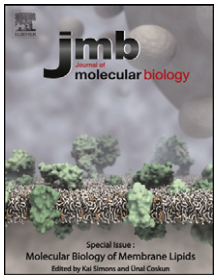

Homeoviscous Adaptation and the Regulation of Membrane Lipids

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http://dx.doi.org/10.1016/j.jmb.2016.08.013
Edited by Ünal Coskun

Abstract

Biological membranes are complex and dynamic assemblies of lipids and proteins. Poikilothermic organisms including bacteria, fungi, reptiles, and fish do not control their body temperature and must adapt their membrane lipid composition in order to maintain membrane fluidity in the cold. This adaptive response was termed homeoviscous adaptation and has been frequently studied with a specific focus on the acyl chain composition of membrane lipids. Mass spectrometry-based lipidomics can nowadays provide more comprehensive insights into the complexity of lipid remodeling during adaptive responses. Eukaryotic cells compartmentalize biochemical processes in organelles with characteristic surface properties, and the lipid composition of organelle membranes must be tightly controlled in order to maintain organelle function and identity during adaptive responses. Some highly differentiated cells such as neurons maintain unique lipid compositions with specific physicochemical properties. To date little is known about the sensory mechanisms regulating the acyl chain profile in such specialized cells or during adaptive responses. Here we summarize our current understanding of lipid metabolic networks with a specific focus on the role of physicochemical membrane properties for the regulation of the acyl chain profile during homeoviscous adaptation. By comparing the mechanisms of the bacterial membrane sensors with the prototypical eukaryotic lipid packing sensor Mga2 from *Saccharomyces cerevisiae*, we identify common operational principles that might guide our search for novel membrane sensors in different organelles, organisms, and highly specialized cells.

Introduction

The lipid and protein composition of biological membranes varies among organisms, tissues, cells, and intracellular organelles. Membrane lipids are amphipathic molecules and can self-assemble into supramolecular structures such as micelles, bilayers, and hexagonal and cubic phases. The most common structure, the lamellar lipid bilayer, has various physicochemical properties including phase behavior, different degrees of fluidity/viscosity, membrane thickness, and bending rigidity that are determined both by the molecular composition and membrane curvature [1]. Biological membranes are functionalized by the incorporation of membrane proteins that serve as receptors, transporters, enzymes, and structural elements. Many crucial signaling processes occur at membrane surfaces. The reversible association of signaling proteins and their specific recognition of target membranes depends on characteristic membrane properties. Therefore, a cell must monitor membrane properties to mount adaptive responses and maintain organelle identities. Lipids have a pivotal role in membrane remodeling processes and their biosynthesis and turnover are tightly regulated.

Although the interplay between the chemical composition and the physicochemical membrane properties, especially viscosity, has been appreciated for decades, it is still largely unknown how cells sense bulk membrane properties to adjust lipid metabolism. Mass

spectrometry-based lipidomics combined with subcellular fractionation or the immunoisolation of organelles have provided comprehensive insights in lipid diversity and lipid distribution [2–5]. Eukaryotic cells and their organelles synthesize hundreds to thousands of lipid molecules differing in their molecular structures, physicochemical properties, and molar abundances. This stunning diversity derives from the combinatorial complexity of the lipid 'building blocks' [6] (Fig. 1a). Glycerophospholipids and sphingolipids have a modular design featuring two apolar hydrocarbon chains (or acyl chains) and a hydrophilic headgroup. The acyl chains are fatty acids (FAs), fatty alcohols, or long-chain bases differing in length and the number and positions of double bonds and hydroxylations (Fig. 1b). The headgroups define the lipid class and are chemically diverse structures spanning from simple structures such as choline to complex oligosaccharide structures.

The proportion of saturated and unsaturated acyl chains in membrane lipids is a key factor determining lipid packing, membrane viscosity, and water permeability [7] (Fig. 2). Poikilothermic organisms including bacteria, cyanobacteria, fungi, plants, and fish that do not control their body temperature increase the proportion of unsaturated acyl chains in membrane lipids to maintain fluidity in the cold [8–13]. However, temperature is not the only factor that explains the unsaturation level of biological membranes. In homeotherms, such as mammals, large variations exist between the acyl chain profiles of several tissues, suggesting that this profile endows cellular membranes with specific properties [14]. Thus, eukaryotic cells establish lipid gradients, with sterols and saturated acyl chains being gradually enriched along the secretory pathway at the expense of monounsaturated acyl chains (Fig. 2b). Neuronal cells accumulate polyunsaturated acyl chains toward the axon tip, whereas less unsaturated lipids show the opposite distribution [15]. Similarly, lipid molecules with saturated and polyunsaturated acyl chains are enriched in the apical plasma membrane of epithelial cells at the expense of monounsaturated acyl chains [3]. Polyunsaturated lipids can adopt different shapes, stabilize co-existing membrane domains, and facilitate membrane deformation normal to the plane of the membrane [14,16]. Their role in shaping physicochemical membrane properties for ultra-fast endocytosis in neurons and signal transduction by mechano-sensitive channels and photoreceptors has only recently gained a fresh emphasis [14].

Little is known about how eukaryotic cells sense and control the acyl chain composition of the membrane lipids. In this review, we summarize our current mechanistic understanding of membrane sensing by the simple eukaryote *Saccharomyces cerevisiae* and compare it with prokaryotic strategies in order to identify common features that might guide our search

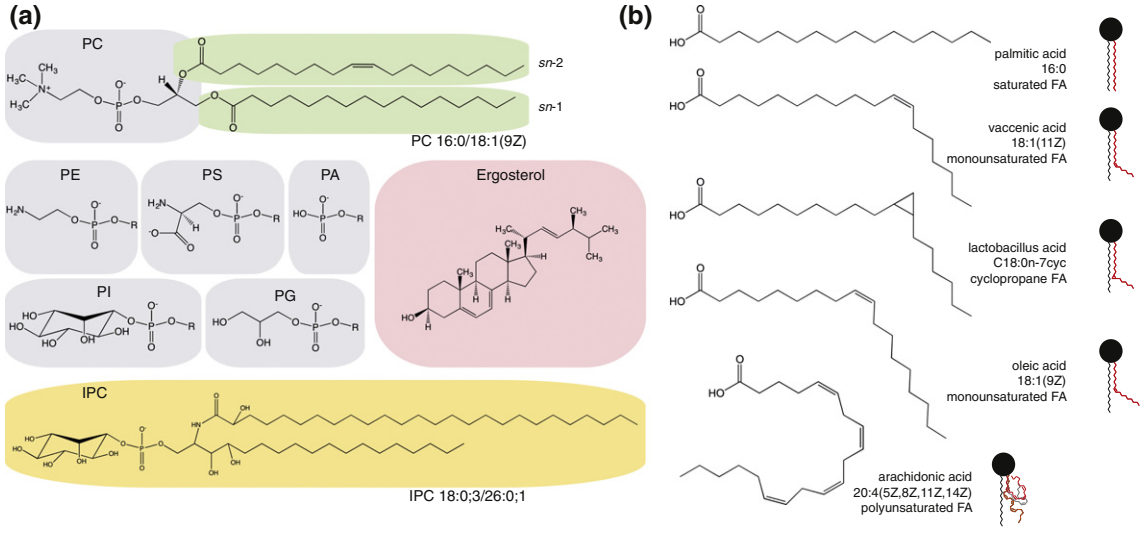

Fig. 1. Membrane lipid and FA complexity. (a) Membrane lipids are subdivided in three major categories: glycerophospholipids (green and gray), sterols (red), and sphingolipids (yellow). Classes of glycerophospholipids are defined by the hydrophilic headgroups (gray) attached to the DAG backbone. The acyl chains in the *sn*-1 and *sn*-2 position (green) of the glycerophospholipids contribute to the species diversity within lipid classes. Also sphingolipids constitute a large category of lipids with diverse headgroups and acyl chains. (b) FAs in bacteria and eukaryotes contribute to lipid species diversity. Palmitic, oleic, and arachidonic acid are common in eukaryotic membrane lipids. Vaccenic acid is important for the homeoviscous response in *E. coli*. Likewise, lactobacillus acid maintains membrane fluidity in *E. coli* under specific growth conditions. Polyunsaturated FAs found are characterized by a remarkable structural flexibility. Different FAs have, when incorporated in membrane lipids and due to their shape (schematically shown), different impact on physicochemical membrane properties.

