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Review

Yeast as a model system for studying lipid homeostasis and function

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ABSTRACT

Lipids are essential eukaryotic cellular constituents. Lipid metabolism has a strong impact on cell physiology, and despite good progress in this area, many important basic questions remain unanswered concerning the functional diversity of lipid species and on the mechanisms that cells employ to sense and adjust their lipid composition. Combining convenient experimental tractability, a large degree of conservation of metabolic pathways with other eukaryotes and the relative simplicity of its genome, proteome and lipidome, yeast represents the most advantageous model organism for studying lipid homeostasis and function. In this review we will focus on the importance of yeast as a model organism and some of the innovative advantages for the lipid research field.

1. Introduction – the study of lipid homeostasis and function – approaches and applications

Lipids are essential eukaryotic cellular constituents. Although their main functions have long been attributed as solely structural components, their role as vital molecules has been increasingly recognized. Today, lipids are considered as important dynamic molecules that play vital roles which extend beyond their roles in membrane structure [1,2]. Therefore the impact of lipids in cell biology has caught the attention of investigators from many different areas, pushing the lipid research field forward. The importance of understanding how lipid homeostasis is maintained has gained much prominence, as new analytical technologies emerge, generating a vast amount of information from studies in model systems and in humans [3,4].

Given their fundamental role in cell biology, it is not surprising that complex mechanisms are employed to robustly maintain lipid homeostasis. The eukaryotic genomes dedicate a considerable proportion to the coding of numerous genes required for lipid metabolism and lipid homeostasis [5]. But genes and transcripts do not always predict the precise levels of active proteins/enzymes and, to fully understand lipid function and homeostasis, it is necessary not only to be able to characterize the genes, proteins and, obviously, lipids involved in the scenario, but also to be able to describe the regulatory system as a whole.

The study of lipids in cell biology (“lipidology”) emerged in the early 1960’s with the first attempts to describe and understand lipid composition of cells [6]. The traditional approaches for the study of cellular lipids relied on analytical techniques with low resolution and sensitivity, such as thin layer chromatography (TLC) – in a time when the characterization of a lipid profile was limited to content of entire lipid classes, addressing only a small fraction of the lipid complexity. With the advent of gas chromatography (GC) and high performance liquid chromatography (HPLC), the field was pushed forward, until the emergence of mass spectrometry as a sophisticated highly sensitive technology able to further deconvolute lipid diversity. Over the last decades, enormous progress occurred in terms of sensitivity and resolution of analytical techniques [7]. Currently, with combined state-of-art techniques, it is possible to virtually identify and quantify the whole lipidome of an organism, cells and organelles [8]. Nonetheless, although global lipid profiling provides extensive characterization and quantification of total lipids, usually extracted from whole cell extracts or organelles, it provides little information on lipid distribution in the cells. Lipids are not homogeneously distributed in the cell, and their synthesis and trafficking are also growing areas of research [9,10].

Many different organisms have been used in studies of lipid biology over the years [2,5,11–15], revealing an enormous variety of lipid structures, with an estimate of several hundred thousand

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distinct lipid species [16]. Still, many important questions remain unanswered on the specific regulatory mechanisms that generate and maintain this diversity of molecules. The most fundamental questions probably are: how do cells sense and adjust their lipid composition? And what are the roles of these different lipid species in cell biology and physiology?

This particularly concerns the physiological consequences of an altered lipid homeostasis. A number of diseases with significant socioeconomic impact (i.e.: obesity, diabetes, atheroschlerosis and Alzheimer's disease) have a putative lipid component in their onset and progress. Hence, understanding the mechanisms that control changes of lipid patterns is of significant diagnostic relevance [17–23].

The major lipid classes in eukaryotes are: glycerophospholipids, sphingolipids and sterols [5,24]. Amongst phospholipid and sphingolipid structures, a remarkable diversity arises from different possible combinations of head-groups and fatty acids, the latter with different chain lengths, hydroxylations and degrees and positions of desaturation.

Noteworthy, lipid metabolism is well conserved among eukaryotes [2,25] making yeast an advantageous model organism for studying lipid homeostasis and function. In this review we will focus on the importance of yeast as a model organism and some of the innovative advantages for the lipid research field.

