Cellular and Molecular Life Sciences (2018) 75:1909–1927
https://doi.org/10.1007/s00018-018-2765-4

REVIEW

Lipids in the cell: organisation regulates function

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Received: 9 October 2017 / Revised: 4 January 2018 / Accepted: 29 January 2018 / Published online: 9 February 2018

Abstract

Lipids are fundamental building blocks of all cells and play important roles in the pathogenesis of different diseases, including inflammation, autoimmune disease, cancer, and neurodegeneration. The lipid composition of different organelles can vary substantially from cell to cell, but increasing evidence demonstrates that lipids become organised specifically in each compartment, and this organisation is essential for regulating cell function. For example, lipid microdomains in the plasma membrane, known as lipid rafts, are platforms for concentrating protein receptors and can influence intra-cellular signalling. Lipid organisation is tightly regulated and can be observed across different model organisms, including bacteria, yeast, *Drosophila,* and *Caenorhabditis elegans,* suggesting that lipid organisation is evolutionarily conserved. In this review, we summarise the importance and function of specific lipid domains in main cellular organelles and discuss recent advances that investigate how these specific and highly regulated structures contribute to diverse biological processes.

Keywords Lipid rafts · Raft-like microdomains · Oxidative stress · Cell signalling · Lipophagy

Abbreviations

ATP	Adenosine triphosphate
Cav-1	Caveolin-1
CDP-DAG	Cytidine diphosphate-diacylglycerol
CL	Cardiolipin
CMA	Chaperone-mediated autophagy
ER	Endoplasmic reticulum
FCS	Fluorescence correlation spectroscopy
FMM	Functional membrane microdomains
FRET	Förster resonance energy transfer
GSH	Reduced glutathione
GSSG	Oxidised glutathione
GP	Glutathione peroxidase
Hsp70	Heat shock protein 70
LDs	Lipid droplets
MT	Metallothionein
mTOR	Mammalian target of rapamycin
$NADP^+$	Oxidised nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NOS	Nitric oxide synthase
PA	Phosphatidic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PI3P	Phosphatidylinositol-3-phosphate
PI-PLC	Phosphatidylinositol-specific phospholipase C
PLEP	Phospholipid exchange protein
PLTP	Phospholipid transfer protein
PrP^c	Cellular prion protein
PS	Phosphatidylserine
ROS	Reactive oxygen species
S1P	Sphingosine-1-phosphate
SCP	Sterol carrier protein
SOD	Superoxide dismutase
SMase	Sphingomyelinase
SPH	Sphingosine
TNFR1	Tumour necrosis factor receptor 1

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Introduction

Lipids are essential components of all cells, playing important roles that include cellular stabilization and signalling. Lipid composition varies across cell types, tissues, and in each organelle, suggesting that different lipid compositions are required for different functions [1]. Increasing evidence demonstrates that lipids are capable of specific organisation in each compartment, and this organisation is essential for regulating lipid functions [2]. Changes in the composition and organisation of lipids can have profound effects on cellular functions, including signal transduction, membrane plasticity, and membrane trafficking [3].

Cholesterol is one of the most important regulators of lipid organisation, and tightly controlled mechanisms maintain cellular cholesterol levels in membranes that regulate cholesterol trafficking [4]. Dysregulation of this balance can lead to disease, such as Niemann–Pick type C [5] or atherosclerosis [6]. Phospholipids (glycerophospholipids and sphingolipids) are another important cellular building block, and modification of their cellular levels also can lead to pathogenic processes, such as Alzheimer's disease.

While studies of the organisation and function of lipids in the plasma membrane continue to increase in the literature, and the definition of lipid rafts is now well established, the concept of nuclear lipid microdomains and mitochondrial raft-like microdomains are not as widely appreciated by the scientific community. This specific organisation of lipids in the nucleus and mitochondria is essential for regulating basal physiological processes, including mitochondrial respiration, regulation of apoptosis, cell proliferation, and transcriptional processes [7–10]. A better understanding of the function of lipids can be achieved by manipulating them at the cellular and subcellular levels via chemical sequestration experiments (i.e., methyl-β-cyclodextrin) or perturbation of biosynthetic enzymes (i.e., statins) [11–14]. However, these methods are not specific to lipid localization and organisation, so a main future research challenge will be to regulate lipid levels in a spatial and temporal manner and to target them in specific organelles.

Initially, lipid rafts were thought to be present exclusively in eukaryotes, with prokaryotes devoid of complex lipid-protein organisation [15]. However, recent evidence suggests that lipid raft-like membrane microdomains, called functional membrane microdomains (FMM), are essential elements of the prokaryotic cell membrane. These microdomains appear to play crucial roles in various cellular processes that are important for their survival [16] including signal transduction, membrane trafficking, and metabolic regulation. Moreover, homologs of Flotillin-1, a specific biomarker of eukaryotic lipid raft, can be found in bacterial membrane microdomains, which further confirm that lipid raft or lipid-protein microdomains are not exclusive properties of eukaryotic cell membranes. Rather, they also are essential structural and functional features of the prokaryotic membrane [17, 18]. In this context, a study using the bacteria, *Borrelia burgdorferi*, found that despite having a different composition, the process of forming lipid rafts is identical in both prokaryotes and eukaryotes [19]. Thus, the presence of lipid rafts or similar membrane microdomains in different model organisms reveals that the formation and functionality of these structures are evolutionarily conserved features of the cells. Given the comparative simplicity of bacterial cells, studies using bacteria as model organisms might help to clarify controversial aspects of the study of eukaryotic lipid rafts.

In this review, we briefly summarise the organisation of lipids (in terms of structure, composition, intra-cellular trafficking, and sorting) in the main cellular compartments to highlight the importance of this organisation for basic cellular processes, including apoptosis, autophagy, and intracellular signalling. We then focus on the development of new methods for studying lipid organisation, explore the limitations of current techniques, and examine the future steps required to enhance our knowledge of these specific cellular microdomains. Investigating these open issues in lipid organisation, distribution, and trafficking will contribute to a better understanding of the molecular and cellular basis of lipid-associated disorders.

Organisation of lipids in the eukaryotic plasma membrane

Subclass of lipid rafts: caveolae and planar lipid rafts

Lipid rafts are highly dynamic, nanoscale (< 200 nm), cholesterol- and sphingolipid-enriched membrane microdomains that are present in all eukaryotic cells. Since the formulation of the lipid raft hypothesis [20], hundreds of studies have reported different roles for these membrane microdomains in the organisation of cell signalling. Lipid rafts can act as concentrating platforms for individual receptors that are activated by ligand binding. If receptor activation takes place in a lipid raft, the signalling complex is protected from non-raft enzymes, such as membrane phosphatases, that otherwise negatively could affect the signalling process.

Lipid raft proteomics is the study of all proteins that use the raft assemblage for proper functioning, and it has been conducted primarily in hematopoietic cells, such as B- and T-cells and in a wide range of cancer cells [21,

22]. The interaction between rafts and cytoplasmic proteins can be seen in actin, which forms protein chains of cadherin–catenin–actin, or CD44–actin. This proteomic approach has quantified approximately 250 raft-associated proteins (Table 1).