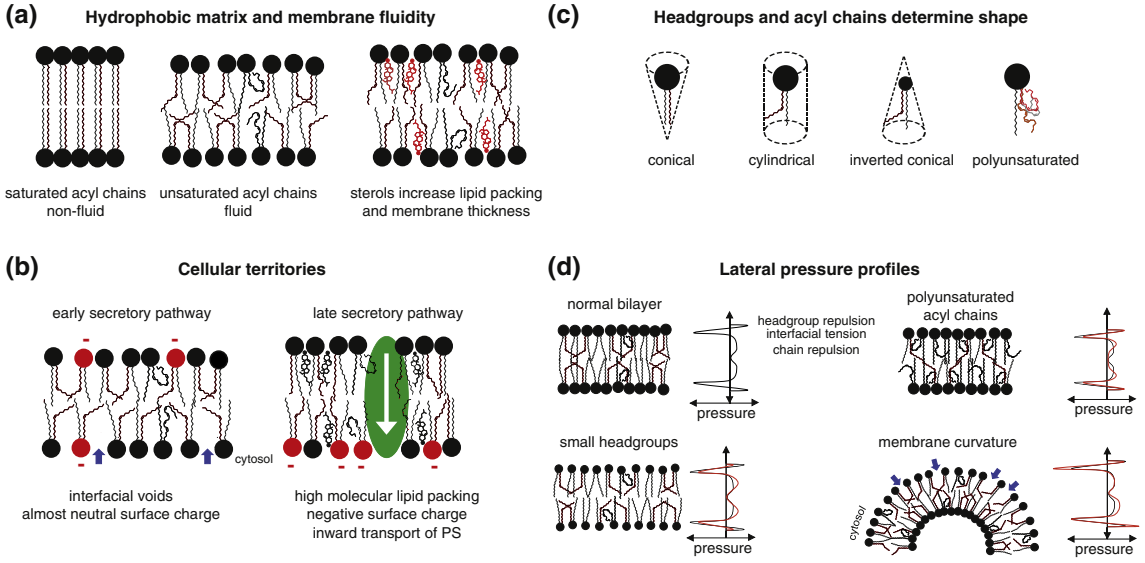

Fig. 2. The lipid composition affects membrane properties including the lateral pressure profile. (a) Lipid acyl chains and sterols constitute the hydrophobic matrix of a eukaryotic membrane and determine membrane viscosity to a large extent. (b) Organelles have characteristic surface properties and a eukaryotic cell establishes two major territories. Organelles of the early secretory pathway exhibit characteristic interfacial membrane voids (blue arrows) and a low density of negative charge on their surfaces. Organelles of the late secretory pathway actively maintain a high negative charge density on their surface and are characterized by a higher lipid packing density due to a higher fraction of saturated acyl chains and high sterol content. (c) The relative volume of the headgroup and acyl chain region determines the molecular shapes of lipids and impact on collective membrane properties. (d) The lateral pressure profile of a membrane affects all incorporated proteins and lipids. The lateral pressure changes at different depths in the membrane. A sharp peak of negative pressure due to interfacial tension is observed at the membrane-water interface, positive lateral pressures are observed in the headgroup and in the acyl chain region. The counteracting pressures in different depths of a bilayer differ by several hundreds of atmospheres and can affect the conformation of embedded proteins. The lipid shape determined by the headgroup and the acyl chains, as well as the membrane curvature have significant impact on the lateral pressure profile.

for novel sensors and those used by mammalian cells to control physicochemical membrane properties.

Biological Membranes and their Physicochemical Properties

Despite a constant exchange of membrane material, cellular organelles maintain characteristic lipid compositions [1,6,17]. In the following, we will provide a short overview of how membrane properties are determined by the lipid compositions and exploited for cellular functions.

Cells can modulate the acyl chain composition of lipids to regulate membrane viscosity (Fig. 2a). The molecular shape of the acyl chains affects lipid packing and the resulting membrane properties. Lipids with saturated acyl chains pack with higher densities and tend to form non-fluid gel phases. Monounsaturated acyl chains have a kinked shape and tend to form fluid bilayers at physiological temperatures. Polyunsaturated acyl chains differ from both saturated and monounsaturated ones by having a larger conformational landscape. The acyl chain length and degree of hydroxylation also affect the melting temperature of a bilayer. Some prokaryotes can synthesize branched acyl chains and/or acyl chains containing cyclic groups such as cyclopropane [18] (Fig. 1b).

Sterols have a planar structure and intercalate between the acyl chains. In this way, they can fluidize gel phases while increasing the acyl chain order and membrane thickness (Fig. 2a). The gradient of sterols along the secretory pathway is actively maintained by directed transport and allows for protein sorting based on the hydrophobic thickness of the transmembrane domains (TMDs) [19–21]. Lipid rafts, fluctuating nano-scale assemblies of sterols, saturated glycerophospholipids, and sphingolipids represent lateral membrane specializations that can be clustered to form signaling active platforms [22,23].

The lipid head groups provide chemical specificity. Some lipids are neutral at physiological pH, while others are negatively charged. As negatively charged phosphatidylserine (PS) lipids are present on the lumenal side of the early secretory pathway [17,24], the corresponding organelles present a relatively low negative charge density on their cytosolic surface (Fig. 2b). On contrary, organelles of the late secretory pathway use lipid flipases to translocate PS, at the