2. Advantages of using yeast as a model system for lipid studies

Saccharomyces cerevisiae is a powerful model organism for studying cell biology. Since 1988, when Botstein and others [26] speculated that yeast would be an ideal model system for modern biology, its use has increased exponentially, eventually becoming a reference organism [27]. A few years after the publication of the complete *S. cerevisiae* genome sequence [28], a vast set of mutants was created, with deletion of nearly all open reading frames [29,30]. Over the years, this yeast has been extensively exploited in different assays improving our understanding of many diverse cellular processes, based on DNA microarrays, gene disruptions, protein localization, protein–protein interactions and functional analysis by genetic interactions [31–34]. In fact, almost every area of cell biology has benefited from the use of yeast as a model system, rendering *S. cerevisiae* as the eukaryotic organism with the best described genetics and metabolism. On the topic of lipids in cell biology, the availability of genome sequences and the collection of data on mutants allowed the characterization of genes and proteins involved in lipid metabolism. But the studies of lipids in yeast were considerably less prominent than the quick burst of large scale genetics and proteomics studies. This hurdle was not due to less appreciation of lipids in cell biology, but rather to technical limitations, given that methods to analyze lipids were laborious and not very sensitive compared to the available genomics and proteomics techniques.

As a model system, yeast has many advantages for the study of lipid biology: (i) convenient experimental tractability, due to simple growth conditions and facile genetic manipulation compared to mammalian cells; (ii) yeast can be cultivated in completely defined medium, allowing a precise control of physical and biochemical parameters, contrary to mammalian cells which usually grow robustly using cultivation with serum containing media (lipid rich media). This is particularly relevant for studying lipid metabolism, since cells can take up molecules as nutrients and fatty acids from the media, making the interpretation of phenotypes less clear; (iii) and the yeast model benefits from a well-curated databases and a large collection of plasmids and genomic libraries that are available; and (iv) the high degree of conservation of many fundamental metabolic pathways between yeast and mammals.

Given the fact that lipid homeostasis is critical for cell physiology, it is not surprising that lipid metabolic reactions also share a high degree of conservation in eukaryotes [2,25]. It is important to note that such similarities are not limited to genetic homology but they also extend to the general architecture of major metabolic/signaling pathways and regulatory mechanisms, despite some differences, especially in the input queues and readouts that must be taken into account [5,35]. Finally, amongst the advantages of using yeast as a model system to study eukaryotic lipid metabolism, there is one key aspect: the simplicity of its lipidome. While yeast has only a few hundred lipid species [36,37], mammalian cells have thousands [24,38], and the task of studying lipid homeostasis in yeast is, obviously, greatly simplified.

As the understanding that lipids are indispensable molecules for physiology arose, studying the mechanisms that control lipid homeostasis and function became a significant topic in cell biology. Many detailed reviews on glycerophospholipids, sphingolipid and sterol metabolism have appeared recently and should be consulted for more detailed information [2,39,40].

3. Lipids homeostasis and function – studies and different approaches

The use of yeast as a model system enabled the discovery and characterization of many genes involved in lipid metabolism. The enzymes involved in the early steps of sphingolipid pathway, for example, were all first identified by yeast genetics [41] and then used to identify the corresponding genes in humans and other organisms.

In attempting to decipher the pathways that regulate lipid homeostasis, researchers have used yeast gene deletion collections in several high-throughput screens, examining the phenotypic effects of gene loss and gene interactions in lipid metabolism, for example: systematic analysis of yeast harboring potential defects in lipid homeostasis [42], a screen that associated sphingolipids and sterols to cell surface delivery [43], genetic profiling of inositol auxotrophic phenotype [44,45] and screening for players in lipid droplet formation [46]. The genetic approaches are still proving to be an enormous aid towards the identification and characterization of genes associated with lipid metabolism. They normally generate extensive datasets that can be used as starting points to the discovery of novel connections between genes and cellular processes (Fig. 1). However, it is important to note that these approaches are rather indirect. Deletion of non-essential genes does not always cause a phenotype, perhaps due to overlapping functions of paralogous genes or compensatory effects during adaptation. This is of special concern when performing screens with mammalian cells, given the high number of genes with multiple paralogs and the complexity of the mammalian lipidome. In an attempt to perform a large-scale screening of protein kinases involved in lipid homeostasis in mammalian cells, Grimard and others [47] perform a siRNA knockdown of 600 human kinases combined with TLC analysis of lipids. Despite the limitations of the approach in terms of sensitivity of lipid profiling, the screen yielded 91 hits with a lipid phenotype, eventually characterizing JNK2 as a new regulator of triglyceride homeostasis. Now, with the currently available lipid analytical techniques all the advantages of yeast as a model system, we believe that the use of yeast-based large scale screenings related to lipid metabolism will greatly accelerate our understanding of lipid homeostasis.