One subclass of lipid rafts, discovered in the 1950s, is found in cell surface invaginations, called caveolae, which are formed by the polymerization of caveolins [23]. Caveolae are involved in endocytosis of different proteins, including albumin. Caveolae also play a role in signal transduction, but they are not essential, because several cell types lack caveolin, including lymphocytes and neurons. In animal models, disruption or deletion of caveolin-1 (cav-1) impairs nitric oxide and calcium signalling in the cardiovascular system, which causes fibrosis and thickening of lung alveoli [24–28]. Biochemical evidence indicates that *CAV1* is a tumour suppressor gene [29] and a negative regulator of many signalling proteins, including v-Src, H-ras, protein kinase A, and MAP kinase [30–34].

On the other hand, cav-1 can function as a tumour metastasis-promoting molecule. Cav-1 is overexpressed in human prostate cancer cell lines, mouse models, and human pancreatic tumours, and it is associated with poor clinical outcomes [35]. Interestingly, within tumour types derived from the same cell type or tissue, cav-1 expression levels are consistently upregulated or downregulated in the majority of cases [36–38]. Cav-1 typically is downregulated in ovarian [39], lung [40], and colon [41] carcinomas, whereas cav-1 is upregulated consistently in bladder [42], esophagus [43], thyroid [44], and prostate [45] carcinomas. The other common type of lipid rafts is planar lipid rafts (also referred as non-caveolar rafts). Planar lipid rafts share many of the features of caveolae (cholesterol- and sphingomyelin-rich cytoskeletal association), but they are not invaginated [46]. In addition to Cav-1 being important for caveolar rafts, flotillin proteins also are an indispensable prerequisite for raft formation in non-caveolar rafts [47]. Flotillins promote the co-assembly of activated and specific GPI-anchored proteins on plasma membrane microdomains, and they promote interaction of signalling molecules, including the Src family kinases [48–50].

Table 1 Main proteins associated with lipid rafts

Protein	References
Caveolin-1	[230–232]
α-Catenin	[233, 234]
β-Catenin	[233, 234]
Fas/CD95	[60, 62, 63, 235]
β-Actin	[230, 231, 236, 237]
Ras	[238, 239]
Integrin β-1	[240–243]
CD44	[244–247]

Pathophysiological function of rafts

Redox signalling

Many studies have demonstrated that lipid rafts play a crucial role in the redox signalling that regulates the pathophysiology of different degenerative diseases [51–53]. Large redox signalling molecules are aggregated into lipid rafts and can produce different types of reactive oxygen species (ROS). The type of lipid raft signalling capable of ROS production has been referred to as lipid raft redox signalling platforms. NADPH oxidase (NOX) is considered the main source of ROS signalling under physiological conditions. Lipid rafts provide an essential platform to aggregate and assemble the necessary subunits of NOX into an active enzyme complex that produces $O_2^{\cdot -}$ and other ROS [54, 55]. There are two types of NOX, known as phagocytic and non-phagocytic. Under physiological circumstances, non-phagocytic NOX expression is very low, and its activity is maintained at a basal level [56]. Unlike ROS produced in phagocytes that are mainly involved in host defences, ROS produced in non-phagocytes primarily serve as signalling messengers that directly or indirectly act on downstream effector proteins, such as protein phosphatase, protein kinase and many transcription factors. Stimulation with specific agonists, such as angiotensin II and platelet-derived growth factor, induces overexpression of non-phagocytic NOX. Assembly of active phagocytic NOX requires translocation of cytosolic subunits $p47^{phox}$ and $p67^{phox}$, as well as *Rac* to the plasma membrane, where these subunits interact with $gp91^{phox}$ and $p22^{phox}$ and associate with other co-factors in the membrane to form a functional enzyme complex. In the assembly and activation of NOX, lipid raft clustering represents an important mechanism that mediates activation [57, 58]. Superoxide production following cholesterol depletion was severely compromised in intact cells and correlated with a reduced translocation of cytosolic phox subunits to the plasma membrane [59]. Moreover, many studies have shown that lipid rafts participate in signalling of cell apoptosis associated with oxidative stress during activation of various death receptors, particularly lipid raft-localised Fas and tumour necrosis factor receptor 1 (TNFR1) [60–63]. Various death factors bind to their receptors in individual lipid rafts in endothelial cells and subsequently stimulate acid sphingomyelinase to produce ceramide from sphingomyelin [64, 65]. Furthermore, redox molecules by themselves can alter the formation of lipid raft platforms. For example, superoxide dismutase (SOD) decreases as $O_2^{\cdot -}$ increases, which forms ceramide-enriched membrane platforms in the membrane of coronary arterial endothelial cells [66]. Other studies have

shown that H_2O_2 activates pro-survival signalling pathways, including activation of PI3 kinase/Akt and ERK1/2, by a lipid raft-dependent mechanism [67].

Host–pathogen interactions

A wide variety of pathogens targets lipid rafts to infect host cells. As signalling for both the innate and adaptive immune responses is initiated in rafts, many pathogens have developed mechanisms to subvert this signalling by co-opting raft-associated pathways [68, 69]. Moreover, entry via lipid rafts can avoid lysosomal fusion and allow pathogen survival. Lipid rafts have been involved in bacterial entry of adhesins, including FimH + of *E. coli* [70], and pore-forming toxins, including proteins of the cholesterol-dependent cytolysins [71–73], and of intra-cellular pathogens, such as *Mycobacterium bovis* [74]. Among viruses that target lipid raft microdomains for entry into the cells, the best characterised has been SV-40, which co-opts these lipid microdomains to enter host cells and becomes established in the ER [75]. Compartmentalization of Ebola and Marburg viral proteins occurs within lipid rafts during viral assembly and budding [76]. Similarly, HIV-1 uses lipid rafts for entry, for signal transduction regulation, and for trafficking of HIV-1 proteins [77, 78]. Indeed, interest in lipid rafts as modulators of host–pathogen interaction has increased because of the discovery of high levels of sphingolipids and cholesterol in the HIV envelope [79]. Caveolae-like structures also have been reported in membranes surrounding the parasite *Plasmodium falciparum*, the agent of malaria [80]. However, despite caveolae and other lipid microdomains being identified as the sites of microbial action, the biological consequences of these interactions require further investigation.

Studying lipid rafts: evolution of techniques

Biochemical strategies

The first evidence for heterogeneity in the plasma membrane came from the observation that various membrane lipids solubilise in different ways. Specifically, the cellular membrane can be separated into two fractions: a detergent-resistant fraction that is enriched in cholesterol and sphingolipids and a detergent-soluble fraction that is not enriched [81]. However, the extraction procedure does not reflect the real composition of the membrane, because many detergents, including Triton X-100, can solubilise proteins associated with the membrane microdomains [82]. As a consequence, new sophisticated methods have been developed. For example, detailed studies of lipid microdomains in animal models have been performed using *Cav-1* null mice [25–27]. Mice lacking CAV-1 show no developmental abnormality or lethality other than an expected lack of cav-1 expression and plasmalemmal caveolae. In addition, cultured fibroblasts from *Cav-1* null mouse embryos also reveal loss of caveolin-2 expression and a hyperproliferative phenotype [26]. Furthermore, lung parenchyma from *Cav-1* null mice shows hyper-cellularity with a thickened alveolar septa and an increased number of vascular endothelial growth factor receptor-positive endothelial cells [26, 83]. The activity of nitric oxide synthetase (NOS) also is upregulated in *Cav-1* null animals, but this activity can be blocked partially with nitro-L-arginine methyl ester NOS inhibitors [84]. On the other hand, the lack of a spontaneous tumour formation and modest proliferation defects found in *Cav-1* null mice are reminiscent of several previously described mouse models lacking inhibitory cell cycle proteins [26]. For example, mice deficient in cyclin-dependent kinase inhibitor do not develop tumours, and their embryonic fibroblasts only displayed a modest proliferative advantage over the wild type [85]. The availability of a viable *Cav-1*-deficient mouse model will allow further investigations that evaluate specific functions of CAV-1 and caveolae organelles in vivo.