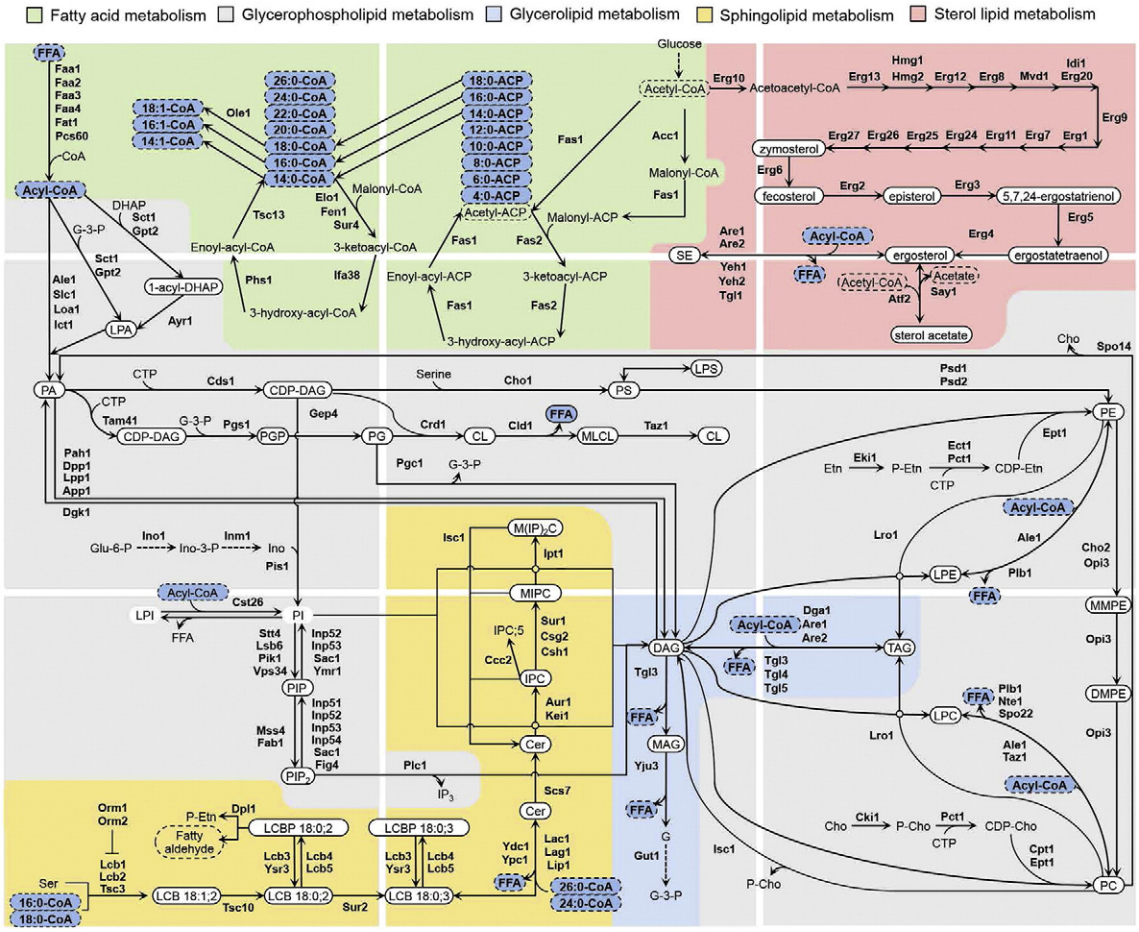

Fig. 3. The lipid metabolic network of *S. cerevisiae*. Metabolism and incorporation of FAs/acyl chains are highlighted with blue background. Lipid classes are highlighted with white background. The network was adapted from [26].

expense of ATP hydrolysis, to the cytosolic membrane leaflet [1,6]. Hence, the surface of these organelles is characterized by a high negative charge density.

Lipids come in different sizes and shapes that are largely determined by the relative volume of the head group and the acyl chains, and water activity (Fig. 2c). Noticeable exceptions, however, are lipids with polyunsaturated acyl chains whose extraordinary high flexibility allow adoption of different shapes [14]. The lipid shape and the molecular packing of the lipids are major determinants of the lateral pressure profile that is experienced by every lipid and membrane protein [25] (Fig. 2d). Regions of high lateral pressure coincide with higher lipid packing densities, while looser packing lowers the local lateral pressure. Another important geometric factor contributing to the lateral pressure profile is membrane curvature. In regions of high positive curvature (e.g., in a small vesicular carriers), the lateral pressure is reduced in the cytosolic leaflet relative to the lumenal leaflet.

Now that the cellular and biochemical importance of acyl chain diversity has gained a fresh emphasis, it is time to revisit key questions regarding the cellular distribution of acyl chains in membrane lipids and their regulation.

Biosynthesis of Membrane Lipids

Membrane lipid biosynthesis in eukaryotic cells is orchestrated by four major metabolic modules: (i) FA metabolism, (ii) *de novo* glycerophospholipid synthesis and acyl chain remodeling, (iii) sphingolipid biosynthesis, and (iv) sterol biosynthesis. These modules are part of a large-scale lipid metabolic network (Fig. 3), defined by enzymes and regulatory factors that control lipid biosynthesis and turnover. Below, we provide a brief summary of the most important pathways involved in synthesis of membrane lipids.

At the center stage of membrane lipid production is the pool of available FAs, determined by *de novo* synthesis, FA uptake, activation, desaturation, elongation, and turnover. *De novo* FA synthesis in

eukaryotes typically produces palmitic acid (16:0). Following activation of FA to FA-CoA esters, they can be subjected to further chemical modifications, including hydrocarbon chain extension by elongases and insertion of double bonds by desaturases to produce unsaturated FAs (UFAs). In combination, the different pathways of FA biosynthesis can produce a wide repertoire of FAs serving as building blocks for making glycerophospholipids and sphingolipids. This palette of FAs is a key determinant of the lipid compositional complexity in biological systems.

The first step in *de novo* glycerophospholipid synthesis is the transfer of an activated FA-CoA unit to the *sn*-1 position of glycerol-3-phosphateto produce 1-lysophosphatidic acid. In *S. cerevisiae*, the most commonly incorporated FAs include palmitic acid (16:0), palmitoleic acid (16:1(9Z)), stearic acid (18:0), and oleic acid (18:1(9Z)) [26]. A second acyltransferase can insert another acyl chain onto the *sn*-2 position of 1-lysophosphatidic acid to produce phosphatidic acid (PA), a key metabolic precursor in the production of glycerophospholipids. The most abundant species of PA in *S. cerevisiae* are PA 16:1/18:1, PA 16:1/16:1, PA 16:0/16:1, and PA 16:0/18:1. However, the acyl chain composition of PA and other lipid molecules is remarkably flexible and depends on the metabolic state of the cell, the culture medium, and the growth temperature [2,26,27]. The recent identification of lysophospholipid acyltransferase enzymes responsible in the regulation of acyl chain diversity at the *sn*-2 position represents a major advance, as it allows for modification of the acyl chain profile of a biological membrane in a more directed manner [28,29].

From PA, numerous metabolic transitions can occur [30,31]. A common route of enzymatic steps involves the conversion of PA to cytidine diphosphate diacylglycerol (CDP-DAG), which in yeast can subsequently be converted to either PS, phosphatidylinositol (PI) or phosphatidylglycerol. PS can be decarboxyated to produce phosphatidylethanolamine (PE). In yeast, and also mammalian liver, PE can be converted, *via* three consecutive methylations catalyzed by methyltransferases, to the major membrane constituent PC. However, PE and PC lipids can also be synthesized by alternative, seemingly redundant pathways. As such, the metabolic intermediate PA can be dephosphorylated by phosphatases (termed lipins in mammals and Pah1/Dpp1/Lpp1/App1 in *S. cerevisiae*) to produce DAG. DAG can serve as a precursor for synthesis of PE and PC by using three consecutive transitions where the metabolic precursors choline and ethanolamine are activated to CDP-choline and CDP-ethanolamine, respectively, followed by transfer to DAG. This set of enzymatic reactions is known as the Kennedy pathway [32].

The second pathway for glycerophospholipid biosynthesis is that of FA remodeling catalyzed by phospholipases, acyltransferases, and transacylases [33]. A particular prominent pathway is the Lands cycle, where phospholipase A_2 removes the FA moiety at the *sn*-2 position of *de novo* synthesized PC lipids (e.g., PC 16:0/18:1), which results in the formation of 1-lysophosphatidylcholine (e.g., LPC 16:0) [33,34]. LPC can be re-acylated at the *sn*-2 position by an LPC acyltransferase using FA-CoA (e.g., activated arachidonic acid (20:4)) to produce a new PC molecule (e.g., PC 16:0/20:4). Also other glycerophospholipids, including PI molecules, can undergo similar enzymatic transitions to have their FA moieties remodeled.

