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Reign in the membrane: How common lipids govern mitochondrial function

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Abstract

The lipids that make up biological membranes tend to be the forgotten molecules of cell biology. The paucity of data on these important entities likely reflects the difficulties of studying and understanding their biological roles, rather than revealing a lack of importance. Indeed, the lipid composition of biological membranes has a profound impact on a diverse array of cellular processes. The focus of this review is on the effects of different lipid classes on the function of mitochondria, particularly bioenergetics, in health and disease.

Introduction

The mitochondrion is a fascinating organelle comprising inner mitochondrial membranes **(IMM)** and outer mitochondrial membranes **(OMM)** that serve distinct and no overlapping functions. Though the lipid composition of these structures has typically received little attention, a recent flurry of publications has revealed that mitochondrial lipids modulate the energy producing and cell death controlling functions of this organelle. Herein, we will discuss the mechanisms linking lipids to changes in cellular bioenergetics and other mitochondrial functions in normal physiology and will explore how disruption of the mitochondrial lip dome influences the progression of cardio metabolic disease.

Oxidative phosphorylation **(OXPHOS)** consists of a sequence of reactions by which energy from electron donors is transduced to generation of a proton motive force that culminates in the synthesis of **ATP**. Inefficiency in the **OXPHOS** system can induce pathology by impairing bioenergetics, creating redox imbalances, and promoting oxidative stress. In particular, compromised mitochondrial function is regarded as a hallmark in obesity-related

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metabolic syndrome [1]. Evidence is rapidly mounting that this and similar disorders are caused by a pathological accumulation of specific intra cellular lipids [2,3]. One consequence of such lipid imbalances is an alteration in the lipid milieu of mitochondrial membranes, specifically the **IMM** wherein the **OXPHOS** enzymes reside. The phenotypes caused by human mutations that perturb **IMM** lipids directly are severe, sometimes fatal, and are characterized by mitochondrial dysfunction, including impaired respiration [4–7]. Therefore, it is reasonable to hypothesize that the **IMM** lipid imbalances associated with metabolic disorders might have significant consequences for mitochondrial electron flux and efficiency also.

The biophysical effects of altered membrane lipid composition on the **OXPHOS** system are complex and multifaceted. The **IMM** primarily consists of a few classes of abundant phospholipids (phosphatidylcholine **[PC]**, phosphatidylethanolamine **[PE]**, cardiolipin **[CL]**, and phosphatidylinositol **[PI])**, with the remainder consisting of other complex lipids that are of relatively low abundance (sphingolipids, cholesterol, and others). The effects of perturbing a few of the individual lipid classes, such as PE and **CL**, on electron transport and **OXPHOS** enzymes are relatively well described [8,9]. However, the various perturbations in multiple classes of lipids that might occur in a complex disorder such as obesity are very difficult to model and therefore the consequences are more poorly defined. Moreover, there are several lipid species (such as ceramides) whose abundances are low but that exhibit potentially profound effects on **OXPHO**S that remain poorly described. In addition to directly influencing protein function via lipid protein interactions, the lipid composition of the **IMM** also modulates cristae morphology [10], mitochondrial fusion and fission [11,12], the assembly of the **OXPHOS** complexes into super complexes [13], metabolite transport, and lipid micro domain formation [14], each of which indirectly affects bioenergetics.

The direct or indirect effects of lipids on the **OXPHOS** system can be compartmentalized into four distinct nodes as follows (Figure 1) [15]: (1) the initial donation of electrons from NADH and succinate to complex I and II; (2) electron transfer from complex I and II to complex III and then complex IV, whereas protons are pumped into the intermembrane space, (3) maintenance of the proton motive force across the **IMM** in the face of mechanisms that carry protons down their con centration gradient back into the mitochondrial matrix (mitochondrial uncoupling), and (4) proton current flux through complex V to drive **ATP** synthesis. In step 1, although the efficiency of electron donation from NADH or succinate to the electron transport system (ETS) is not known to be affected, the supply of NADH and succinate is completely controlled by the rate of import of substrates such as pyruvate via the mitochondrial pyruvate carrier or fatty acids via carnitine palmate transferase and their oxidation in the tricarboxylic acid cycle. These import processes can be affected by **IMM** lipid composition. In step 2, electrons can prematurely ‘jump’ to molecular O_2 or other ac- captors before doing so in a highly controlled manner in complex IV and their propensity to do so appears to be dramatically increased when the efficiency of electron transport is low. For example, electron stalling in complex III is thought to lead to reverse electron transfer in complex I, which then enables electrons to reduce O_2 thereby contributing a large fraction of the reactive oxygen species burden [16]. The oxidants that result initiate the oxidative stress cascade and also have important

signaling roles. Membrane lipids may directly affect electron transfer efficiency within each respiratory complex, as well as by modulating electron transfer between the complexes. This is profoundly affected by formation of ETS super complexes and the lateral infusibility of electron carriers, which are modulated by **IMM** lipid composition. In step 3, the proton gradient may be modulated by opening/closing of cristae junctions or other membrane dynamics events, which can produce subdomains of the intermembrane space that can achieve a higher proton concentration. In contrast, the proton gradient can become dissipated by proton current flux independent of complex V, which is commonly referred to as 'proton leak'. Both of these can be affected by the **IMM** lipid composition. In fact, the predominant uncoupling protein (UCP1) is thought to be directly activated by lipids [17,18]. Step 4 is stoichiometric ally fixed [19], and its activity is dependent on total cellular work (DG_{ATP}). There is currently no evidence that **IMM** lipids have positive or negative effects on the energetic efficiency of **ATP** production per proton flux.