One useful approach in raft research involving cell signalling has been the manipulation of lipid raft constituents [46, 86]. This leads to protein dissociation from rafts that allows for straightforward detection using common methods used to analyse raft associations, including small-angle neutron scattering and Raman spectroscopy, which is a label-free technique that has been applied successfully to monitor changes in membrane domain composition [87, 88].

These methodologies have contributed greatly to our understanding of raft function in vivo and have highlighted the importance of lipid rafts as an entry platform for different types of viruses and bacteria [76, 89–92]. For example, the most widely used drugs used to disrupt rafts are β-cyclodextrins and cyclic oligosaccharides that remove cholesterol from the plasma membrane. The previous studies using these agents have shown that membrane cholesterol depletion induces apoptotic death in some cells [93], but they also can inhibit Fas-induced apoptosis, which is signalled through lipid rafts [62, 94]. Cholesterol is not the only component that can be targeted to limit bacterial and viral entry, as ceramide also is involved in the fusion of endosomes with lysosomes during the internalization of microbial pathogens in mammalian cells [95, 96]. An extensive review by Bagam and colleagues summarises the importance of ceramide rafts for bacterial and viral infection [97]. Therefore, new lipid raft-targeting agents have been used to systematically investigate how membrane composition affects cell signalling (Table 2).

Biophysical strategies

A variety of artificial membranes have been developed over the past few years, with the aim to simulate the

Table 2 Lipid raft-targeting agents

Drug candidate	Mechanism of action	References
Methyl-β-cyclodextrin	Cholesterol depletion	[11, 14, 86, 94, 132]
Dynasore	Cholesterol depletion/sequestration	[71, 192]
Pore-forming agents	Cholesterol sequestration	[248, 249]
Filipin, nystatin, amphotericin	Cholesterol sequestration	[250–252]
Statins	Inhibition of cholesterol biosynthesis	[12, 13, 253, 254]
Fumonisins, myriocin, lipoxamycin	Inhibitors of sphingolipid metabolism	[255–258]
Sphingomyelinase	Cholesterol displacement by ceramide	[259–261]

basic functions of a cell membrane, including biomimetic monolayers [98], suspended and supported lipid bilayers [99–101], tethered lipid bilayer [102–104], and giant unilamellar vesicles [105, 106]. Each has advantages and disadvantages with regard to ease of formation, membrane lifetime, and physiological limits of transmembrane proteins incorporation. These artificial membranes have provided important information on membrane structure/function and the nature of membrane–protein interaction [107]. Domains with raft-like properties were found to coexist with fluid lipid regions in different model membranes, including planar-supported lipid layers and giant unilamellar vesicles formed from equimolar mixtures of phospholipid–cholesterol–sphingomyelin [108]. Even though most of experiments have been performed in lipid-only systems, the incorporation of integral membrane proteins into artificial systems recently has been developed to better mimic the plasma membrane. A limitation of these techniques is that the artificial membranes occasionally show a high protein/lipid ratio that does not reflect the constitution of actual biological membranes protein content, which can be as high as 25% [109]. A possible solution to this problem is the use of giant plasma membrane vesicles, which are cell-derived plasma membrane vesicles that retain lipid and protein diversity typical of the cellular plasma membrane but do not contain an actin cytoskeleton connection [110–112]. Thus, there is a big opportunity to expand the range of applications of artificial membranes and to further improve their similarity to biological membranes.

Advanced microscopy strategies

Because lipid rafts are defined as nanoscopic domains (< 200 nm), they cannot be studied using the traditional microscopy methods. Co-localization of the cholera toxin with lipid rafts and visualization with confocal microscopy has been used extensively as a method to study these membrane microdomains [71, 113–115]. More advanced optical tools have been developed recently to investigate the direct dynamics of membrane organisation, including Förster's resonance energy transfer (FRET) microscopy. The FRET methodology employs the use of fluorescently labelled isoforms of biomolecules to detect extremely short-range interactions between the labelled species. It is a non-invasive methodology, because fluorescent tags necessary to observe FRET do not alter the function and distribution of the proteins associated with lipid rafts. It has been applied to both model membranes and live cells [116, 117]. Different reviews provide detailed descriptions of this technique for the study of lipid rafts [118, 119]. Fluorescence correlation spectroscopy (FCS) is another method that has been used extensively to characterise model and cellular membranes. FCS measures small fluctuations in fluorescence intensity in a defined volume. It provides accurate information about different parameters, including diffusion coefficients, intra-molecular dynamics, or molecular interactions, and it has been used in association with many imaging methods, such as laser scanning confocal microscopy or two-photon microscopy. Detailed reviews on the use of this technique for lipid rafts characterization also are available [120–122]. Electron microscopy has the necessary resolution to perform studies on lipid rafts, but, because it requires cell fixation and staining (limiting steps in lipid visualization), it is not the preferred technique. Currently, fluorescence microscopy remains the most advanced method for visualizing lipid membrane microdomains, and efforts are focused on optimizing fluorescent labels to further improve its precision.

Open issues and future perspectives

While the composition, regulation, and roles of lipid rafts have been thoroughly studies and brilliantly reviewed recently by Sezgin et al. [123], several aspects of raft structure and function require further clarification. For example, it is of primary importance to understand the exact composition of lipid rafts and how they vary in different cell types. Is cancer cell phenotype always characterised by a different composition of lipid rafts? How does this composition affect cell signalling and the resistance of tumour cells to chemotherapeutic agents? Answers to these questions are essential to understand the dynamic organisation of lipid bilayers and to develop an effective strategy based on lipid raft-targeting agents. The question of how single lipid rafts are crosslinked to form clustered rafts also is important for

understanding how lipid microdomains affect signal transduction. Real-time imaging and progress in microscopy will allow a better investigation of signalling complexes under normal conditions and after cholesterol depletion. The lack of specific fluorescent labels continues to limit investigations of membranes, because the behaviour of lipids is strictly dependent on their amphiphilic properties and molecular packing, both of which are affected by fluorophores [124]. Thanks to advances in microscopy, a recent study showed that molecules move in and out of lipid rafts at unexpectedly fast rates (i.e., sphingomyelins spend only 12–50 ms inside the lipid rafts before and after extracellular immunostimulation) [125], which can explain difficulties studying these membrane microdomains.

Novel mass spectroscopy methods are available to analyse proteins and lipids in membranes. A study combining super-resolution fluorescence and high-resolution ion mass spectrometry allowed the sphingolipid distribution across the plasma membrane to be characterised. Specifically, reduction in cellular cholesterol was shown to decrease the number of sphingolipid domains in the plasma membrane, whereas disruption of the cytoskeleton completely eliminated them [126]. Resolving controversies related to the organisation and dynamics of membrane microdomains will require the complementary use of lipidomic and proteomic analysis, in silico membrane modeling, and microscopy methods using probes with high spatial and temporal resolution.