The acyl chain profile differs between lipid classes. PI lipids are the main membrane constituent with saturated FA moieties in *S. cerevisiae* [2]. *De novo* synthesized di-unsaturated PI 16:1/16:1 and PI 16:1/18:1 molecules are rapidly remodeled to mono-unsaturated PI 18:0/16:1 and PI 18:0/18:1 by the putative 2-lysophosphatidylinositol acyltransferase Cst26 [35]. In most mammalian cells, PC lipids are more abundant than PI lipids and typically feature saturated (e.g., 16:0) and mono-unsaturated (e.g., 18:1) acyl chains, while PI lipids contain at least one polyunsaturated (e.g., 20:4) acyl chain. The mechanisms underlying the regulation of the acyl chain composition are just starting to emerge [28].

Sphingolipid biosynthesis involves a series of reactions where serine and FA-CoAs are combined to produce diverse long-chain bases differing in hydrocarbon chain length, number of double bonds, and hydroxyl groups. These long-chain bases (e.g., sphingosine) can be N-acylated with diverse FA-CoA moieties to produce ceramide molecules. Ceramides can subsequently serve as precursors for synthesis of important membrane lipids including inositol-containing sphingolipids in yeast and sphingomyelins and more complex sphingolipids such as gangliosides in mammals [6].

Sterol biosynthesis is mediated by an evolutionary conserved pathway that involves around 30 enzymatic steps [36]. In mammals and yeast, this pathway gives rise to cholesterol and ergosterol, respectively, which typically make up about 20%–30% of all cellular lipids. Notably, a metabolic branch point in the pathway of sterol biosynthesis is responsible for the production of isoprenoids, which serve as precursors for molecules such as ubiquinone, prenyl membrane anchors, and dolichol-based molecules required for N-linked glycosylation.

Today we have to come to grasp that the blueprint of cellular lipid biosynthesis is not merely a collection of independent linear pathways (as outlined above) but a highly complex and dynamically regulated network [2,6,31]. This architecture allows cells to integrate and co-regulate enzymatic conversions across the different lipid metabolic modules and to exchange lipid building blocks between them. One example is the production of inositol-containing sphingolipids in yeast

where the inositol-phosphate headgroup from PI molecules is transferred to ceramide to produce inositol-containing sphingolipids and DAG (the orthologous reaction in mammals transfers choline-phosphate from PC to produce sphingomyelin) [37].

The interconnectivity of lipid metabolic pathways implies that "specific" genetic or pharmacologic perturbations are prone to induce ripple effects on seemingly unrelated lipid metabolic pathways. A powerful avenue for monitoring the absolute abundance of all cellular lipid molecules (i.e., the cellular lipidome) and their turnover is using mass spectrometry-based lipidomics combined with flux analysis [2,38]. When complemented with quantitative proteomics, to determine lipid enzyme levels and post-translational modifications, this technology can be used for monitoring lipid metabolic activity and studying regulatory mechanisms across the entire lipid metabolic network [26].

Regulation of Membrane Lipid Metabolism

The rate by which lipids are synthesized and turned over can be regulated by a wide range of mechanisms. These mechanisms fall into three principal categories: mass action, enzyme activity, and subcellular enzyme localization.

The first important determinant of lipid metabolic flux is the law of mass action. In cellular systems, this mechanism is commonly at play when a lipid metabolic precursor becomes exhausted or is not delivered to the subcellular location harboring the lipid enzyme. When choline is depleted from the growth medium, for example, cells cannot use the Kennedy pathway for PC biosynthesis and are forced to use the PE methylation pathway. Another example is the insertion of double bonds into FAs and sterols by desaturases that require molecular oxygen (O_2) [9,39]. Subjecting cells to hypoxia ceases the production of UFAs and sterols [36,40].

The second crucial determinant of lipid metabolic flux is enzyme abundance and activity, which is governed by a plethora of molecular mechanisms that span transcriptional and translational control [31,41], enzyme stability and controlled degradation [42], post-translational modifications (e.g., phosphorylation) [43], protein–protein interactions, and allosteric regulation (e.g., protein–lipid interactions) [41]. A remarkable feature of transcriptional regulation is that a single transcription factor can regulate a whole set of genes and thus mediate a coordinated rewiring of the lipid metabolic network. A prominent example for such a metabolic switch is the control of membrane biogenesis by the transcriptional repressor Opi1 [31]. When Opi1 enters the nucleus, it not only attenuates the expression of *INO1*, rate limiting for the biosynthesis of PI, but also affects the expression of a number of genes involved in PS, PE, and PC biosynthesis. Another prominent example of transcriptional regulation is mediated by the mammalian sterol response element binding proteins SREBP-1a, -1c, and -2 controlling the expression of several hundreds of genes and coordinating sterol, FA, and glycerolipid metabolism in mammals [41].

The third determinant of lipid metabolic flux is subcellular compartmentalization. A key textbook example is the coordinated regulation of *de novo* FA synthesis by the FA synthase in the cytosol and the breakdown of FAs by β-oxidation in mitochondria. Conditions with increased activity of the acetyl-CoA carboxylase (e.g., high glucose) result in an elevated production of malonyl-CoA, which allosterically inhibits the carnitine palmitoyltransferase and thus prevents mitochondrial import and β-oxidation of FAs. Another example is the localization of enzymes responsible for the biosynthesis of inositol-containing sphingolipids (in yeast) and sphingomyelin (in mammals) to the Golgi apparatus [1]. The regulation of lipid synthesis can also occur by the reversible association with lipid metabolic enzymes with the membrane surface of cellular organelles. An example hereof is CTP:phosphocholine cytidylyltransferase (CCT), which contains an amphipathic helix recruiting the enzyme to the ER membrane and the surface of lipid droplets to activate PC biosynthesis [44,45]. The increased binding and activity of CCT at membranes with a low PC:PE ratio represents an important homeostatic mechanism to control the cellular level of non-bilayer forming PE lipids and to maintain membrane integrity. On the surface of growing lipid droplets, CCT stabilizes the monolayer and prevents lipid droplet coalescence [44,46]. Similarly to CCT, Pah1 and its mammalian orthologs lipins 1 and 2 use amphipathic helices to target PA-enriched membranes *via* electrostatic interactions and generate DAG from PA to control membrane and lipid droplet biogenesis [31,47]. Both enzymes, CCT and Pah1, explore the interfacial properties of their target membrane for proper subcellular localization and regulating of the enzyme activity. Thus, the regulated recruitment of key metabolic enzymes to specific subcellular membranes is determined by surface properties that depend on the lipid composition and membrane curvature.

Sensing of Membrane Properties

The complex and dynamic system of cellular membranes is under constant surveillance by dedicated membrane sensors that serve to maintain membrane function and homeostasis [48]. Given the key importance of the acyl chain composition of lipids in shaping membrane properties and organelle identity, it is crucial to control their abundance and subcellular distribution [14].