In this review, we shall summarize our limited understanding regarding the lipid composition of mitochondrial membranes, how these lipids are generated and transported into mitochondria, and their effects on physiology.

Lipid composition of mammalian mitochondria

The lipid composition of mitochondrial membranes is distinct from any other organelle. In liver, mitochondria consist of 34–55% PC, 19–36% PE, 12–23% CL, 5–8% PI, 1% phosphatidylserine (PS), 1e2% lysophospholipids, 1e3% sphingomyelin (SM), and 1e2% phosphatidic acid (PA) [20]. In comparison, skeletal muscle mitochondria consist of ~40% PC, ~30% PE, ~15% CL, ~7% PI, ~3% PS, ~3% Lysol-PC, and ~2% SM [21]. The variability in reported data, potentially arising from differences in mitochondrial purity and mass spectrometry procedures, makes it difficult to conclude whether there are meaningful differences in mitochondrial membrane lipids between different tissues. It is also unknown whether there are tissue-dependent relationships between membrane lipid composition and mitochondrial functions [15].

Unlike the plasma membrane, cholesterol and SM are not major constituents of mitochondria. Because these lipids are largely responsible for the formation of micro domain in the plasma membrane, it is unclear to what extent mitochondrial membranes form lipid domains analogous to 'lipid rafts' formed at the cell surface. Nonetheless, it is clear that some lipid classes segregate to form domains of a particular curvature or orientation. Mitochondria are highly abundant in PE and CL, both conically shaped no bilayer lipids that stabilize the negatively curved inner leaflet in cristae (Figure 2). CL is highly abundant only in mitochondria and has been traditionally used as a marker of mitochondrial content. The lipid composition of mitochondria is kept distinct from other organelles by the hydrophilic barrier of the cytosol, specific lipid carriers at mitochondrial contact sites, and mitochondria resident lipid-synthesizing enzymes.

Mitochondria consist of two phospholipid bilayers: the **OMM** and the **IMM**. The **OMM** is lipid-rich, relatively smooth, and highly fluid, whereas the **IMM** is protein rich, extensively folded, and highly compartmentalized. The phospholipid composition of the liver **OMM** is

44–59% **PC**, 20–35% **PE**, 5–20% **PI**, with the remaining phospholipid pool consisting of **PS, PA, CL**, and lysophospholipids [20]. By contrast, the **IMM** consists of 38–45% **PC**, 32–39% **PE**, 14–23% **CL**, 2–7% **PI**, with the remaining consisting mostly of **PS, PG**, and lysophospholipids. In the following context, we discuss the pathways of phospholipid and sphingolipid biosynthesis, and their potential roles in modulating mitochondrial bioenergetics.

Glycerophospholipids

Pathways for phospholipid biosynthesis differ in some aspects between mammalian cells and unicellular or genism's such as yeast (Figure 3). In both cases, the endoplasmic reticulum (ER) is the major hub for phospholipid biosynthesis. As intermediates in the pathway of triglyceride synthesis, which also occurs at the **ER, PA**, and diacylglycerol **(DAG)** serve as precursors for the remaining seven classes of phospholipids **(PC, PE, CL, PI, PS, PG**, and **CDP-DAG**). In yeast, PA is the predominant precursor, which is converted to **CDP-DAG** by the enzyme Cds1 **(ER**) or Tam41 (mitochondria). In yeast, all classes of phospholipids can be derived from **CDP-DAG**. In contrast, mammalian cells appear to not possess the ability to generate PS from **CDP-DAG** (reaction mediated by Cho1), making the **PA/CDP-DAG** pathway precursors only for **CL, PI**, and. **PG** Instead, **PC, PE, PS** are generated through the Kennedy Pathway, by which **DAG** is converted to **PE** or **PC** by reaction with **CDP**-ethanolamine or **CDP**-choline, respectively [22]. **PE** or **PC** can then be converted to **PS** by **PSS1** or **PSS2** [23,24]. Yeast also has the Kennedy pathway, but appears to not possess the **PSS1** and **PSS2** enzymes to synthesize **PS** from **PE** and **PC**. It is unclear how or why mammals relegate the synthesis of **PC, PE, PS** to the **CDP** ethanolamine or **CDP**-choline route, rather than also having the **CDP-DAG** route.

It is believed that **ER**-synthesized phospholipids are a major contributor to the lipid content of mitochondria. In both mammals and yeast, phospholipid molecules are thought to be transported into mitochondria primarily at mitochondrial-associated membranes sites of the **ER**, which are sites of close apposition between mitochondria and **ER** that have a unique protein composition and function [25–27]. However, mutants for mitochondrial associated membranes assembly do not demonstrate gross impairment in mitochondrial membrane biogenesis, suggesting an alternate route [26]. Instead, lipids may also enter mitochondria through lysosomes via vacuole and mitochondria patch (**clamp**) [28,29]. Phospholipids are transported across these contact sites by class-specific lipid carriers such as **PRELID1**/**Ups1 for PA** [30,31], **STARD7** for **PC** [32], and **PRELID3b/ Ups2** for **PS** [33,34]. For reasons that are currently unknown, **PE** generated at the **ER** does not seem to be a significant contributor to mitochondria lipid content [35]. Rather, mitochondrial **PE** is generated by **PS** decarboxylase (**PSD**) which resides in the **IMM** [8,36,37]. **CL**, a phospholipid mostly unique to mitochondria, is also generated in the **IMM** by a sequence of reactions [38,39] that involves combining two phospholipid intermediates (**PG** and **CDP-DAG**) to generate a four-acyl chain phospholipid, which is catalyzed by **CL** synthase **(CLS**). Many of the phospholipids, but especially **CL**, undergo trans acylation reactions that appear to be critical for their proper function in mitochondria as human mutations in this pathway are lethal [4].