Concerning the functional architecture of lipid pathways, *S. cerevisiae* was employed to characterize the distribution of enzymes involved in lipid metabolism. In a screening using green fluorescent protein (GFP)-tagged proteins involved in lipid metabolic reactions, Natter et al. [48] described the ER as a central stage

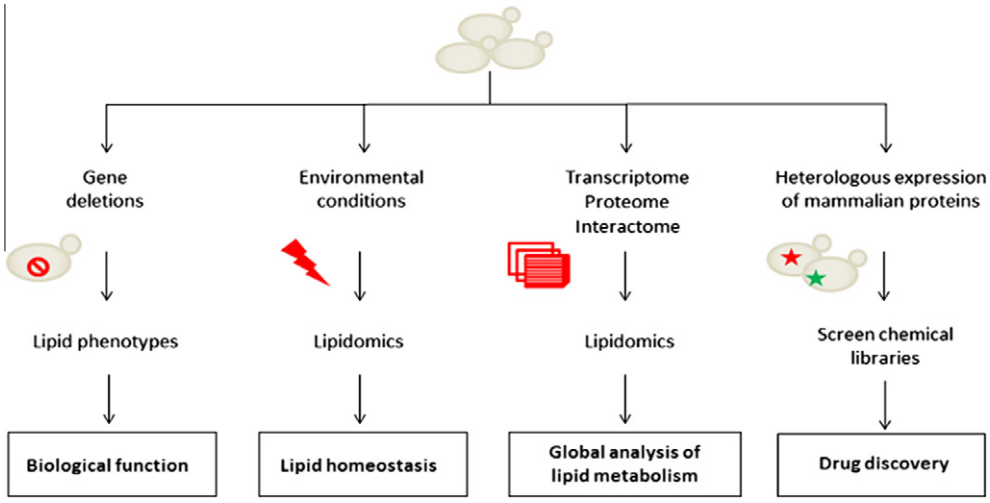

Fig. 1. Different approaches to study lipid homeostasis and function using yeast as a model system. Genes involved in lipid metabolism/regulation have been identified and characterized through yeast genetic screens. Lipidomics analysis reveal the global changes of lipid profiles in different conditions, and the integration of other "omics" datasets generated from high-throughput yeast based screenings will help to unravel global mechanisms of lipid homeostasis. Expression of human proteins in yeast to create target-based screens to facilitate discovery of compounds that interfere with lipid homeostasis.

of lipid metabolism together with lipid droplets and peroxisomes, but they also found a significant number of these proteins in other membrane compartments as mitochondria, Golgi, vacuole and vesicles.

Apart from understanding the distribution of lipid biosynthetic machinery in the cell, another key aspect of the study of lipid homeostasis and function is the pattern of lipid distribution in subcellular compartments. Cells synthesize a large variety of lipids, the vast majority of which is made at the ER and further translocated to specific compartments. Using cell fractionation techniques, characterization of subcellular membranes has been accomplished [49,50], and, although some mechanisms of membrane trafficking, inter-organellar contact sites and vesicular and non-vesicular transport have been proposed to account for this heterogeneity in lipid distribution [51–54], the dynamic nature of the differential lipid composition is still far from being understood.