Membrane sensors are either integral membrane proteins or soluble proteins that associate reversibly with the membrane to explore surface properties. Members of the second class must distinguish between organelles to fulfill their function. A recurring theme in membrane recognition is the use of modular, low-affinity interactions that cooperate to provide specificity [49]. Some proteins can target specific membranes by binding to low abundant lipids, such as phosphoinositides, that are restricted to certain compartments. Another strategy involves post-translational lipid-like modifications that are only exposed for membrane targeting under certain conditions [50,51]. A third mode of membrane binding facilitates sensing of physicochemical properties such as membrane curvature, electrostatics, and lipid packing [17,52]. This mode of sensing is of particular interest, as it can be harnessed to modulate collective membrane properties and to orchestrate large-scale cellular responses.

The early secretory pathway exhibits characteristic lipid packing defects that can be recognized by cytosolic proteins with an amphipathic lipid packing sensor (ALPS) motif [17,48]. The ALPS motif is unstructured in solution but can fold into a helical structure in the presence of membrane exhibiting specific lipid packing defects. The residues on the hydrophobic site of the folded ALPS motif are bulky or aromatic (leucine, phenylalanine, or tryptophan) and ideally suited to insert into packing defects arising from a mismatch of membrane curvature and the shape of the lipids. The hydrophilic side is composed of small and uncharged residues (glycine, serine, and threonine). Helix-destabilizing residues such as glycines and prolines are also found in the ALPS motif, probably preventing premature helix formation in the absence of a membrane [17,53]. These properties render ALPS motif containing proteins particularly sensitive to lipid packing defects.

Selective membrane binding has been reported for an increasing number of proteins organizing the secretory pathway and lipid metabolism. Charged residues on the hydrophilic side of the amphiphatic helix can render proteins more sensitive to the surface charge density of the target membrane and allows for a regulated membrane recruitment of the above-mentioned CCT1 and Pah1 to control PC synthesis and lipid droplet biogenesis, respectively [44]. Because the interfacial properties of membranes vary considerably between compartments (e.g., electrostatics, lipid unsaturation), some membrane-adsorbing amphipathic helices show striking features in their chemistry, which accentuate their sensing properties. For example, the amphipathic helix of α-synuclein, which targets the late secretory pathway, has a positively charged polar face mediating electrostatic interactions with the membrane surface and a hydrophobic face composed of small hydrophilic residues (valine, alanine, and threonine), which adsorbs preferentially to highly curved and polyunsaturated membranes [53,54]. Numerous proteins that preferentially associate with highly curved membranes of the late secretory pathway contain BAR domains, which are characterized by a banana shape with a positively charged surface interacting with the negatively charged membrane surface [55]. A classical example is endophilin, which is involved in various forms of endocytosis [56]. In summary, conditionally membrane-associated proteins can sense specific membrane properties and transduce these to fulfill central regulatory functions in the biosynthesis of lipids or in membrane traffic. By targeting membrane voids, amphipathic helices exhibit the inherent property to sense both membrane curvature and acyl chain composition at the same time [57].

Much less is known about integral membrane sensors involved in adaptive responses. It is clear, however, that the activity of many integral membrane proteins depends on the membrane environment [58,59]. Mechano-sensitive channels, for example, sense physical changes of the membrane and their activation is tuned by the acyl chain composition of membrane lipids [59]. Knowledge about the regulation of physicochemical membrane properties lags behind. In the following paragraphs, we will focus on acyl chain sensing and remodeling in simple model organisms in order to identify common features that might be used also in higher eukaryotes. Importantly, integral membrane proteins can sense deep within the hydrophobic matrix of the membrane and might report on the acyl chain composition of a membrane without being biased by the membrane curvature.

Homeoviscous Adaptation—a Collective Response to a Simple Perturbation

Even before the fluid mosaic model of the lipid bilayer was proposed [60], the first observation of acyl chain remodeling for thermal regulation had already been reported. At elevated temperatures *Escherichia coli* synthesize and incorporate an increased proportion of saturated acyl chains into membrane lipids [61] and maintain membrane viscosity over a broad range of growth temperatures [11]. This phenomenon was termed homeoviscous adaptation and has been described throughout various poikilothermic organisms. Remnants of the homeoviscous response have been observed even in warm-blooded organisms with a constant body temperature: the lipid composition in the outer extremities in the arctic reindeer, for example, is adapted to maintain membrane fluidity and function at low temperatures [62].

It is important to understand that the homeoviscous adaptation is not an isolated event focused exclusively on the lipid acyl chains. The cellular lipidomes of *S. cerevisiae* cultured at different temperatures differ significantly in their glycerophospholipid and

sphingolipid composition, as well as in their content of sterol [2,27]. The contribution of individual organelles to this adaptation remains to be systematically investigated. Until now, most studies have characterized the homeoviscous response with a specific focus on the acyl chain composition [8,63]. With contemporary quantitative lipidomics technology, it will be possible to follow the complex lipidome changes during the homeoviscous response in unprecedented detail.

Changes in temperature are not the only cue that cells have to cope with. The membranes of unicellular organisms, for example, are facing dramatic changes in surface tension and the lateral pressure profile during osmotic stress. In the following, we will discuss mechanisms employed by *E. coli*, *Bacillus subtilis*, *Synechocystis*, and *S. cerevisiae* to sense and control membrane viscosity.

Bacterial Strategies of Sensing—*E. coli*

The lipid composition of the *E. coli* plasma membrane is relatively simple and comprises three major lipid classes: PE, phosphatidylglycerol, and cardiolipin. The most abundant acyl chains of these lipids are palmitic (16:0), palmitoleic (16:1(9Z)), and vaccenic acid (18:1(11Z)) [18]. *E. coli* regulates membrane viscosity primarily *via* adjusting the incorporation of saturated and UFAs (Fig. 4a). FabA is the key enzyme for UFA biosynthesis *via* an anaerobic pathway introducing a *cis* double bond into a 10-carbon chain intermediate. In order to generate the most abundant UFAs found in