Metabolic insults perturb the phospholipid milieu in key metabolic tissues. In the liver, progression of nonalcoholic fatty liver disease coincides with robust changes in the cellular lipedema in mice [40–42] and in humans [43,44]. However, although some studies have linked these lipid signatures to changes in mitochondrial function [40,41], there are currently no reports that describe lipidomic changes that occur specifically in the mitochondria. In skeletal muscle, obesity or exercise induces changes in the cellular lipedema in mice [45,46], rats [47,48], and humans [49,50]. Similarly, it is unclear to what extent these changes reflect or elicit changes to the mitochondrial lipidome. We recently reported perturbations that occur in the muscle mitochondrial lipidome in response to exercise or inactivity [8] with changes in **PE** and **CL** being the common features. In mouse brown adipose tissue, cold exposure promotes a disproportionately large increase in **CL** content, suggestive of mitochondrial phospholipid remodeling [39]. It is unknown whether these changes in mitochondrial phospholipids represent an adaptive (or maladaptive) mechanism to support the bioenergetics or other metabolic demands in these situations or whether they are merely epiphenomena of metabolic rewiring that occurs upon these interventions.

What is the evidence that mitochondrial phospholipids affect the bioenergetics of the organelle? Phospholipids are known to directly interact with the enzymes of the **ETS** and to affect their activities. **CL** is tightly and specifically bound to Complex I and sustains its structural integrity [51], whereas **PC** and **PE** are more loosely bound and modulate the catalytic activity of complex I. Similarly, **PE** binds to complex II [52], **PE** and **CL** to complex III [53,54], **PC, PE**, and **CL** to complex IV [55], and **CL** to complex V [56]. **PE** and **CL** are also important for the individual **ETS** complexes to assembly into super complexes [57,58], which are believed to facilitate the efficient transfer of electrons. In addition, **PE** and **CL** also regulate proton leak by interacting with **UCP**1 [17,18] and the **ATP/ADP** carrier [59,60]. **IMM** lipids also indirectly affect bioenergetics by modulating cristae morphology [11], the formation of cristae junctions [10], as well as mitochondrial fusion, fission, and mycophagy [11,12,61], all of which can affect the establishment and maintenance of the proton gradient (J_M). It is also conceivable that mitochondrial lipids regulate mitochondrial substrate flux through metabolite transporters of the mitochondrial carrier family and carnitine palmitoyltransferase-1 [62]. Thus, phospholipid composition of the **IMM**, particularly the content of **PE** and **CL**, has strong implications for the activity and efficiency of **OXPHOS**.

Human mutations that promote loss of mitochondrial **PE** or **CL** are characterized by oxidative stress and have devastating pathological consequences. Recent studies identified families with mutations in the ***PISD*** gene (which encodes the **PSD** enzyme) that lead to severe mitochondrial dysfunction and are associated with congenital cataracts, short stature, facial dysophism, platyspondyly, ataxia, and intellectual disability [5,6]. Whole-body deletion of the mouse gene encoding the **PSD** enzyme causes embryonic lethality [36]. Mitochondria from embryonic fibroblasts of these mice are swollen, rounded, and fragmented, consistent with the idea that **PE** is important for cristae development and overall mitochondrial morphology. In **CHO** cells, reducing **PSD** expression decreases the activities and/or abundance of **ETS** complex I, II, and IV, super complex formation, and **ATP** synthesis [37]. Skeletal muscle specific loss of **PSD** promotes rapid loss of muscle mass

[8,63] that culminates in diaphragm failure and lethality [8]. In the liver, a defect in mitochondrial **PS** transfer promotes nonalcoholic steatohepatitis [64]. Together, these studies demonstrate that mitochondrial **PE** is absolutely required not only for normal mitochondrial function but also for prenatal and postnatal overall health.

There are two known human genetic defects attributed to defects in enzymes of mitochondrial **CL** metabolism. Barth syndrome is an **X**-linked genetic disorder that is caused by a mutation in the **TAZ** gene, which encodes a **CL** transacylase [4]. Affected individuals lack mature **CL** and have abnormal mitochondrial cristae and reduced oxidative capacity in heart and skeletal muscle [65]. A mouse model of Barth syndrome recapitulates many of the human disease phenotypes [66]. Sengers syndrome is an autosomal-recessive disorder caused by a mutation in the gene that encodes the mitochondrial acyl glycerol kinase (**AGK)** enzyme [7]. Individuals with Sengers syndrome suffer from congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance, and lactic acidosis. It is thought that the disease-causing mutation leads to a loss of **AGK** enzymatic activity, decreasing the synthesis of mitochondrial **PA**, an important precursor for **CL** synthesis [7]. It is noteworthy that these defects in **CL** deficiency likely reflect a combination of effects on **OXPHOS** system, as well as on mycophagy and apoptosis [67,68] Other studies in genetically modified mice have also linked CL biosynthesis to mitochondrial function [69,70]. Adipose tissueespecific inactivation of CLS caused robust metabolic abnormalities that culminated in defects in adipose tissue thermogenesis and whole-body glucose homeostasis [39]. It is likely that other tissues will also have interesting and important phenotypes upon perturbation of CL synthesis and remodeling.