Organisation of lipids in eukaryotic mitochondria

Raft-like microdomains and regulation of mitochondrial apoptosis

Lipid microdomains are not confined to the plasma membrane. Rather, they are present in other raft-like microdomain compartments, including mitochondria [9], endoplasmic reticulum (ER) [127], and nuclei [7]. Raft-like microdomains appear to be involved in a series of mitochondrial functions, including oxidative phosphorylation, ATP production and membrane "scrambling", which participate in cell death pathways and recruitment of proteins to the mitochondria. Due to recruitment of CD95/Fas and tumour necrosis factor family receptors to plasma membrane lipid rafts and recruitment of specific proapoptotic Bcl-2 family proteins to mitochondrial raft-like microdomains, lipid rafts play a key role in receptor-mediated apoptosis of T-cells [10].

Raft-like microdomains are analogous to lipid rafts on the plasma membrane, in that they represent preferential sites on the mitochondrial membrane where key reactions can be catalysed. CD95/Fas triggering recruits Bcl-2 family proteins, including truncated Bid, t-Bid, and Bax. Cardiolipin (CL) is an activation platform for caspase-8 translocation to mitochondria, it is a mitochondrial receptor for Bid, and it regulates oligomerization of Bax and insertion into the mitochondrial membrane [128]. In addition, the mobilization of cytochrome c, another key apoptotic protein, is regulated strictly by its interaction with CL. Therefore, CL is an essential constituent of functional domains, localised at contact sites between the inner and outer mitochondrial membranes, from which it regulates apoptosis by integrating signals from a variety of apoptosis-inducing proteins [128, 129]. The importance of CL is underlined by the observation that some bacterial proteins involved in cell division and oxidative phosphorylation are bound tightly to CL. Thus, targeting CL is emerging as a new antimicrobial strategy [130].

Other studies have suggested that proapoptotic members of the Bcl-2 family are associated with mitochondrial fission proteins during apoptosis. More specifically, after triggering apoptotic stimuli, molecules involved in mitochondrial fission are recruited into raft-like microdomains. The importance of this process is related to the fact that raft-targeting compounds, like methyl-β-cyclodextrin, reduce mitochondrial fission and apoptosis [131]. Treatment with methyl-β-cyclodextrin prevents mitochondrial depolarization and cytochrome C release and impairs mitochondrial bioenergetics [132]. As a consequence, the effects of regulating mitochondrial homeostasis and apoptosis suggest that raft-like microdomains could play a role in the pathogenesis of diseases with mitochondrial alterations, such as amyotrophic lateral sclerosis or Parkinson's disease, as well as cancer, where apoptotic triggering is a main therapeutic goal [133–135]. Of remarkable importance is the observation that during apoptosis, the cellular form of prion protein (PrP^c) can undergo intra-cellular re-localization, via ER-mitochondria-associated membranes and microtubular networks, to mitochondrial raft-like microdomains [136, 137]. This observation could imply that PrP^c may participate in the prion neurodegenerative cascade through mitochondria-mediated events and suggests a new potential therapeutic target based on the prevention of mitochondrial mis-localization of PrP^c to protect cells from mitochondria-mediated apoptosis. Further studies are needed to clarify the function of prion protein in mitochondria and during physiological conditions, as PrP^C was found in brain mitochondria in 6–12 week old wild-type and transgenic mice in the absence of any disease [138].

Mitochondrial lipids and oxidative stress

Oxidative stress and the resulting lipid peroxidation are involved in many pathological conditions, including inflammation, atherosclerosis, neurodegenerative diseases,

and cancer [139–141]. The term "oxidative stress" is used to describe imbalances in redox couples, such as those reduced to oxidised glutathione (GSH/GSSG) or NADPH/$NADP^+$ ratios. Alteration of these ratios determines overproduction of molecules that are enriched with one or more oxygen atoms that generally are considered to be markers of oxidative stress.

ROS are mainly responsible for the alteration of macromolecules, which is known as oxidative stress. ROS are generated as intermediate products of cellular metabolism primarily in the mitochondria, and include free radicals, such as superoxide anion ($O_2^{\cdot-}$), perhydroxyl radical ($HO_2\cdot$), hydroxyl radical ($\cdot OH$), and nitric oxide (NO). To prevent damage from ROS, cells possess several antioxidant enzymes, such as superoxide dismutases, that are located mainly in the mitochondria and convert superoxide into hydrogen peroxide. Catalase further catalyses the decomposition of hydrogen peroxide into water and oxygen.

Reactive intermediates produced by oxidative stress can alter membrane bilayers and cause lipid peroxidation of polyunsaturated fatty acids [142, 143]. Lipid peroxidation and breakage of lipids with the formation of reactive compounds can change permeability and fluidity of the membrane lipid bilayer and dramatically alter cell integrity and cell homeostasis (Fig. 1). Lipid peroxidation is particularly harmful to mitochondria, which contain CL as a main component

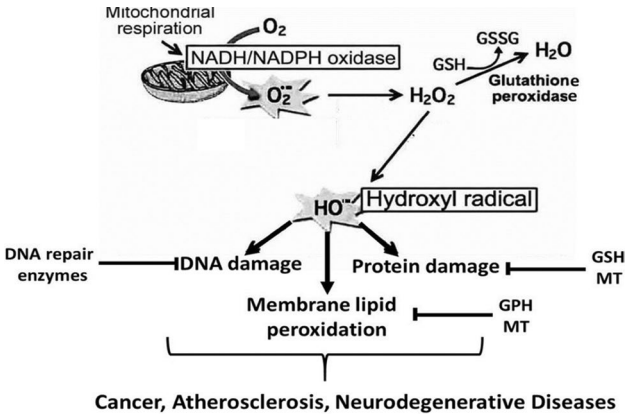

Fig. 1 ROS generation and lipid peroxidation. The main source of reactive oxygen species (ROS) is mitochondrial respiration: electrons leak from complexes I and IV and produce superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). The cellular detoxification system (SOD and GPx) scavenges ROS and converts them into final products (H_2O and O_2). If ROS are augmented considerably, the Fenton reaction can produce hydroxyl radicals ($HO\cdot$), the most damaging form of ROS. Hydroxyl radicals are responsible for damage to DNA and proteins, as well as membrane lipid peroxidation. Cells have developed protective mechanisms against these cellular threats, including DNA repair enzymes and enzymes, such as GPx, SOD, and MT. However, pathologies like cancer, atherosclerosis, and neurodegenerative diseases can arise when a serious imbalance between ROS production and ROS scavenging persists in an organism

of the inner mitochondrial membrane, because this lipid is required for activity of cytochrome oxidase [144]. Oxidative stress decreases CL levels to a larger extent than other lipids, decreasing cytochrome oxidase activity [145]. CL has been shown recently to provide an essential mitochondrial activating platform for caspase-8. More specifically, the role for CL in 'raft-like' microdomains could be to anchor caspase-8 at contact sites between inner and outer membranes, facilitating its self-activation, Bid cleavage, and apoptosis [128].