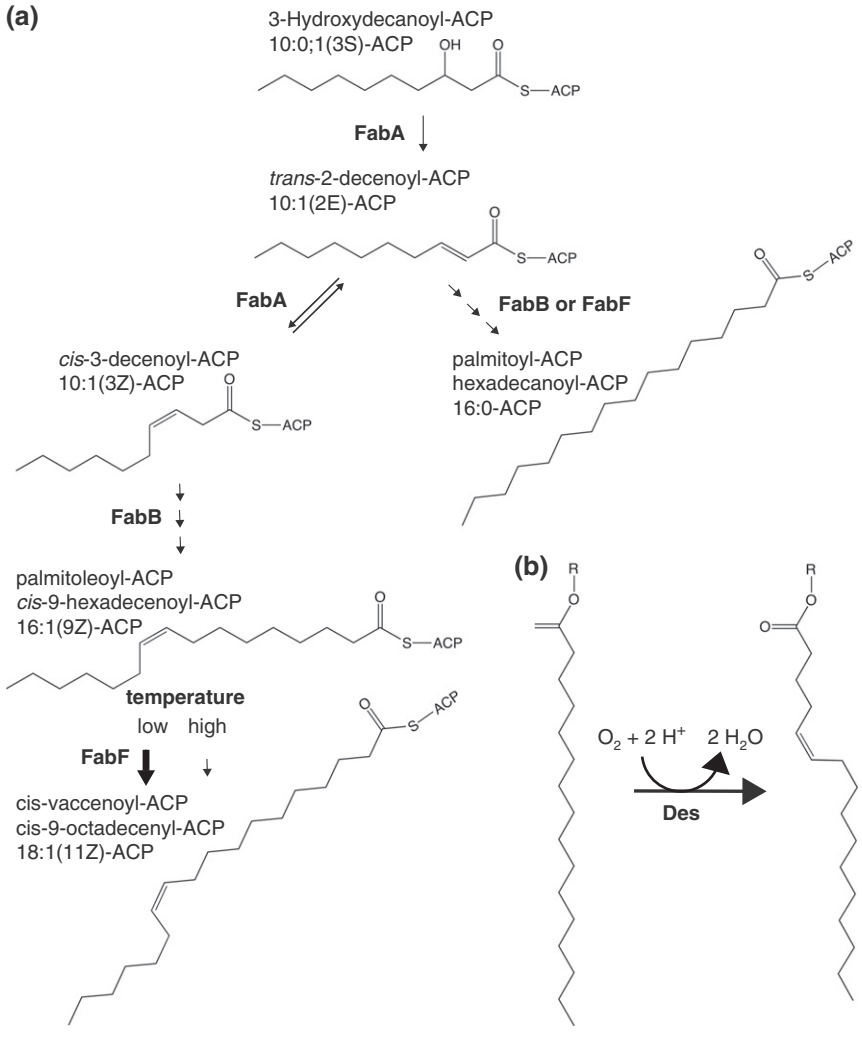

Fig. 4. Generation of unsaturated lipid acyl chains in bacteria. (a) The production of UFAs in *E. coli* is dependent of FabA, which introduces a double bond in a 10-carbon intermediate bound to an acyl carrier protein (ACP) and catalyzes a *cis–trans* isomerization between 10:1(2E)-ACP and 10:1(3Z)-ACP. The elongases FabB and FabF are required to generate abundant saturated and unsaturated acyl chains for membrane lipids. The production of 18:1(11Z)-ACP by FabF is particularly temperature dependent. (b) *B. subtilis* uses the oxygen-dependent lipid desaturase Des acting directly on the acyl chains of membrane lipids by introducing a Δ5 double bond.

membrane lipids, FabB and/or FabF elongate this intermediate. When the growth temperature of *E. coli* is increased, the proportion of lipids with unsaturated 18:1(11Z) chains decreases (Figs. 1b and 4a), while the proportion saturated 16:0 acyl chains increases [64]. Cells lacking the elongase FabF are not able to regulate the synthesis of 18:1(11Z) in a temperature-dependent fashion [65]. It was concluded that thermal regulation of membrane fluidity was an intrinsic property of FabF, a soluble protein, and that acyl chain remodeling in *E. coli* is critically dependent on FabF [66]. However, a closer look at the original data shows that cells lacking FabF still adjust the proportion of saturated and unsaturated acyl chains in membrane lipids with the growth temperature [65]. This suggests that thermal regulation in *E. coli* affects a step upstream and possibly the expression of fabA and fabB. The expression of these genes is controlled by the transcriptional repressor FabR, which binds to regulatory sites within their promoters. Binding of FabR to these sites is promoted by 16:1(9Z)-CoA and 18:1(11Z)-CoA and antagonized by 16:0-CoA [67]. Crystal structures of DesT, an ortholog of FabR from *Pseudomonas aeruginosa*, revealed the molecular basis of ligand controlled DNA binding [68]. This elegant mechanism reveals how UFA synthesis can be adjusted to the available pool of saturated and UFA-CoA. However, it does not explain the remarkable remodeling of the acyl chain profile during thermal adaptation. Thus, the hunt is on for an elusive membrane sensor controlling the production of UFAs in *E. coli*.

Substantial lipid acyl chain remodeling occurs also when *E. coli* enters stationary phase. When cultivated under optimal conditions up to 40% of the acyl chains in membrane lipids contain a cyclopropane group [69,70] rendering the cells more resistant to freezing and acid stress [71,72] (Fig. 1B). Cyclopropane FAs (CFAs) are produced by methylation of pre-existing UFAs by the CFA synthase. They are chemically more stable than a double bond without hampering membrane fluidity [71]. The CFA synthase can reversibly associate with synthetic liposomes and this interaction is controlled by the acyl chain composition of the membrane [73]. While saturated lipid acyl chains hinder membrane binding of the CFA synthase, unsaturated lipid acyl chains support it. Although the underlying molecular mechanism is not known, it is

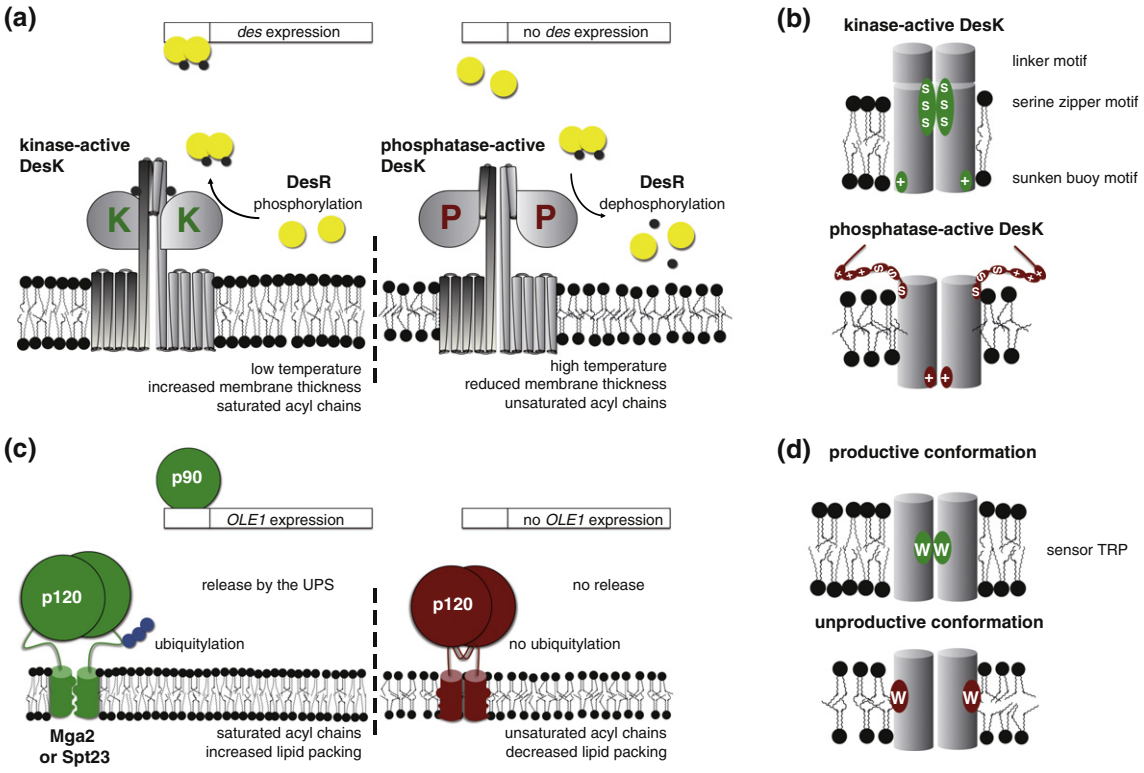

Fig. 5. Sensing and regulating membrane viscosity. (a) Schematic representation of the DesK/DesR system from *B. subtilis* regulating the expression of *des* encoding for a lipid desaturase. (b) The structural elements contributing to membrane sensing by DesK are highlighted. (c) Schematic representation of the OLE pathway from *S. cerevisiae* mediating the activation of Mga2 and Spt23. Alternative rotational configurations of the dimeric TMHs are transmitted to the site of ubiquitylation to control the proteolytic transcription factor activation. (d) The sensory tryptophan residue (W) is indicated to underscore the rotational movements in the TMH region.

tempting to speculate based on precedents in eukaryotes [74] and prokaryotes [75,76] that the synthase uses an amphipathic helix to mediate membrane association when sufficient unsaturated acyl chains are available in the membrane.