The physiological consequences of changes in other lipid classes are more difficult to interpret, as such changes are often not specific to mitochondria. For example, because mitochondrial **PC** is generated by enzymes located in the **ER**, it is not possible to specifically eliminate or modulate mitochondrial **PC**. Deletion of the genes encoding the **ER** enzymes that synthesize **PC** perturbs phospholipid composition throughout the cell, leading to confounding and complex phenotypes. Nevertheless, they can provide some insights regarding the roles of these lipids in mitochondria. A rare congenital muscular dystrophy disease in humans is caused by homozygous or compound heterozygous mutations in the gene encoding the choline kinase-β [71].

These individuals have reduced **PC**, abnormally enlarged mitochondria, and early-onset muscle wasting, muscle weakness, and hypotonic. A defect in choline kinase-β also leads to muscular dystrophy in mice, with swollen mitochondria that have lower membrane potential [72]. Altogether, it is clear that mitochondrial phospholipids play an essential role in supporting, and perhaps controlling, mitochondrial respiratory flux and efficiency. In turn, it is of significant interest to identify mechanisms by which **IMM** phospholipids are themselves modulated in response to metabolic insults and thereby regulate **OXPHOS** flux and efficiency or other aspects of mitochondrial function.

Sphingolipids

Despite being minor constituents of the mitochondrial liposome [73], sphingolipids particularly C16 ceramides potently affect cellular bioenergetics (Figure 4). They accrue in inner and outer mitochondria membranes under conditions of nutritional overload, serving as signals of lipid excess that influence the properties of this important organelle [74].

In mammals, sphingolipid production involves a 4-step biosynthetic cascade that converts fatty and amino acids into ceramides, the precursors of the most complex sphingolipids (e.g. **SMs**, gangliosides, etc.). In the first step in the pathway, serine palmitoyltransferase condenses serine and palmitoyl- **CoA** to 3-ketodihydrosphingosine, which is a transient intermediate. The enzyme 3-ketodihydrosphingosine reductase rapidly converts this molecule into dihydrosphingosine to create the basic structure of the sphenoid backbone. One of six (dihydro)ceramide synthases (**CERS1–6**) adds a variable acyl chain to the scaffold, with each enzyme differing in substrate specificity and tissue distribution [75]. The **CERS**6 enzyme produces the mitochondrial **C16** ceramides that predominantly influence function of the organelle [76]. The final reaction, catalyzed by dihydroceramide desaturase 1 **(DES1**), inserts a double bond into dihydroceramides to produce the more abundant ceramides [77]. In selected tissues such as the skin and the gut, a different desaturase isoform **(DES2**) inserts a hydroxyl group, rather than inserting the double bond. This reaction produces phytoceramides that are important for barrier function.

In most tissues, ceramides are the first major species of sphingolipid to accumulate and are major regulators of cellular metabolism and energetics. Though de novo sphingolipid synthesis occurs predominantly in the **ER**, scattered reports suggest that some enzymes in the pathway reside in mitochondria, including **CERS1,2,4**, and **6** and neutral sphingomyelinase [78]. Therefore, mitochondrial sphingolipids might be regulated independently of other organelles in response to signaling or metabolic cues.

SM, the most abundant sphingolipid, is a cylindrical lipid that includes a choline head group (Figure 2) [79]. Though the head group is similar to **PC**, the hydrophobic regions are very different. For one thing, they frequently have a mismatch in acyl chain lengths. This leads to interdigitating of the fatty acids which is implicated in forming ‘metastable’ membrane micro domains [80]. They have a much higher melting temperature than **PC**, for example. Moreover, the ceramide moiety of **SM** binds cholesterol much more tightly than glycolipids bind cholesterol. This likely contributes to the phase separation that promotes the formation of rafts. In the absence of the choline head group, the ceramides displace cholesterol and pack tightly with one another, leading to the speculation that they form stable membrane platforms, and perhaps even channels in the mitochondrial outer membrane [81,82].

Studies conducted in cultured cells or isolated mitochondria demonstrate that ceramides inhibit electron transport chain activity and induce formation of reactive oxygen species. The initial experiments were carried out with the no physiological short-chain ceramide analog **C2**-ceramide [83,84], but similar observations were later made using naturally occurring sphingolipids or following experimental manipulations to influence the endogenous sphingolipid pool. For example, Zig don et al. [85] found that treating isolated mitochondria

with **C16** ceramide, sphingosine, or sphingosine led to inhibition of complex IV. By comparison, very long chain ceramides (e.g. **C24**-ceramides) failed to affect complex activity. Richer et al. [86] determined that overexpression of **CERS6**, which produces mitochondrial **C16** ceramides and inhibits complex II. Similarly, inhibition or knockdown of sphingomyelinase synthase-2 led to accumulation of ceramides and impairments in mitochondrial respiration [87]. In many of these studies, the interventions were shown to also decrease the **IMM** potential and **ATP** production and increase reactive oxygen species.