4. Lipid visualization

The role of most lipid species in cellular membranes is thought to be mainly as passive structural components or for specific lipid species as factors involved in recruitment of soluble proteins to membranes. While the early models of cellular membranes were oversimplified representations of the lipid bilayer, it is now known that cellular membranes are composed of an enormous variety of lipid structures and proteins that are non-randomly distributed. The observations that sterols and sphingolipids have the capacity to partition together in the plasma membrane with specific proteins have raised the possibility of the existence of membrane micro domains, formally hypothesized as lipid rafts [55]and/or lipid shells [56], platforms in the plasma membrane that are proposed to be enriched in sphingolipids, sterols and proteins. The underlying molecular concept of the raft hypothesis is that the interaction of sphingolipids and sterols is favored by their physical properties and creates lateral heterogeneities in the membrane, driving the appearance of ordered/disordered domains. The raft hypothesis has been revisited many times in the last 20 years [57–59]. Yet, the proof for the existence of rafts and their dynamic assembly in the biological context has been a target of dispute [60–62] and despite all the efforts, the important question - whether lipid rafts play a role as physiologically relevant entities – still remains vague. The lipid shell hypothesis postulates that protein transmembrane domains show specific interaction with lipids and these lipids have low affinity interactions with other lipids organizing a lipid shell of distinct composition around membrane proteins [56]. Molecular dynamics studies [63] seem to support the basic tenant of this hypothesis. If protein–protein interactions were to occur this could lead to a structure that would be very difficult, is not impossible, to differentiate from that proposed in the lipid raft hypothesis.

Controversies in the field, however, stimulated interest and prompted progress in development of techniques to allow the study of these domains, eventually contributing to our understanding of membrane dynamics. With the development of high temporal and spatial super resolution microscopy, it was shown that both proteins and lipids are not freely moving in the membrane, but that their diffusion is occasionally restricted in specific domains [64,65]. These experiments use single particle tracking, which is visualized generally in two dimensions by the available techniques. Potential problems with these experiments stem from the fact that most cell surfaces are not flat and that movement in the z-axis is therefore not efficiently detected [66]. Therefore, there is still a high level of skepticism in the field that is partially due to the lack of tools to visualize these domains in vivo and partially due to the lack of direct functional evidence that these domains exert an effect on signaling pathways and cell physiology in general. The challenge of visualizing lipids remains difficult because of the limited availability of appropriate probes to visualize naturally occurring lipids in the living cell context. Lipid-binding proteins, such as lysenin [67] and cholera toxin [68] have been used to study membrane domains, but they provide little information about the lipid composition and structure of these domains. More specific probes used to study lipid domains comprise analogues of sterols [69], phospholipids [65] and fluorescent tagged proteins. However, fluorophores usually have an enormous impact on the behavior of the lipid as shown by differential partitioning of the cholesterol fluorescent analogue dehydroergosterol (DHE) and 25-NBD-cholesterol, which localize preferentially to liquid ordered and disordered domains, respectively [69].

A promising tool to study the distribution of lipids in the cell is the development of antibodies against specific lipids. Antibodies against the lyso(bis)phosphatidic acid (LBPA) [70] phosphatidylglucose [71], ceramides [72], and even an antibody that recognizes ceramide/cholesterol enriched domain have been described [73]. Novel technologies are currently being developed using yeast as

a model, such as targeted evolution of bicyclic peptides to specifically bind sphingolipids [74,75]. This will putatively allow the visualization of specific lipids in the cellular context, massively improving our understanding of membrane organization and intracellular trafficking.

5. Intracellular trafficking

Eukaryotic cells have a highly complex system of intracellular trafficking. Proteins and most of lipids are synthesized and matured in the ER and Golgi and further transported to different subcellular compartments and to the cell surface, ultimately affecting global membrane composition. Endocytosis also plays a role in the plasma membrane composition, by selective internalization of membrane patches in the form of endosomes, which will eventually follow different routes to degradation or recycling [76,77]. By controlling the composition of plasma membrane, cells regulate their ability to respond to their environment, in processes including nutrient uptake, intercellular signaling and morphogenesis. Intracellular trafficking is a highly conserved process between yeast and mammals, and studies in yeast have largely contributed to the identification and characterization of genes involved in the secretory [78] and endocytic pathways [79], which, later on, allowed their mammalian homologues to be characterized [77]. Yeast research still promotes critical advances in the intracellular trafficking field, especially with regard to the regulatory mechanisms that govern this machinery. Of special interest, is the collection of mutants defective in secretory, vacuolar and endocytic pathways [80]. Intracellular trafficking has long been studied from the protein perspective, but more recently, lipids have emerged as equally important players in this process. In yeast, the delivery of many plasma membrane proteins requires the proper synthesis of sphingolipids and sterols [81]. Based on studies showing the presence of plasma membrane proteins in detergent-resistant membrane fractions [82], a mechanism of protein-lipid delivery has been proposed suggesting that membrane protein sorting occurs in the trans-Golgi network in parallel with lipid sorting – preferentially with sphingolipid and sterols – generating an enrichment of those lipids in the plasma membrane [10,83].