Open issues and future perspectives

Despite a significant progress in identifying key protein factors that control mitochondrial morphology through regulation of fission and fusion, advances in signalling of mitochondrial lipids is the essential step to understand the regulation of mitochondrial homeostasis [146]. In addition to the reported role as an activator of apoptosis, CL is important for recruitment of fusion- and fission-promoting proteins to mitochondria, including α-synuclein, which is involved in the pathogenesis of Parkinson's disease [147].

Another major lipid component of mitochondrial membranes is phosphatidylethanolamine (PE). PE plays important roles in mitochondrial fusion, as yeast lacking Psd1 develop PE deficiency in mitochondrial membranes, with an abnormal mitochondrial morphology characterised by extensive fragmentation and reduced ATP synthesis [148]. Given the important role of mitochondrial morphology in many cellular and physiological processes, further research on the roles of lipids in fusion and fission may produce novel findings to help explain the pathogenesis of mitochondrial diseases. Special attention should be dedicated to research on the mechanisms by which lipids interact with mitochondrial surface proteins, how they regulate each other and factors capable of influencing fusion and fission processes. These advances will provide essential knowledge for future development of new types of therapeutics.

Organisation of lipids in the eukaryotic endomembrane system

Role of lipids in autophagy regulation

Autophagy is an essential cellular pathway mediating lysosomal degradation of defective organelles, long-lived proteins, and protein aggregates [149, 150]. Autophagy involves a complex sequence of membrane remodelling and trafficking events, including the formation of autophagosomes, which engulf portions of cytoplasm at specific subcellular locations, and their subsequent maturation into autophagolysosomes by fusion with the endolysosomal compartment.

An emerging role for lipids and their metabolizing enzymes is their control of this cellular process.

Specifically, lipids control the autophagic process via key steps: (1) lipids regulate signalling cascades by converging into the mammalian TOR (mTOR) pathway, which negatively regulates the initiation of autophagy; (2) lipids act as membrane-bound localised signals that regulate membrane dynamics by recruiting cytosolic protein effectors that mediate membrane expansion and vesicle transport; and (3) lipids control membrane dynamics by influencing the physical properties of lipid bilayers, including curvature and fluidity [151]. For example, phosphoinositides, like PI3P, are a group of phosphorylated derivatives of phosphatidylinositol that mediate recruitment of cytosolic proteins controlling autophagosome maturation [152]. Class I PI3Ks and their product PI(3,4,5)P_3 are essential for the regulation of the mTORC1 pathway signalling cascade [153]. The vast majority of lipids involved in regulation of autophagy are phospholipids, and their importance is related to their capacity to influence the structure of lipid bilayers and to control assembly of protein scaffolds responsible for important steps in autophagy [154].

However, other types of lipids are involved in the control of autophagy. Ceramide activates autophagy by inhibiting the class I PI3K/Akt pathway and causes Bcl-2 to dissociate from beclin-1 through Bcl-2 phosphorylation induced by JNK [155]. Furthermore, ceramide also regulates death-associated protein kinase, which triggers autophagic cell death in different types of cancer cells [156, 157]. The role of ceramide in autophagy also could be structural, since ceramide increases negative membrane curvature and co-localises with autophagosomes. Sphingosine-1-phosphate also induces autophagy by inhibiting the mTOR complex independently of Akt and causes only slight accumulation of beclin-1. Furthermore, cholesterol has a role in so-called chaperone-mediated autophagy (CMA) [151]. Figure 2 provides a brief overview of how lipids influence autophagy. Cytosolic proteins containing a KFERQ motif are recognised by Hsp70, which facilitate protein unfolding and delivery to LAMP-2A, forming a translocation pore in the lysosomal membrane. Localization of LAMP-2A to lipid microdomains enriched in cholesterol and sphingolipid in the lysosomal membrane seems to regulate its function in this particular type of autophagy [158]. Cellular cholesterol levels negatively correlate with CMA activity, whereas disruption of microdomains with cholesterol extracting agents, such as cyclodextrin, activates CMA [159]. Modification of cellular cholesterol levels also can regulate macroautophagy: cholesterol depletion activates macroautophagy in several cell types, as determined by increased levels of lipidated LC3, the main marker of this type of autophagy. Although cholesterol depletion inactivates mTOR, hypercholesterolemia seems to activate mTOR signalling, suggesting that cholesterol may regulate autophagy through the mTOR pathway [160, 161].

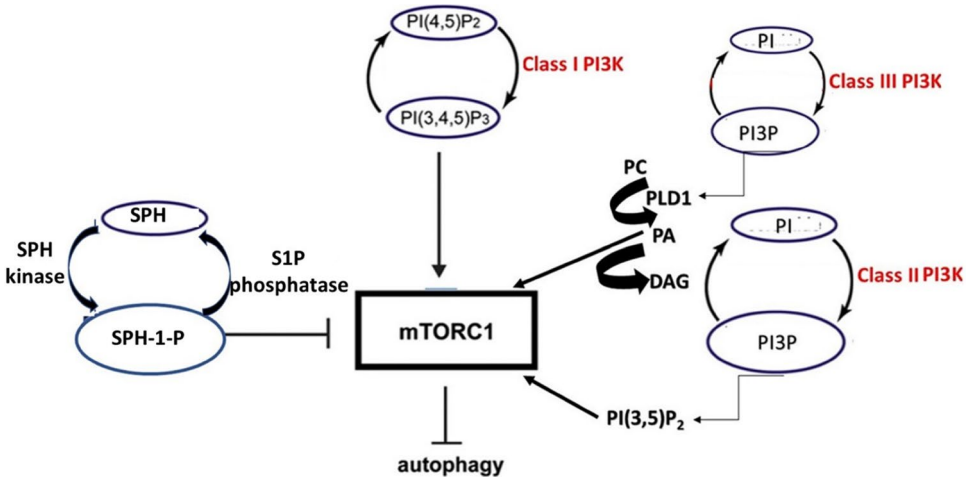

Fig. 2 mTORC1 modulates cellular response to nutrients and is the key suppressor of autophagy. Lipids and lipid enzymes play a major role in mediating mTORC1 regulation. The class I PI3K product PI(3,4,5)P3 activates mTORC1, as well as the class II PI3K products PI(3,5)P_2 and PA. In the absence of nutrients (starvation), mTORC1 signalling shuts down, and lipids, such as PI3P, PA and sphingosine-1-phosphate act as positive modulators of autophagy. Specifically, sphingosine 1 phosphate (produced by sphingosine kinase 1) contributes to mTORC1 inhibition, whereas PI3P, PA, and DAG (produced by class III PI3K) modulate signalling and membrane remodelling to support autophagy activation. PI(3)P can be synthesised from PI by class II and class III PI3-kinases. 3-Phosphatases can convert PI(3)P back into PI, and PI(3)P can be converted into PI(3,4)P_2 and PI(3,5)P_2 by 4-kinases and 5-kinases, respectively. S1P phosphatase can convert sphingosine 1 phosphate into sphingosine

Lipophagy: the missing link between autophagy and lipid metabolism

Recently, it has been proposed that the contribution of autophagy to cellular energy balance may not only be dependent on the capacity to degrade misfolded proteins (which are a relatively inefficient source of energy), but also on other components, such as free fatty acids and sugars. In this form of lipid metabolism, named lipophagy, triglycerides and cholesterol are taken up by autophagosomes and delivered to lysosomes for degradation by acidic hydrolases [162]. Free fatty acids generated by lipophagy from the breakdown of triglycerides are used during mitochondrial β-oxidation. The amount of lipid degraded by lipophagy varies in response to extracellular nutrient levels. Furthermore, the ability of cells to regulate the amount of lipid for autophagic degradation depends on nutritional status, demonstrating that this process is highly selective. Triglycerides and cholesterol are stored in specialised cellular organelles called lipid droplets (LDs). As a consequence of starvation, hepatocytes and other cell types including neurons display an increased association of the autophagy marker LC3 with LDs [163].