Similar findings were also reported in mice undergoing experimental manipulations, targeting the sphingolipid pool. (a) Knockout of the mouse ceramide transport protein **CERT**, which carries ceramides from the **ER** to the Golgi for **SM** production, increases mitochondrial ceramide levels while reducing complex IV activity [88]. (b) **CERS2**-null mice undergo massive remodeling of the hepatic sphingolipid pool, including a marked upregulation of **C16**-ceramides. These animals exhibit impairments in complex II and IV, increased oxidative stress, and a profound hepatopathy. (c) Mice lacking one copy of the Cers2 gene exhibit impairments in hepatic complex I, II, and IV and susceptibility to hepatic insulin resistance and steatosis, upon challenge with a high fat diet [86]. In this latter study, the effects of **CERS2** haploinsufficiency were reversed by treating with the serine palmitoyltransferase inhibitor muricin, suggesting that the accumulation of C16-sphingolipids drove the mitochondrial pathology. (d) Mice lacking **CERS6** in the whole body, liver, or brown adipocytes have increased mitochondrial oxygen consumption and protection from hepatic steatosis and insulin resistance [76,89]. (e) Pharmacological inhibition or genetic depletion of **CERS**1, the major **CERS** isoform in skeletal muscle, increases muscle respiratory capacity [90,91].

The mechanism(s) by which C16 ceramides influence the **IMM** and respiratory complex activity remain elusive. Researchers have speculated that **C16** ceramides, which have unique biophysical properties [81], might influence the hydrophobicity and other biophysical properties of the membrane and therefore influence complex activity or stability [92]. However, little experimental support for this idea has thus far emerged. Scientists have also recommended that the lipid might serve as an allosteric effector of individual respiratory chain complexes. Using a biotin-labeled **C6**-ceramide coupled with a proteomics approach, Kota et al. [93] identified a complex IV subunit as a putative ceramide binding protein, but the functional consequences of this interaction have not been elucidated. Hammer Schmidt et al. [89] used a **SILAC**-based proteomics approach to identify sphingolipid-binding proteins, using the functional sphingosine analog pacSph to identify proteins that interacted with sphingolipids produced by **CERS6** but not **CERS5**. They identified mitochondrial fission factor as a downstream effector linking ceramides to an alteration in mitochondrial morphology and diminution of respiratory capacity [89]. Indeed, they determined that **CERS6** depletion produced fused, very large, and highly efficient mitochondria; mitochondrial fission factor was essential for these effects [89]. These studies support prior observations by Smith et al. [94], who reported that fission was requisite for the impairment in oxygen consumption caused by short-chain ceramide analogs in vitro. Nonetheless, these ceramide effects on fission are unlikely to fully explain the effects of the lipid on cellular

bioenergetics, as sphingolipids have acute effects on the isolated organelle that cannot be downstream of effects on mitochondrial **OMM** dynamics.

When ceramide levels increase to even higher levels, they cause profound mitochondrial impact that go beyond energetic impairment to cell death. Highly elevated **C16-**ceramide induces mitochondrial outer membrane permeabilization **(MOMP)**, thus promoting cytochrome c release to drive apoptosis [95]. Birbes et al. [96] found that overexpressing a mitochondrial targeted sphingomyelinase, which produces mitochondrial ceramides, induced apoptosis in culture lines. By comparison, overexpressing sphingomyelinase constructs directed to other organelles had no effect. Jain et al. [97] similarly demonstrated that diverting **ER** ceramides from the Golgi apparatus to the mitochondria (i.e. by overexpressing a mitochondrial-targeted ceramide transfer protein) induced Bax-dependent apoptosis. Convincingly, inhibition of enzymes in the ceramide biosynthesis pathway negates the effects of many proapoptotic stimuli [78].

Several mechanisms have been proposed to link mitochondrial ceramides to **MOMP**. Ceramides have been shown to promote translocation of the proapoptotic protein **BAX** to mitochondrial membranes, functioning synergistically with the protein to induce oligomerization that promotes **MOMP** [98]. Voltage-dependent anion channel 2 has also been identified as a mitochondrial ceramide binding protein that enhances **MOMP** [99]. Siskind et al. [100–103] have also reported that **C16** ceramides are sufficient to form channels that directly allow passage of molecules such as cytochrome c. The channels rely on the formation of hydrogen bonds between ceramides, which cannot occur between the dihydroceramides [103,104]. The ant apoptotic protein **BCLXL1** disrupts formation of these ceramide channels [105–107]. Ceramides have also been shown to alter signaling pathways that provide ant apoptotic signals, such as the prosurvival protein kinase **Akt/PKB** [108], but it is unclear whether these effects emanate from ceramides localized in mitochondria.

Although the paragraphs aforementioned discuss sphingolipid actions on mitochondria using mammalian models, several groups have additionally probed this interaction in yeast. Notably, yeast sphingolipids are considerably different than those found in mammals, comprising phytoceramides in place of ceramides and mannose diinositolphosphoceramide in place of complex lipids such as SM and gangliosides. Nonetheless, deletion of genes involved in sphingolipid production in Saccharomyces cerevisiae often alters mitochondrial morphology and function, producing phenotypes such poor growth on no fermentable carbon sources or by the appearance of petite colonies [109,110]. Additional work is necessary to determine downstream mechanisms and to tease out the functional differences between yeast sphingolipids and their mammalian counterparts.

These ceramide actions have clinical implications in the context of obesity or inflammation. For example, inhibition of ceramide production in mice reverses insulin resistance and hepatic steatosis [74]. These actions are attributable, at least in part, to changes in mitochondrial energetics. Moreover, ceramide induction of apoptosis contributes to diabetes and heart failure, by killing pancreatic beta cells and cardio myocytes, respectively [94]. Because of these observations, pharmaceuticals targeting ceramide biosynthesis are emerging as attractive potential therapies for a broad spectrum of cardio metabolic disorders.