Lipids are not just involved in trafficking as platform components, they also have a critical role in protein remodeling during their maturation process towards the plasma membrane. The transport of GPI-anchored proteins to the plasma membrane is a good example of the roles of lipids in the intracellular trafficking. GPI-anchored proteins are synthesized in the ER and further submitted to remodeling of their lipid moiety before transport to Golgi. During the remodeling, the initial glycerophospholipid moiety is replaced by a ceramide structure or very long chain saturated diacylglycerol [84], an important step in concentration into ER-exit sites for the formation of GPI-anchored protein containing vesicles [85]. Despite some differences between yeast and mammals concerning the precise structures and the location of the remodeling, there is good evidence of the conservation of the cellular strategy for accomplishing this process. A recent study on mammalian cells also showed that interventions at the multiple steps of GPI-anchored protein synthesis and remodeling differentially affect the levels of glycosphingolipids, suggesting that the transport of very long chain ceramides from ER to Golgi is coordinately regulated by the metabolism of GPI-anchored proteins [86].

Another example of the role of lipids in intracellular trafficking comes from the trans-Golgi vesicular transport. Phosphatidylinositol-transfer proteins (PITPs) are highly conserved proteins that mediate the transfer of phosphatidylinositol or phosphatidylcholine monomers between membranes. It was through yeast genetics that the first insights into the biological role of PIPTs came to light. In yeast, the major PIPT is encoded by the essential *SEC14* gene, which is necessary for the protein transport from the Golgi via vesicular trafficking [87]. Basically, the physiological function of Sec14p is to coordinate PI and PC metabolism, generating an adequate lipid environment in Golgi membranes that is permissive to vesicle biogenesis. The dysfunction of Sec14p not only impairs protein trafficking in the trans-Golgi, but it also deranges lipid metabolism in general, eventually affecting the biogenesis of secretory vesicles [88]. Subsequent, studies in mammalian cells have demonstrated that dysfunctional PIPTs are of great medical interest, as they lead to neurodegenerative processes, defects in nutrient absorption and glucose homeostasis [89].

6. Lipid asymmetry

One important aspect of membrane composition in cell biology is the lipid asymmetry. Sphingolipids and phosphatidylcholine (PC) are mainly found in the exoplasmic leaflet, whereas the aminophospholipids phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) are mainly found in the cytoplasmic leaflet. This differential distribution of phospholipids in the two leaflets of the plasma membrane is crucial for cellular processes such as polarization during cell budding and mating in yeast [90] and the internalization step of endocytosis [91]. The enzymes responsible for the translocation of phospholipids from the outer to the inner leaflet of the plasma membrane in yeast belong to the type 4 subfamily of P-type ATPases [91], and two of them, Dnf1 and Dnf2, were recently shown to govern phospholipid flipping with Cdc50, by regulating the activity of Cdc42 – a highly conserved protein that regulates cellular morphogenesis [92]. Interestingly, sphingolipid homeostasis also seems to be involved in this dynamic control of phospholipid distribution, by indirect control of Fpk1, a kinase that stimulates the activity of the flippases [93].

We are just starting to understand how a eukaryotic cell controls these dynamic changes at the plasma membrane in order to maintain the proper balance among the different lipids.

7. Functional interaction – ergosterol and sphingolipids – one good example

It is likely that lipids often function collectively rather than as single molecules in the cell. This is evidenced by the interconnection in the lipid biosynthetic and degradation pathways - through the use of common metabolic substrates and/or intermediates [94], giving place to the conception of a highly dynamic and interconnected network [95,96]. It is clear now that studies in lipid metabolism must be conceived and interpreted in a global manner, as interventions in one pathway can have secondary effects in other classes.