The finding that autophagy regulates lipid metabolism suggests an effective role for lipophagy in modulating lipid stores in adipocytes [164]. There are two types of differentiated adipocytes: white adipose tissue and brown adipose tissue. White adipocytes function as a lipid storage deposit to prevent lipotoxicity, while brown adipocytes have reduced capacity for lipid storage but a high rate of lipid metabolism through β-oxidation. Inhibition of autophagy inhibits the accumulation of triglycerides in white adipocytes and decreases expression of important regulators of adipogenesis. On the other hand, adipose-selective knockout of essential autophagy genes in mice significantly reduces adipocyte LD content and fat tissue mass. It is possible that autophagy directly regulates expression of one or more transcriptional regulators of adipogenesis or that autophagy may promote adipogenesis through cytoplasmic remodelling.

Open issues and future perspectives

Despite the demonstration that main lipid classes (sterols, fatty acids, phospholipids, and sphingolipids) are implicated in autophagy control, the molecular basis behind their involvement is still poorly understood. New biological approaches, such as lipidomics, hopefully will clarify the specific role of lipids in autophagy and lipophagy. New discoveries in the field may enable the potential use of autophagy modulation as a therapeutic weapon [165, 166].

Recent findings that lipids are a substrate for autophagic degradation, and that autophagy has an essential role in lipid metabolism, provide an opportunity for a complete understanding of diseases, such as type II diabetes and atherosclerosis. Key factors to clarify are how lipid composition of autophagic membranes could influence autophagic processes, how lipid signalling in autophagy can be controlled and modified, and the basis for the connection between dietary lipid uptake, lipid breakdown by lipophagy and metabolic disorders [165, 167]. Even though lipophagy appears to be a common pathway in many cells, this conclusion still requires confirmation. Another remarkable point is that very little is known about genetic differences that lead to individual variation in autophagic efficiency, but their existence may explain heterogeneity in manifestations of diseases marked by increased LD accumulation, such as steatohepatitis. Further investigations of lipophagy are likely to increase our understanding of the role of LD breakdown in cell physiology and pathology [167].

Organisation of lipids in eukaryotic nuclei

Lipid microdomains in cell nuclei

Many studies have demonstrated that the lipid component is present in various subnuclear compartments, where it plays different roles [168]. In the nuclear membrane and nuclear matrix, lipids regulate fluidity, while they participate in cellular signalling in chromatin [7, 8]. Furthermore, lipids, such as phosphatidylcholine and sphingomyelin, are linked with cholesterol. Composition and localization of lipids change throughout the cell cycle under the action of different enzymes, including sphingomyelinase (SMase) and phospholipase C. Similar to lipid rafts in cell membranes, lipid microdomains in nuclei are associated with proteins and a small amount of DNA and double-stranded RNA to form an intra-nuclear complex that is not extracted with nuclear membrane and chromatin purification techniques [169]. These nuclear lipids affect cellular functions of intranuclear signalling molecules and by modifying subnuclear structures. Activation of SMase changes sphingomyelin and cholesterol levels, which modifies fluidity of the nuclear membrane and allows nuclear-cytoplasm efflux of mRNA [170]. Because nuclear-cytoplasmic exchange is a basic cellular process of eukaryotes, these lipids are essential for healthy cells [171, 172]. Similar to the plasma membrane, where three pools of cholesterol are present (labile, sphingomyelin-linked cholesterol, and essential pool) [4], two pools of cholesterol co-exist in chromatin: a sphingomyelin-free cholesterol pool that does not change during cellular proliferation and a sphingomyelin-linked cholesterol pool that can change during the S-phase of the cell cycle in relation to SMase activation [168].

Nuclear lipid signalling

One of the most well-studied nuclear lipid signalling complex is the polyphosphoinositol lipid complex, which, together with the enzymes that synthesise them, forms the intra-nuclear phospholipase C complex (PI-PLC) signalling system that generates diacylglycerol and inositol-1,4,5-triphosphate [173, 174]. Diacylglycerol is believed to be essential for recruiting protein kinase C to the nucleus to phosphorylate intra-nuclear proteins, while inositol triphosphate increases nucleoplasmic Ca^{2+}. Phospholipase A_2, PI-3 kinase, and different PKC isoforms are involved in nuclear signalling, and the main metabolic product of sphingolipids, such as sphingomyelin, is ceramide generated by sphingomyelinase. Evidence suggests that the ratio of ceramide to diacylglycerol is a form of regulatory control that is critical for homeostatic nuclear properties [175–177]. Nuclear lipid signalling has an advantage over plasma membrane signalling in that lipids or their metabolites interact directly with nuclear factors, influencing transcription, and enzyme activities [178, 179]. For example, acidic phospholipids, such as CL, strongly affect the activities of different nuclear enzymes [170, 180]. It also should be noted that DNA and acidic phospholipids—in addition to many other nuclear proteins, including histones and DNA/RNA polymerase—share a common phosphatidylinositol consensus-binding sequence motif, indicating that nuclear lipid and protein interactions are essential to basic nuclear phenomena, including transcription, nuclear transport, and chromatin organisation.

Open issues and future perspectives

Lipids play both functional and structural roles in the nucleus and plasma membrane. Many aspects of these roles still need to be clarified, including specificity of nuclear signalling compared to the cell membrane, or how altered lipid compositions could affect nuclear signalling. Many studies point out that imbalances in the lipid signalling network can contribute to the pathogenesis of human diseases, including inflammation, atherosclerosis, and cancer [172]. These pathways are mostly studied in isolation due to their complexity, but many signalling lipids and downstream targets are common to multiple signalling pathways, resulting in highly inter-connected lipid signalling networks [3]. Despite their complexity, signalling lipid-generating enzymes are targeted pharmacologically to counteract the progression of different diseases. The limit of this therapy is based on the poor predictability of in vivo responses, which is related to an incomplete understanding of the dominant and permissive properties of interacting lipid signalling pathways. Further advances in this therapy are likely to improve time-resolved methods for monitoring lipid signals, including lipidomic studies to unveil the function of lipids in the plasma membrane and mitochondria.