Concluding remarks

Membrane lipids are abundant and fairly difficult to both analyze and manipulate with precision. As a result, our understanding of the biological impact of these common molecules dramatically lags behind other small and large biological molecules. We have clear evidence that the quantity and quality of membrane lipids have profound effects on mitochondria, particularly on the complex processes that underlie OXPHOS. We are still at the very beginning in our development of a mechanistic understanding of these effects. As that knowledge evolves in concert with an enhanced ability to quantitate mitochondrial lipids in different physiological and pathophysiological states, we will be better able to define (and hopefully someday use) these complex interactions to manipulate mitochondrial efficiency and function.

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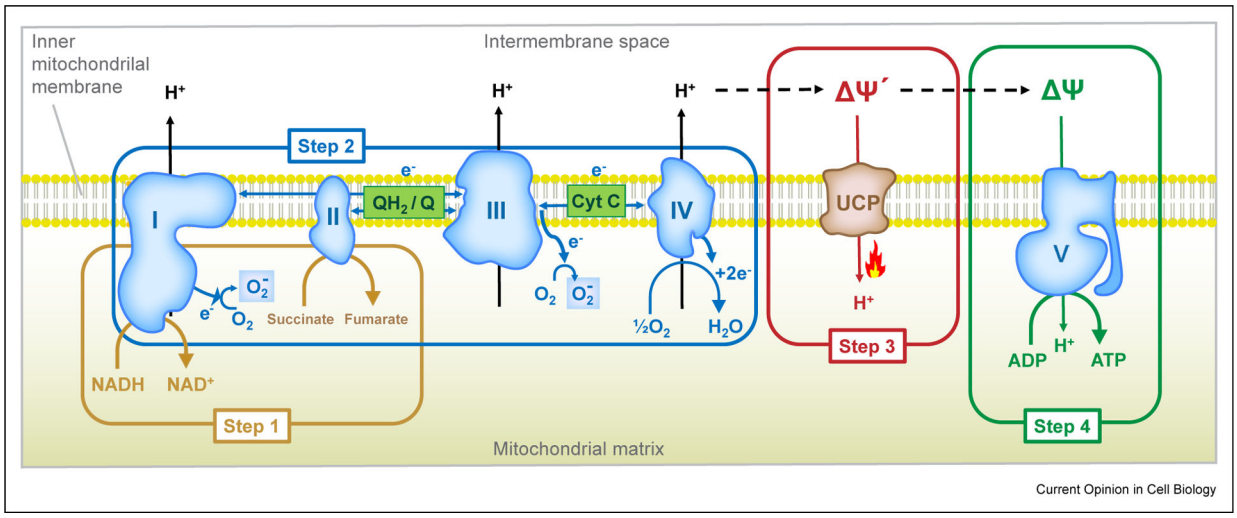

Figure 1. Four steps of ETS energy transduction.

Step 1: NADH or succinate donates electrons to complex I or II, respectively. Step 2: Electrons undergo sequential chain reactions within complex I whose action drive its proton pumping. Complex II does not possess a proton pumping activity. Both complex I and II donate the electrons to coenzyme Q and transfers them to complex III. Complex III also channels energy from the electrons to pump protons and hand them over to cytochrome C that carries them to complex IV. Energy from the electrons is again extracted in complex IV to pump protons, after which they are accepted by molecular oxygen to become water. Step 3: Protons pumped by complex I, III, and IV becomes permeated in the intermembrane space. The proton gradient across the inner mitochondrial membrane is determined by the number of protons pumped divided by the volume of the intermembrane space which can be affected by closing of cristae junctions or mitochondrial fusion/fission. Proton gradient can also become dissipated by uncoupling proteins. Step 4: Cellular work (ADP/ATP) drives the complex V to channel the proton motive force for ATP resynthesis. Cyt C, cytochrome C; QH_2/Q, qunol/quinone; UCP, uncoupling protein; ETS , electron transport system.