The relationship between different lipid classes have been long discussed in terms of membrane structure, controlling the fluidity and preferential association of different lipids that would facilitate the emergence of membrane domains (argued to be important for activity of membrane associated proteins). It was only recently, however, that the functional relationship of different lipid classes was proven. The study of Guan et al. [97] is the most convincing evidence of a functional interaction of sphingolipids and sterols. Using an unbiased lipidomics analysis of mutants with gene deletions in late steps of ergosterol biosynthesis, it was shown that deletions of *ERG* genes led to the absence of ergosterol and accumulation of the corresponding metabolic intermediates. But most remarkable was the observation of dramatic changes in the sphingolipid composition of these *erg* mutants. One important lesson from this study is the observation that cells can sense the presence of aberrant sterol structures and adapt their sphingolipid composition accordingly, most likely in an attempt to maintain important cellular functions (Figure 2). One example showing that interruption of this

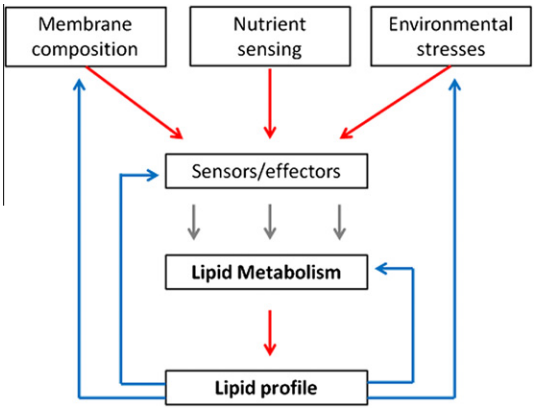

Fig. 2. Regulation of lipid homeostasis. Changes in membrane fluidity or curvature are perceived by cells and translated into pathways to induce compensatory changes in the lipid profile. The lipid profile is also affected by nutritional status and abiotic stresses, such as temperature and osmolarity. Adaptive changes occur via genetic and biochemical mechanisms, and changes in the lipid profile also alter the capability of cells to respond to external stimuli.

adaptation causes a novel phenotype is the reduced ability of the *erg2scs7* double mutant to grow in the presence of inhibitors of the TORC1 signaling pathway, such as caffeine and rapamycin, despite normal TORC1 signaling activity [98].

Many questions remain open after this study: how do cells sense and control their lipid composition? Which are the molecular sensors that signal the alterations in membrane lipid composition to trigger the changes in lipid abundance? How is this process controlled?

Recent studies in yeast have shed some light on the mechanisms of control of sphingolipid biosynthesis. It was known that the TORC2 complex, and the associated proteins Slm1/2, exerts part of their functions in cell growth through the regulation of the sphingolipid pathway [99,100]. Recently, a feedback mechanism was discovered that controls sphingolipid metabolism [101]. Two homologous proteins – called Orm1p and Orm2p, have been characterized as negative regulators of the enzymatic activity of serine palmitoyl transferase, the first committed step in the synthesis of sphingoid bases. The Orm proteins are regulated by a protein kinase cascade that involves the Ypk1 kinase [102]. Subsequently, bridging the knowledge that sterol mutants with altered sphingolipid composition have decreased TORC2 activity, it was proposed that TORC2 signaling involves a lipid homeostatic control [103]. In an elegant series of experiments, it was shown that Slm1/2 proteins, which are sequestered on eisosomes during exponential growth, leave eisosomes upon membrane stresses (either mechanical or induced by inhibition of SL biosynthesis) to associate with and activate TORC2 complex. TORC2, in turn, phosphorylates Ypk2p which phosphorylates the Orm proteins. This revealed a homeostatic mechanism in which TORC2 signaling pathway responds to membrane stresses, possibly by sensing plasma membrane curvature at eisosomes, and mediates compensatory changes in lipid synthesis.

8. Lipid homeostasis – lipid sensors

Sterols are not evenly distributed in all cellular compartments. They are synthesized in the ER and distributed towards the plasma membrane, where their concentration is increased. Understanding the processes that govern sterol distribution in the cell is of great interest, as its levels in the plasma membrane are critical for vital processes, such as endocytosis and most likely in creating an efficient membrane barrier. Clearly, cells maintain the levels of sterols in the membrane under tight control, and the imbalance in this processes can lead to several dysfunctions [97,104]. Oxysterol binding proteins are a well conserved family of protein from yeast to man, that are considered putative sterol sensors and regulators of sterol distribution in the cell. The characteristic of this family is the presence of a ligand binding domain that accommodates different sterols. In yeast, the OSH family is composed of seven members (OSH1-OSH7). Deletion of all OSH proteins is lethal, but expression of any of the seven isoforms restores cell viability [105]. Some of these proteins contain pleckstrin homology domains, are membrane associated and are enriched at membrane contact sites, i.e. junctions of ER with other organelles, where they supposedly perform their function of sterol transfer between organelles [53] as well as another function as regulators of phosphoinositide metabolism [106].