The molecular sensors underlying the homeoviscous adaptation in *E. coli* remain largely unknown, but there is evidence that the biosynthesis of UFAs is tightly regulated. Given that bacteria use two-component systems as multifunctional sensors to adapt to environmental cues, we speculate that one or several histidine kinases might sense and regulate membrane viscosity in *E. coli*.

Bacterial Strategies of Sensing—*B. subtilis*

The membrane of *B. subtilis* harbors DesK as a sensory system controlling glycerophospholipid desaturation. DesK is a dimeric histidine kinase with five transmembrane helices (TMHs) and a cytosolic kinase/phosphatase domain [10] (Fig. 5a). In the cold, DesK switches from a phosphatase-active to a kinase-active state [77]. It undergoes autophosphorylation and activates DesR, a transcriptional regulator of the *des* gene. The Δ5-Des lipid desaturase encoded by the *des* gene converts saturated lipid acyl chains to unsaturated ones (Fig. 4b). When membrane fluidity is restored, DesK switches back to its phosphatase-active state, dephosphorylates and inactivates DesR, and stalls the production of the lipid desaturase. A series of studies have shed light on the sensing mechanism of DesK and established a critical role of membrane thickness. DesK switches to the kinase active state at low temperature or when the proportion of saturated acyl chains is increased [10,78]. Both conditions induce membrane thickening. Thus, membrane thickness might serve as a proxy for both the ambient temperature and the lipid composition.

The mechanism of membrane sensing by DesK has been studied using a minimal sensor composed of a single, chimeric TMH fused to the kinase/phosphatase effector domain. The activity of this dimeric and minimal construct mimicked the activation of full-length DesK, implying that the key sensory elements were preserved [79]. Three structural elements were identified that cooperate to regulate DesK (Fig. 5b): (i) the sunken buoy motif close to the water–membrane interface in the N-terminal part of TMH1 [79], (ii) a serine zipper motif in the C-terminal part of the TMH5 [80], and (iii) a linker motif in the juxta-membrane region that connects the membrane embedded sensor region with the effector domains [81]. A cold-induced increase of membrane thickness hides polar residues (the buoy) of the sunken buoy motif in the hydrophobic core of the membrane and cooperates with the serine zipper motif to stabilize a specific rotational arrangement of the dimeric TMHs. The linker motif on the cytosolic side might undergo a folding-unfolding transition and help to transmit the signal when membrane thickness increases [80]. All three structural elements cooperate in stabilizing alternative, rotational arrangements of the dimeric TMH. The signal is propagated *via* an elongated 2-helix bundle to the kinase/phosphatase domain, switching it between the kinase- and phosphatase-active states [10].

A difficult question is the mode of action by which unsaturated lipid acyl chains generated by the Δ5-Des lipid desaturase switch DesK back to its phosphatase-active state. Is it a change in membrane thickness induced by lipid desaturation or a change of the lateral pressure profile? An interdisciplinary approach addressing the role the lipid acyl chain composition both *in vivo* and *in vitro* paired with molecular dynamics simulations is most promising to reveal new insights into the physiological stimuli underlying the activation and inactivation of DesK.

Bacterial Strategies of Sensing—*Synechocystis*

Similar to *B. subtilis*, phototrophic cyanobacteria use a two-component system to regulate the expression of a desaturase and to control membrane viscosity. The histidine kinase 33 (Hik33) from *Synechocystis* was identified as key regulatory element for the cold-inducible expression of the *desB* gene encoding a desaturase [82,83]. Importantly, Hik33 controls not only FA desaturation but also more than two dozens of cold-inducible genes, suggesting that the homeoviscous adaptation is part of a larger cellular program [82].

Hik33 contains several conserved structural domains: A HAMP domain (named for the typical occurrence in *h*istidine kinases, *a*denyl cyclases, *m*ethyl-accepting chemotaxis proteins, and *p*hosphatases) [84], a leucine zipper domain that may have a similar role in signal transduction to the 2-helix bundle in DesK [8], and a PAS domain (occurring in *P*er, *A*rnt, *S*im sensor proteins) acting as a light-sensitive module in Hik33 [85]. The HAMP domain contains two adjacent helical regions transducing cold stress signals *via* coordinated structural changes. The structural characterization of an archaeal HAMP domain suggested that signal transmission is mediated by homo-dimeric, four-helical, parallel coiled-coils that can switch between two alternative modes of inter-helical packing [86]. An increased molecular lipid packing was suggested as the prime signal for Hik33 activation [8], but the underlying sensor mechanism remains to be established. A systematic screen for structural elements affecting Hik33 function with a specific focus on the TMD would represent a logic step toward a better understanding of the homeoviscous adaptation in this phototrophic bacterium.

A eukaryotic Mechanism of Sensing—*S. cerevisiae*

Eukaryotic cells have compartmentalized biochemical processes in organelles. A stressed eukaryotic cell faces the problem of maintaining membrane viscosity without destroying organelle identity and function. This poses an additional challenge to the homeoviscous adaptation and highlights a specific demand for tightly controlled membrane surveillance systems.

The genome of *S. cerevisiae* encodes only a single and essential FA desaturase, Ole1, introducing a Δ9 double bond in CoA-activated 16:0 and 18:0. The level of Ole1 is tightly controlled by several inter-dependent mechanisms [9,87,88]. Loss of *OLE1* expression is lethal within few cell divisions accompanied by severe morphological changes of cellular organelles [89]. The expression of *OLE1* is controlled by two transcription factors embedded in the ER-membrane *via* a C-terminal TMH: Mga2 and Spt23 [9]. For activation, these transcription factors are released from the ER-membrane *via* a pathway referred to as the OLE pathway (Fig. 5c). The membrane-bound precursors of ~120 kDa (p120) are recognized by the E3 ubiquitin ligase Rsp5 and become ubiquitylated and proteolytically processed by the cytosolic proteasome. This releases an active transcription factor of ~90 kDa (p90) that enters the nucleus and induces the expression of *OLE1* [90].

OLE1 is not the only target of regulation by Mga2 and Spt23. Highly expressed genes involved in ribosome biogenesis, glycolysis, and lipid metabolism are controlled by these transcription factors [91]. Mga2 has been implicated in the hypoxic response, adaptation to oxidative stress, and zinc homeostasis [92,93]. Moreover, Mga2 and Spt23 have been implicated in the regulation of sterol biosynthesis [94]. The concomitant loss of *MGA2* and *SPT23* causes synthetic lethality that is reversed by UFA supplementation [89]. Thus, despite the broad spectrum of target genes, *OLE1* appears as the most critical target of Mga2 and Spt23 regulation.

The activation of Mga2 and Spt23 is controlled by the membrane environment and can be suppressed when the growth medium is supplemented with UFAs [90,95]. The sensor mechanism of dimeric Mga2 involves dramatic rotational motions of the TMH contributing to the dimer interface [95]. Due to these motions, the dimeric TMHs explore a large conformational space forming a continuum of dimer interfaces. The alternative rotational orientations are not separated by high-energy barriers such that minute changes in the energy landscape, e.g., changes in membrane lipid saturation, can substantially affect the balance between alternative conformations. A membrane with a high proportion of saturated acyl chains stabilizes a productive rotational orientation allowing for downstream transcription factor activation, while that with a high proportion of unsaturated acyl chains stabilizes Mga2 in a non-productive orientation [95] (Fig. 5c and d).