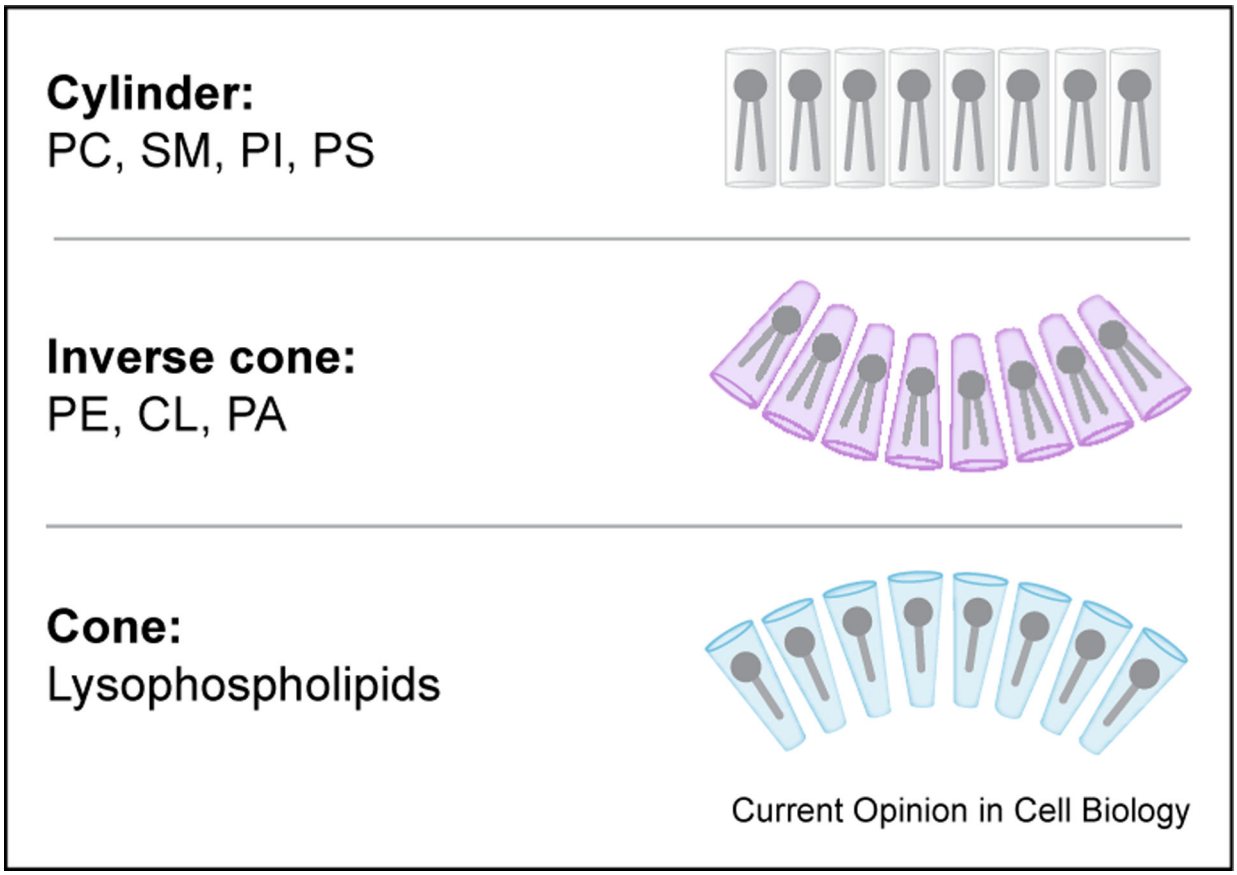

Figure 2. Shapes of lipids and their effects on membrane curvature.

Cylindrical lipids such as PC, SM, PI, and PS produce the planar portion of the membrane bilayer. In contrast, PE, CL, PA, and lysophospholipids are cone-shaped nonbilayer forming lipids that produce positive or negative curvature in the membranes. PC, phosphatidylcholine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; CL, cardiolipin; PA, phosphatidic acid.

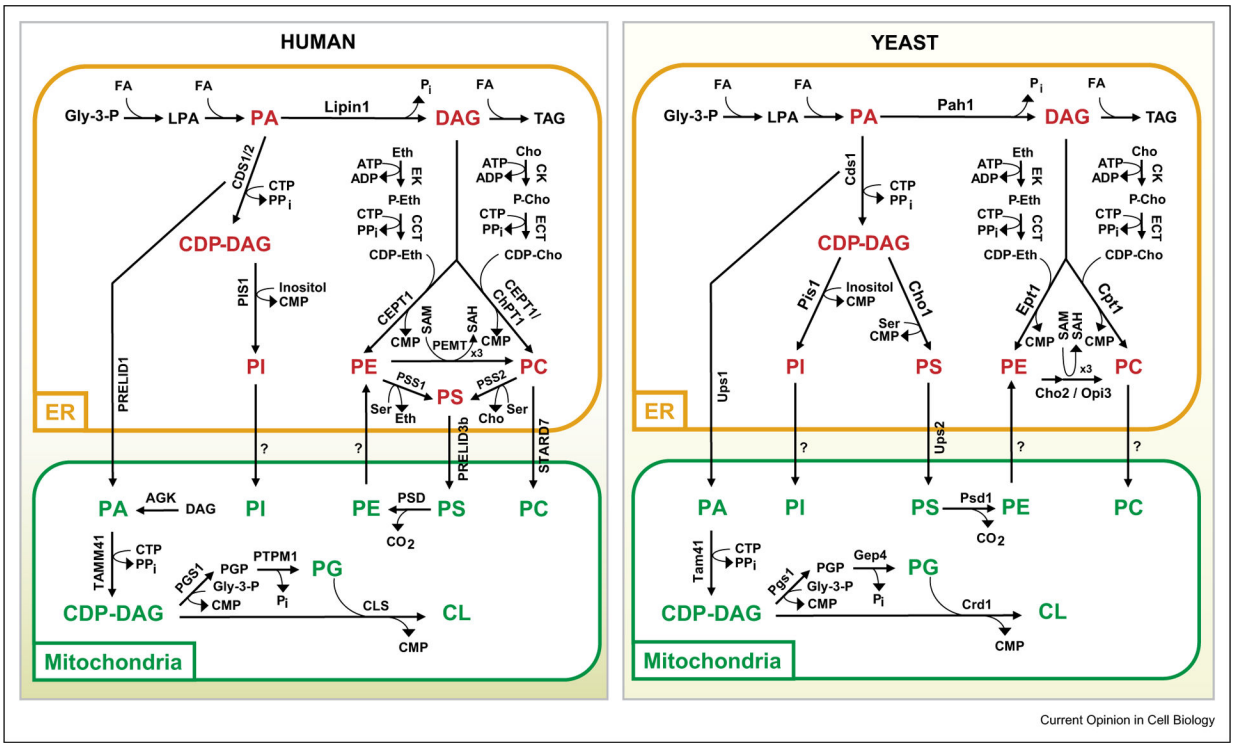

Figure 3. Pathways for phospholipid biosynthesis in human and yeast.