9. Phosphatidic acid and the regulation of phospholipid biosynthesis

Concerning phospholipids, the scenario is not less complicated. Cells have hundreds of different phospholipids that are distributed over all the cellular membranes. Phospholipid biosynthesis relies on a sophisticated regulatory circuitry, that involves nutrient sensing, lipid-protein interactions and transcriptional regulation of genes involved in the biosynthetic pathways of different phospholipids [40]. A few years ago, it was reported that the phospholipid phosphatidic acid (PA) has a central role in phospholipid homeostasis [107]. PA itself is a central metabolite in the phospholipid metabolism, being a precursor for most phospholipids [40]. Its mode of action involves the association with the transcription repressor Opi1p, which is bound to the ER membrane via association with Scs7p. Opi1 localization is however dependent on intracellular inositol levels, which in turn control PA availability. Opi1p controls the expression of many genes in phospholipid metabolism, and it is likely that this regulatory circuitry controls phospholipid metabolism in many different conditions [108]. Recently, it was shown that the lipid-protein interaction between Opi1p and PA is influenced by pH, altering the electrostatic properties of the lipid, which is recognized by the effector [109]. Despite this fundamental discovery unveiling a global transcriptional control on a variety of phospholipid biosynthetic enzymes, many questions still remain on the topic and many other regulatory pathways are yet to be discovered.

10. Protein lipid interactions

In order to understand the function of cell membranes and biological processes performed by membrane proteins, it is fundamental to understand how the lipid composition affects protein folding, enzymatic and other activities. To address these questions, we need detailed information about the lipid environment in which these proteins are inserted [110]. One recent attempt to unravel protein-lipid interactions was a screening done with *S. cerevisiae* to catalog these interactions [111]. They developed nitrocellulose arrays that contained sets of lipids of the main lipid classes in yeast and determined the binding profiles of soluble proteins. Several novel interactions were reported, many of which could not be inferred from canonical lipid-binding domains, indicating that our understanding of lipid-protein interaction is still on its infancy.

11. Maintenance of lipid homeostasis

One of the major challenges in the assessment of the roles of lipids in cell biology is the diversity of lipid molecules and the variety of cellular processes that can be affected by lipids. Only a few studies have described the changes of lipids under different environmental conditions or drug treatments in a systematic way

[50,112] and the lack of standardized protocols for lipid extractions and lipid annotation makes the comparison of such studies very difficult.

The cell's lipid composition is maintained by coordinated pathways of synthesis, distribution and degradation of phospholipids, sphingolipids and sterols [5,113,114]. These interconnected networks constitute a highly dynamic biological core that allows cells not only to adapt their lipid profile to environmental challenges such as heat stress, osmotic stress and nutrient availability [115–119], but also to sense the levels of the different lipids and adjust their lipid composition to preserve cellular functions (Fig. 2) [97]. The importance of a lipid homeostasis at the systemic level is demonstrated by the fact that many important diseases such as obesity, diabetes, atheroschlerosis and Alzheimer's disease have a lipid component postulated to affect their onset and/or progression. Even though the physiological consequences of an altered lipid homeostasis have gained more attention the regulatory mechanisms involved in the control of the lipid profile of a cell or an organism are still largely unknown [16–20].

As a model system for understanding lipid homeostasis, *S. cerevisiae* allows for more detailed study of the underlying molecular mechanisms compared to what can be carried out in isolated human cells. With the ease of manipulation, yeast is an ideal model to study regulatory networks, where the system as a whole needs to be considered.

12. Drug screens

S. cerevisiae is currently the best characterized model organism to monitor drug sensitivity [120]. Despite being a simplified model as compared to mammalian cells, it still provides a biologically intact system, in which the different cellular effects of a chemical compound can be assessed in the context of a living cell (Fig. 1). Yeast is particularly well suited for high throughput methods because of its short life cycle, growth in liquid or solid media, without elaborate technical restrictions or expensive media. For drug screening approaches, yeast is probably the most convenient model organism to assess the effects of different compounds on proteins and pathways in a genome-wide scale [121,122]. Maybe the most valuable feature of yeast as a system for drug screenings is the possibility to express human protein homologs in yeast, making it valuable tool for drug discovery. A recent yeast-based assay identified drugs active against human mitochondrial disorders [123].