Intra-cellular lipid trafficking

Studies of lipid trafficking and the recognition of its importance have been overshadowed by a predominant focus on protein trafficking. However, intra-cellular lipid sorting and translocation are significant steps in many metabolic processes that affect the progression of various diseases [181]. The question of how the plasma membrane acquires its most important structural element, lipids, is an ongoing research area. There are three major possible mechanisms to transfer or sort lipids in the intra-cellular region: transport by vesicles along with sorting proteins, translocation of lipid monomers through the cytosol by lipid carrier proteins, and intermembrane lateral diffusion through transient interconnection at points of membrane contacts [182]. A specialised class of proteins called phospholipid transfer proteins or phospholipid exchange proteins (PLEPs) is involved in subcellular translocation of lipids [183, 184]. PLEP-mediated transfer of lipids is extremely fast, with almost no lag detected between the synthesis and transfer of PE [185]. On the contrary, the lag between protein synthesis and translocation to the membrane is almost an hour. Several identified sterol carrier proteins (i.e., SCP-1 and SCP-2) transport cholesterol and enzymatically convert intermediates of cholesterol biosynthesis into cholesterol [186]. The major functions of such SCPs include participation in microsomal conversion of lanosterol to cholesterol, transport of cholesterol from cytoplasmic LDs to mitochondria, translocation of cholesterol from the outer to inner mitochondrial membrane, regulation of pregnenolone production, stimulation of hydroxycholesterol production, and facilitation of phosphatidylinositol exchange between natural and artificial membranes [186–188].

Recent evidence supports the presence of three pools of cholesterol in plasma membranes: a labile pool of cholesterol that is depleted when cells are deprived of cholesterol, a sphingomyelin-bounded cholesterol pool that is not labile, and an essential pool of cholesterol that is necessary for cell viability [4]. Cellular cholesterol homeostasis depends on the balance between membrane sequestration of cholesterol or cholesterol metabolism and the uptake of low-density lipoprotein-derived cholesterol or cholesterol synthesis via the mevalonate pathway [189, 190]. Many studies have investigated the ability of different inhibitors to influence cholesterol concentration and trafficking among different compartments, although this is a continually developing field [86, 132, 191–193].

The ER is a major region of phospholipid and neutral lipid biosynthesis. Bulk transfer of lipids is executed through 50–70 nm transition vesicles, bulging out from part-rough,

part-smooth transition elements of the ER. This transfer can be either ATP-dependent, which is specific, or ATP-independent, which is thought to lack specificity [194–196]. Major lipid species found in transitional ER and transition vesicles are di- and triglycerides, phosphatidylcholine, and sterols. The Golgi apparatus—but not the ER or plasma membrane—functions as an acceptor, whereas only the transitional ER, but not conventional rough ER, serves as an efficient donor. Notably, triglycerides, which are the major ER lipids, do not undergo ATP-dependent transfer from the ER to Golgi apparatus [195].

Most lipids are synthesised in the ER, except CL and PE, which are synthesised at the inner membrane of mitochondria. Both the inner membrane and outer membrane of the mitochondria maintain dynamic lipid composition by concerted synthesis and trafficking. Several pieces of evidence support the hypothesis of rapid and bidirectional trans-bilayer movement of PC, PE, and CL across the outer and inner mitochondrial membranes [197].

However, a few studies have investigated nuclear translocation of lipids. Phospholipid transfer protein (PLTP) is found in several cell types, including neuron, ovary, and kidney cells; in addition, it is involved in transport and distribution of phospholipids and cholesterol within the nucleus [198, 199]. In addition, PLTP is involved in nuclear transport of α-tocopherol, which regulates RNA synthesis. Importantly, PLTP is a key component of one of the pathways that regulates cell survival in breast cancer [200]. Although nuclei contain many other lipids involved in signalling pathways (e.g., inositol lipids, diacylglycerol, and phospholipids) [170], little is known about their trafficking and transport mechanisms, so further studies are required.

Organisation of lipids in different organisms

Bacteria

Bacterial membranes consist of distinct lipid species that differ in molecular structures and physicochemical properties, similar to eukaryotic cellular membranes [61]. In addition, bacterial membranes display a phenomenon known as lipid ordering, in which lipid constituents coalesce into microdomains [201]. Therefore, lipid ordering in bacterial membranes is thought to be similar to the process that occurs in eukaryotic cells [202]. Studies have demonstrated that bacteria possess widely distributed lipid rafts and that signal transduction cascades and protein transport is organised into functional membrane microdomains (FMMs) established by distinct lipids [17, 18, 20, 203, 204]. In addition, polyisoprenoid lipid synthesis and co-localization with flotillin-like proteins in membranes are related to assembly of FMMs that are present in bacteria. Among the different functions, bacteria flotillin maintains FMM architecture and acts as a protein scaffold to select proteins that are required to promote interactions among lipid rafts [15, 205]. This process is similar to the role played by eukaryotic flotillins. Bacterial membranes present a large diversity of amphiphilic lipids [206], including common lipids, such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL), and less common lipids, such as phosphatidylinositol (PI) and phosphatidylcholine (PC) [207]. Furthermore, studies suggest complexity in the proposed pathway for synthesis of phospholipids and phosphorus-free membrane lipids. Mizoguchi et al. used the effects of changes in the environment on lipid composition to show that survival of bacteria under unfavourable conditions is influenced by adaptation to environmental change [208]. Therefore, this adaptation will affect nutrient levels, metabolism, pH, and oxygen levels in membrane lipid composition. In general, bacterial membrane fluidity decreases with decreased temperatures by increasing bacterial content of unsaturated fatty acids or fatty acids with similar properties [209]. Relative increases in temperature increase fluidity and discontinuities in the membrane by increasing membrane content of saturated fatty acids. These studies provide the building blocks for additional research on bacterial lipid modifications.

Despite experimental evidence for the existence of membrane microdomains in bacteria and their similarities with eukaryotic lipid rafts, there are some controversies regarding the structure and function of FMM. Although it is known that co-localization of flotillin-homolog proteins, FloT and FloA, and sensor protein kinase, KinC, is an important feature of FMM and is essential for bacterial biofilm formation [210], recent studies in *B. subtilis* revealed that activation of KinC does not require FloA and FloT [211]. Similarly, another study has shown that FloA and FloT did not function as molecular scaffolds in FMM; rather, they formed protein microdomains of a specific size [212]. In contrast, evidence from another study, using the same experimental model, revealed that the bacterial membrane contained two different types of FMM: one type of FMM was associated with scaffold protein FloA and selectively participated in cellular processes, such as cell envelop turnover and metabolism, and the second type of FMM harboured both FloA and FloT and was involved in cellular adaptation to stationary phase [213].

Thus, despite highly similar structural organisation, FMM and lipid rafts possess different functional characteristics. For instance, FMM is involved primarily in the oligomerisation of different FMM-related proteins, whereas lipid rafts contain proteins that are associated with signal transduction and membrane trafficking [17].

Saccharomyces cerevisiae

Phospholipids are the primary component of *S. cerevisiae* membranes, which also contain glycosphingolipids, ergosterol, and proteins [214]. Furthermore, a glycerol-3-phosphate backbone with two fatty acid chains esterified to positions 1 and 2 defines the fundamental structure of these phospholipids: the amphipathic nature of molecules bonded to the phosphate group forms membrane bilayers. Nutrient transport, pH homeostasis, and cellular signalling are achieved by being embedded in integral and peripheral membrane proteins. In the yeast cell membrane bilayer, phospholipids are distributed to maintain membrane surface potential and membrane protein activity. In addition, lipid translocases are a class of proteins that retain the asymmetry of lipids and play a significant role in cell cycle progression, endocytosis, and cell polarity [215, 216].