Synthesis of phospholipid branch out of the pathway of triglyceride biosynthesis which occurs at ER. All glycerophospholipids are generated from PA or DAG. In yeast, all phospholipids can be generated from PA, whereas mammals do not possess the ability to generate PE, PC, PS from PA. Rather mammals rely on the Kennedy pathway for their syntheses. Lipids generated at ER are transported to mitochondria by class-specific lipid carriers. Mitochondria are highly enriched in CL and PE, aided by presence of IMM-resident enzymes that generate these lipids. AGL, acylglycerol kinase; CCT/Pct1, CTP:phosphocholine cytidylyltransferase; CDP, cytidine diphosphate; CDS1/2/Cds1/ TAMM41/Tamm41, CDP-diacylglycerol synthase; CEPT1, choline/ethanolamine phosphotransferase; Cho, choline; ChPT1/Cpt1, choline phosphotransferase; CK/Cki1, choline kinase; CLS/Crd1, cardiolipin synthase; DAG, diacylglycerol; ECT/Pkt1, CTP:phosphoethanolamine cytidylyltransferase; Eth, ethanolamine; EK/Eki1, ethanolamine kinase; Ept1, ethanolamine phosphotransferase; ER, endoplasmic reticulum; FA, fatty acid; Gly-3-P, glycerol 3-phosphate; Lipin1/Pah1, PA phosphatase; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT/ Cho2/Opi3, PE methyltransferase; PG, phosphatidylglycerol; PGP, PG phosphate; PGS1/ Pgs1, PG synthase; PI, phosphatidylinositol; PIS1/Pis1, PI synthase; PS, phosphatidylserine; PSD/psd1, PS decarboxylase; PSS1/2/Cho1, PS synthase; PTPM1/Gep4, PGP phosphatase; PRELID1/Ups1/ Mdm35, mitochondrial PA transfer protein; PRELID3b/Ups2/Mdm35, mitochondrial PS transfer protein; STARD7, steroidogenic acute regulatory protein–related lipid transfer domains 7; TAG, triacylglycerol.

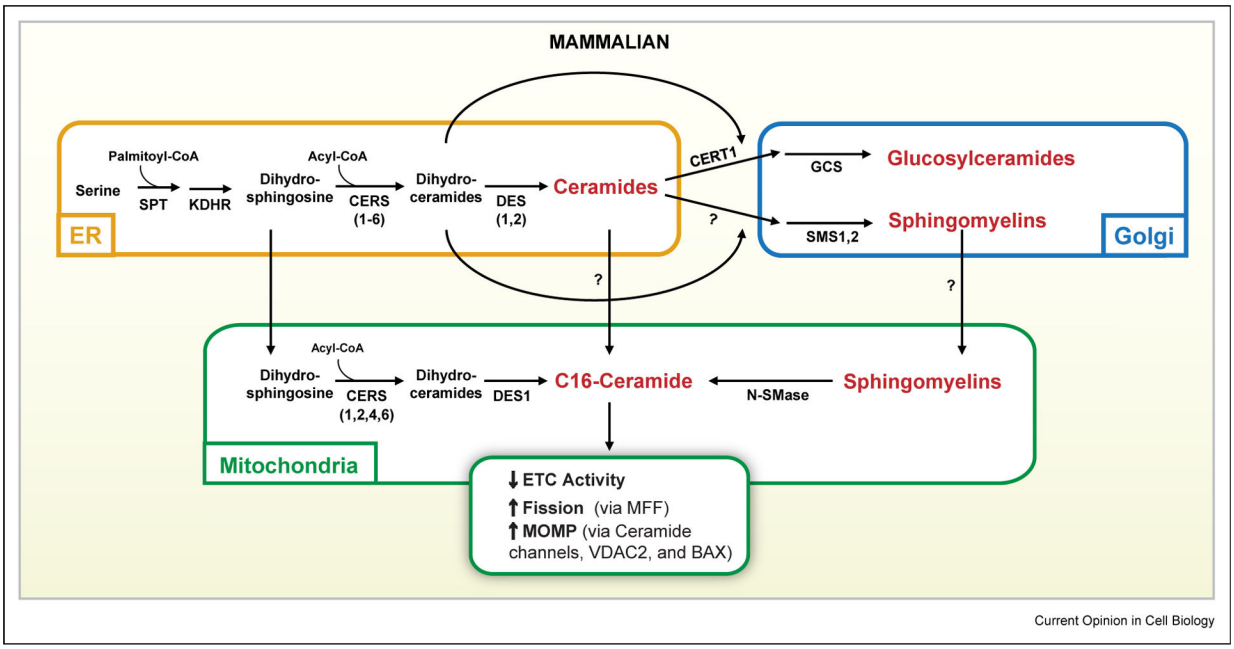

Figure 4. Schematic depicting the influence of sphingolipids on mitochondrial function.

The early steps of the sphingolipid biosynthesis pathway occur in the ER, where acyl-CoAs and amino acids combine to form the dihydroceramides and ceramides that are scaffolds for complex sphingolipids. These lipids then move to the Golgi apparatus, where additional modifications produce the majority of species comprising the cellular sphingolipidome. A subset of studies suggests that some of the early biosynthetic steps may occur in mitochondria, though this remains and active area of investigation and debate. SPT, serine palmitoyltransferase; KDHR, 3-ketodihydrosphingosine reductase; CERS ceramide synthase; DES, dihydroceramide desaturase; SMS, sphingomyelin synthase; GCS, glucosylceramide synthase; N-SMase, neutral sphingomyelinase; ETC, electron transport chain; MOMP, mitochondrial outer membrane permeabilization.