The dynamic behavior of the sensory TMHs seems to rely on three structural features conserved both in Mga2 and Spt23: (i) The TMHs are particularly rich in aromatic residues distributed over the whole TMH, thereby facilitating helix:helix interactions in several relative rotational configurations. (ii) A conserved proline residue introduces flexibility to the helix backbone that maximizes the interfacial area between two rotating monomers. (iii) A conserved tryptophan residue situated deep within the lipid bilayer senses fluctuations in lipid packing caused by unsaturated acyl chains, which generate transient membrane voids (Fig. 5d). If these voids occur more frequently, the bulky tryptophan side chain can be accommodated, thereby stabilizing an orientation of the TMH, where the tryptophane points toward the membrane environment. In a more densely packed bilayer, the bulky tryptophan is less adapted to the membrane environment, thereby rotating to 'hide' in the dimer interface.

Membrane saturation sensing by Mga2 relies on specific surface properties of the sensory TMHs. In contrast to the prokaryotic DesK, Mga2 is not sensitive to membrane thickness. Eukaryotes including *S. cerevisiae* might have established a distinct sensing mechanism for a specific reason. The membrane of the ER as the entry point of the secretory pathway is tuned to accept proteins with TMDs of different thicknesses and the ER lipid composition minimizes the energetic costs of a hydrophobic mismatch [19,21]. This might explain why eukaryotic cells had to establish a new, sensitive sensing mechanism that does not rely primarily on membrane thickness.

Common themes in the Homeoviscous Response

From the few examples of homeoviscous adaptation in enterobacteria, bacillaceae, cyanobacteria, and fungi, we might be in better position to identify new sensors in the near future and to decipher their mode of action. We propose that the following lines are likely to apply to many different organisms.

Membrane sensing

The homeoviscous adaptation relies to a large extent on *de novo* membrane lipid biosynthesis and acyl chain remodeling. Increasing evidence suggests that prokaryotes and eukaryotes use both soluble proteins that monitor sensing membrane surface properties and integral membrane proteins exploring the hydrophobic membrane core. It is tempting to speculate that most, if not all cells, use a direct and

stringent feedback mechanism with integral membrane sensors to maintain membrane viscosity. This does not exclude accessory mechanisms sensing specific, soluble lipid metabolites exemplified by the FA-CoA binding transcription factor FabR from *E. coli* and its orthologue DesT from *P. aeruginosa* [18]. Highly specialized cells such as neurons or photoreceptor cells in higher eukaryotes would have to use specific sensory and regulatory circuits for their specific needs.

Signal integration

There is substantial crosstalk between stress responses and pathways relevant for the homeoviscous adaptation. Given the global impact of membrane viscosity on cellular homeostasis and signaling, it is not surprising that the homeoviscous adaptation integrates various inputs. Many forms of cellular stress such as hypoxia, cold-, heat-, osmotic-, or oxidative stress perturb membrane properties and possibly the membrane lipid composition. In this light, membranes serve as platform for signal integration. The effects of hypoxia on the membrane lipid composition, for example, accumulate over time due to an impaired biosynthesis of sterols and UFAs. This remodeling of the acyl chain composition can take minutes to hours. Thus, the membrane lipid composition contains information of oxygen availability over a relatively long period of time and is harnessed to control the expression of desaturases as part of a widespread, transcriptional response for adaptation.

Rotation-based mechanisms

The sensing mechanisms of DesK and Mga2 suggest that rotational motions of the TMHs are key triggers of the downstream responses. Rotation-based mechanisms underlying signal transduction are not unprecedented in prokaryotes and eukaryotes [86,96–98]. It is new, however, that signal transduction can be triggered by subtle changes in the acyl chain composition of membrane lipids. The rotational motions of interacting α-helices seem particularly sensitive to small perturbations in their energetic landscape and ideally suited as membrane sensing mechanism.

Position of sensing and pressure profiles

The sensors of the homeoviscous adaptation use specific structural elements to probe the bilayer and the position of these elements determine the sensitivity of the system. They can be situated deep within the lipid bilayer, rendering it particularly sensitive to the acyl chain packing but less sensitive to membrane thickness. Here, Mga2 serves as a first paradigm [95]. Other sensors use structural elements situated closer to the cytosol–membrane interface rendering them particularly sensitive to membrane thickness as observed for DesK from *B. subtilis*. Yet another group of sensors use amphipathic helices to probe lipid packing, surfaces properties, and membrane curvature [17]. The examples discussed here show that cells use structurally very different sensors to probe the packing properties of membranes at various depths. As such, they can probe the chemical and structural diversity of the membrane lipid acyl chains. The interplay of distinct classes of sensors exploring surface properties and/or the hydrophobic core of a membrane is particularly interesting in eukaryotic cells, where the lateral pressure profile of the membrane critically contributes to organelle identity and function.

Summary and perspectives

The homeoviscous adaptation identified in prokaryotes has been inspiring to investigate and understand some principles underlying collective adaptation of membrane lipid composition. Clearly, an acyl chain centric view on the homeoviscous response has its limitations and the general validity has been critically discussed here and elsewhere [63]. Eukaryotic cells have different constraints than prokaryotes. Notably, the ER and the plasma membrane have different functions: The former is a factory for lipid biosynthesis and membrane protein integration, while the plasma membrane serves as a barrier. Lipids with monounsaturated acyl chains are crucial for ER function, but hamper plasma membrane integrity. Consequently, their homeostasis cannot follow the same rules.

The concept of lipid-specific shapes is valid for most but not all lipids. Lipids with polyunsaturated acyl chains resemble contortionists and are involved in forming specialized cellular structures [54]. The formation and maintenance of membranes with unique physicochemical properties is crucial for cellular function and necessitates sophisticated mechanisms of sensing. Membrane sensors can sense the packing properties of membranes either at the interface, for example, by long amphipathic helices, or within the hydrophobic core of the membrane by the packing-dependent rotation of sensory TMHs.

The complex and dynamic nature of biological membranes requires an emphasis on multidisciplinary approaches spanning cell biology, biochemistry, mass spectrometry-based lipidomics, and computational biology in order to identify novel membrane sensors in different organelles that sense and communicate membrane properties to control large-scale cellular adaptations. Subcellular lipidomics combined with lipid flux analysis will be an invaluable tool for such undertakings in the future. We are convinced that there are still a large number of unidentified membrane sensors in different

organelles surveying physicochemical membrane properties to regulate the lipid metabolic network.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (ER608/2-1 and SFB807 "Transport and Communication across Biological Membranes"; R.E.) and the VILLUM FONDEN (VKR023439; C.S.E.). The authors acknowledge Susanne Wolf for her help in preparing the figures.

Received 24 June 2016;
Received in revised form 5 August 2016;
Accepted 8 August 2016
Available online 14 August 2016

Keywords:
Homeoviscous adaptation;
Membrane homeostasis;
Regulation of lipid metabolism;
Lipid packing;
Membrane fluidity

Abbreviations used:
FA, fatty acid; TMD, transmembrane domain; PS, phosphatidylserine; UFA, unsaturated fatty acid; PA, phosphatidic acid; CDP-DAG, cytidine diphosphate diacylglycerol; DAG, diacylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; CCT, CTP:phosphocholine cytidylyltransferase; ALPS, amphipathic lipid packing sensor; CFA, cyclopropane fatty acid; TMH, transmembrane helix.

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