to the regulation of global sphingolipid levels by Orm family proteins. It was also shown that excess ceramide in the ER is converted into acylceramide and transferred into lipid droplets[143]. Therefore, sphingolipid homeostasis is maintained through regulation of synthesis, transport and compartmentalization into lipid droplets.

From these examples, it is clear that there is no universal way to sense and maintain lipid composition. Rather, multiple strategies have been developed to sense and to respond to changes in lipid composition.

Sensing cellular state through lipid composition. Lipid composition seems to reflect cellular metabolic status. A systematic analysis of yeast kinase mutants identified the involvement of the major nutrient-sensing pathways, AMP-activated protein kinase (AMPK; Snf1 in yeast) and TOR in the regulation of GPL acyl-chain length[16]. AMPK is a sensor of energy status and inhibits lipogenesis (among many other pathways) under starvation. Loss of AMPK activity increases lipogenesis, which also results in longer acyl chains of GPLs, including PtdA[144]. Interestingly, the interaction of yeast Opi1 (the inhibitor of GPL synthesis; see above) with PtdA depends on fatty acid composition, with C16 being preferred over C18 (REF. 144). Therefore, in the absence of AMPK activity, the longer acyl chains in PtdA reduce ER binding of Opi1, which promotes inhibition of GPL synthesis and prevents overproduction of GPLs under highly lipogenic conditions. Thus, the acyl-chain composition enables the discrimination of whether the PtdA increase is due to reduced GPL synthesis or overactive lipogenesis (in which case further GPL synthesis is undesirable). In addition, under glucose deprivation the pH of cytoplasm drops. Consequently, binding of Opi1 to PtdA (which becomes protonated at lower pH) is lost and Opi1 migrates into the nucleus, where it represses transcription[145]. This use of the pKa of the phosphate on PtdA allows rapid coordination of lipid synthesis with the cellular metabolic state. Similarly, in mammals, PtdA generated through *de novo* synthesis regulates the nutrient sensor mTOR complex 2 both negatively[146] and positively[147], which might reflect the acyl-chain sensitivity of mTOR signalling[148]. The purpose of this sensing is unclear but might help in the coordination of cell growth and nutrient availability, using PtdA composition as a source of information about the metabolic status of the cell (FIG. 5b).

Although the concept of lipid composition as a source of information on nutritional status still needs to be firmly established, it is already clear that many intermediates of lipid metabolism affect biological functions. For example, the precursor of PtdA, lyso-PtdA, regulates mitochondrial fusion[149] and sphingosine regulates lysosomal calcium signalling and biogenesis[150]. Many nuclear receptors, such as PPARα, recognize GPLs with specific acyl chains as ligands to regulate transcription, although they often recognize lipids from other classes as well, which makes it difficult to understand intuitively how they maintain lipid homeostasis[101–103]. These sensing properties might also help to coordinate the cellular metabolic state with appropriate transcriptional responses. Therefore, it is likely that lipid composition is sensed to affect many biological functions, because it is an indicator of cell status. It is important to study lipid sensing in a broader context to understand how cells utilize various sources of information to coordinate cellular status (for example, nutrient availability and differences in energy sources) and biological processes (for example, cell growth, division and death).

Conclusions and perspectives

Although our knowledge of lipid metabolism and function has improved, we have so far revealed only the tip of the iceberg. We have only a limited understanding of the biological consequences of slight structural differences in lipids, but the known cases suggest that small structural

Tor complex 2
(TORC2) Protein kinase complex that contains the target of rapamycin subunit that responds to nutritional and other signals and acts as a central regulator of protein and lipid synthesis and cell proliferation.

AMP-activated protein kinase
(AMPK). An important protein kinase that senses energy status by binding to AMP and that is activated upon glucose deprivation to regulate several biosynthetic pathways.

pKa
The negative $\log_{10}$ of the acid dissociation constant of a molecule.

changes will be very important. Many of these cases were unpredictable when the research started, suggesting that exciting new findings lie ahead. One approach to discover more lipid functions would be to interfere with lipid homeostasis, find resulting phenotypes, dissect their mechanisms and reconstitute the phenomenon to prove it. Increased lipidomics coverage and information about metabolite fluxes is critical to understand lipid metabolism and interfere with it. Integrating metabolism into mathematical models[151] and a complete understanding of feedback responses will be necessary to allow making predictions about how a specific lipid composition can be achieved. To further improve the studies of lipid composition, approaches enabling modification of lipids with higher spatiotemporal resolution using chemical biology tools for photorelease[150] or photoactivation[152] of lipids will be of great use. They might be particularly useful for detailed studies of the role of compartmentalization in lipid homeostasis, of which we currently know very little. Physics will continue to be important for understanding the effect of altered lipid composition on membrane properties. To tackle the unpredictability of lipid functions, comprehensive and unbiased approaches will be useful for detecting and explaining lipid-related phenotypes. For example, high-throughput lipid–protein interaction analysis[6], genome-wide genetic screens[153] and systematic lipidomics[16] have been done so far, but further breakthroughs are required. As mentioned earlier, proteome-wide identification of proteins affected by lipid composition would be highly informative. Reconstitution might be done *in vitro*, in heterologous *in vivo* systems but also *in silico* using molecular dynamics simulations[20,43]. Molecular dynamics simulations not only help us understand how lipids behave when executing their functions but could also predict what would happen if lipid composition is altered, which in turn can be further investigated experimentally. All of these arguments contend that interdisciplinarity will be key to understanding lipid diversity.

The most accurate summary of our current understanding is that we are only beginning to find out what membrane lipids do. Unexpected results are among the most exciting output of scientific research, and we believe that lipid biology will continue to meet with much excitement as many lipid functions are thus far unexplored and cannot be easily predicted.

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