PA, PC, PE, and PI, with minor amounts of cytidine diphosphate-diacylglycerol (CDP-DAG), are considered important yeast phospholipids, but palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1) are common yeast fatty acids. An analysis of lipid membrane organisation was performed to determine the ethanol tolerance of *S. cerevisiae* using different approaches, including lipidomics [217, 218]. Ethanol-induced membrane perturbations have a number of potential effects, including a significant reduction in membrane thickness due to lipid inter-digitation that caused alteration to membrane-associated protein distributions [217, 219]. These studies are essential for understanding how yeast cell lipid composition and function change over the course of fermentation, as levels of ethanol rise as yeasts convert sugar to ethanol.

Drosophila

Laurinyec et al. described a system to study the effect of lipid composition in *Drosophila* and its synthesis or remodelling processes [220]. Post-meiotic stages of spermatogenesis are sensitive to slight changes in gene products, and further transcriptional activity is completed by cyst entry in meiotic division. Lipid constituents of the cell membrane, such as PC and cholesterol, function according to their structural roles. However, several other lipid constituents, such as PE and phosphatidylserine (PS), function based on both structural and signalling roles. Pleiotropic effects occur due to mutations in metabolic lipid enzymes, as they function in several *Drosophila* organs and developmental processes [221, 222].

In addition, an interesting lipidomic study of *Drosophila* analysed the fatty acid composition of phospholipids, and showed that *TRF2* and *TAF9* expression varied in composition and size of LDs [223]. *TRF2* and *TAF9* affected transcription of a common set of genes that affected phospholipid fatty acid composition, including peroxisomal fatty acid β-oxidation-related genes. These findings suggest that the LD phenotype in *TRF2* mutants can be restored by overexpression of target genes [223].

Caenorhabditis elegans

Several studies of lipid profiling in *C. elegans* show that the organisms store fats as triglycerides [224–226]. Comparisons of lipid profiles suggest that the spectrum of mono-, di- and triglycerides in *C. elegans* is similar to that in mammals. Studies also indicate that stored body fat in *C. elegans* is formed by conversion of fatty acids found in bacteria to triglycerides [227–229]. Zhang et al. (2012) devised a strategy to diversify the worm lipidome by desaturating fatty acids, which provides excess signalling molecules to control organismal physiology [226]. *C. elegans* possesses cellular strategies for spatial and temporal control over energy flow using subcellular compartmentalization to prevent localization of fats. These processes are necessary for long-term storage of fat in the same compartment. Furthermore, *C. elegans* intestines synthesise, store, and mobilise lipids, and this regulatory mechanism balances transfer between fat storage and use in restricted compartments. In addition, Wahlby et al. derived a precise pathway for biochemical temporal control for the accumulation and breakdown of triglycerides in *C. elegans* [224]. Currently, several investigations have used genetic and biochemical approaches to control fat storage in worm intestines.

Conclusions

Lipid organisation in tightly regulated structures is evolutionarily conserved: cells and organisms have developed extraordinarily sophisticated mechanisms to control lipid composition and organisation in each cellular compartment. Although there are myriad of studies on the organisation and function of lipid rafts in the plasma membrane, the function of nuclear lipid microdomains and mitochondrial raft-like microdomains has not been investigated to the same degree (Fig. 3). A thorough study of this specific organisation raises many questions, including: is it possible to target specific lipid microdomains in the plasma membrane to modify network signalling of cells? How do these changes affect basic physiological processes, such as metabolism, apoptosis and cell proliferation? And is it realistic to develop therapeutic drugs that target specific lipids in specific compartments? With the help of innovative approaches, like lipidomics and advanced microscopy techniques, answering these questions becomes possible. Indeed, the discovery of the very short residence time of sphingomyelins in raft domains represents an outstanding

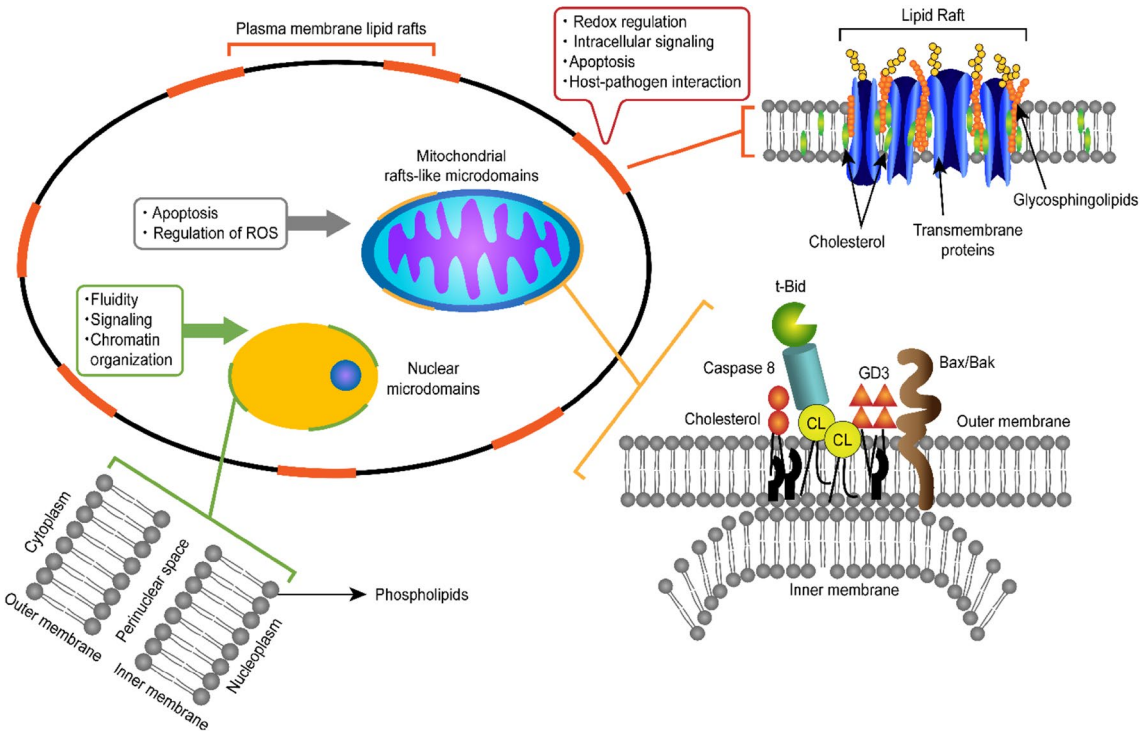

Fig. 3 Lipid organisation is present in different compartments and has specific functional roles. Lipids acquire specific organisation in each compartment, and this organisation is essential for regulating cell function. Lipid rafts in the plasma membrane function as a concentrating platform for different receptors and consequently regulate a plethora of functions, including intra-cellular signalling, interaction with the extracellular milieu, and proliferation. In the mitochondria, cardiolipin-enriched raft-like microdomains, at the contact sites between inner and outer mitochondrial membranes, represent specialised portions of the mitochondrial membrane where t-Bid is recruited and determines Bax oligomerisation. Nuclear rafts are important for transcription, nuclear transport, and chromatin organisation. Adapted from Sorice et al. [128]

shift in the field that has allowed effective characterization of the mechanisms of cell membrane signalling and invasion of various pathogens. These advances will pave the road for development of effective therapies targeting disorders associated with lipid metabolism and lipid storage.

Compliance with ethical standards

Conflict of interest I am unaware of any potential conflict of interest, including professional or financial affiliations that might be perceived as biasing the manuscript.

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