Ceramides and sphingolipids play fundamental roles in cell biology and have been implicated in different types of cancers. Many efforts have been applied to explain the role of ceramides and sphingolipids in normal and diseased cells, but intervening in ceramides biosynthesis in humans can be challenging, as there are six different ceramides synthases (CerS), each of which has a different specificity to the length of the fatty acyl coenzyme A they use as substrate [124]. The CerS are differentially distributed in various tissues, indicating that different subsets of ceramides could be made in specific tissues to meet specific physiological needs [125]. Despite all the efforts to characterize these enzymes, much remains to be understood about the precise roles of the different ceramides and sphingolipids made by each of the CerS. The use of specific drug inhibitors would greatly facilitate the study of each of these enzymes, but the available inhibitors against CerS (Fumonisin B, Australifungin) are rather unspecific towards the different homologs. In two recent studies, the yeast ceramides synthases (*LAG1* and *LAC1*) were replaced by each of the human CerS, retaining their specificity [126,127]. This is an ideal situation for screening drug inhibitors against CerS. By a high throughput comparative screening, it should be possible now to test and identify compounds that specifically inhibit each of the enzymes [74].

Chemical genomics is also a valuable approach to study the mechanism of action of bioactive chemicals that may have human therapeutic significance [121,128]. In the particular case of lipid metabolism, two successful studies were designed in yeast and uncovered important connections of the dysfunctional processes and lipid biosynthesis. The inhibitor of cancer cell invasion, dihydromotuporamine C (dhMoTC), was first discovered in a phenotypic screen for small molecules that inhibited invasion of carcinoma cells. Because of the difficulties of identifying the target of this drug, the authors relied on a genome-wide drug-induced haplo insufficiency screening to identify its mode of action, which was eventually shown to target sphingolipid biosynthesis [129,130]. Another promising example for the use of yeast screenings to unravel pathological mechanisms of actions, was the identification of fatty acid elongases as essential players to cope with alpha-synuclein toxicity – a hallmark of Parkinson's disease [131].

13. Lipidomics – advances and challenges

It is clear now that studies in lipid metabolism must be conceived and interpreted in a global manner, as interventions in one pathway can have effects in other lipid classes. In this sense, the emergence of lipidomics was of vital importance to study lipid homeostasis and function. The recent technical advances have empowered us to perform global lipid analysis. Although it is still not possible to quantitatively analyze all lipid species at once, many different methods are available, offering high sensitivity and resolution for global lipid profiling [132,133]. The modern approaches for lipid analysis are dominated by mass spectrometry, commonly ESI-MS combined with a previous separation by liquid chromatography or gas chromatography to satisfy the demands for specific solutions given the structural diversity of lipids. MALDI is another ionization technique suitable for lipid analysis, and unlike ESI, it can analyze the analyte directly from the solid phase, i.e. TLC plates [134]; or directly from tissue sections, in imaging mass spectrometry [135], or directly from isolated organelles [8].

In lipidomics, the mass spectrometry approach is used not only for qualitative, but also for quantitative analysis, which can be either relative or absolute, depending on the method. Global lipidomics (shotgun lipidomics) usually performs relative quantification, while targeted approaches as MRM-MS allow for quantitative analysis of specific lipid species. Absolute quantification of lipids, however, is dependent on the availability of internal standards, which are still few. Ideally, internal standards should be structurally similar to the analyte (i.e. a stable isotopically labeled version); however, this is not practical for lipidomic analysis and usually a mix of selected representatives of lipid classes is used.

The yeast global lipidome have been elucidated by different groups [36,37]. Despite a few characterization studies of lipidomic changes over a range of environmental conditions, to date, there is no comprehensive catalog of how cells adapt their lipidome to different environmental conditions [50,112]. One of the major future challenges for lipidomics will be the interpretation of the enormous datasets with complex lipid profiles that are emerging from lipidomic studies (Fig. 2). Undoubtedly, there are many potentially interesting lipid patterns, and therefore, advances in computational and statistical approaches is of vital importance [136].

14. Mathematical modeling

Some attempts have been made to develop mathematical models of the metabolic fluxes of lipid metabolism, using *S. cerevisiae* as the model system. The most comprehensive examples come from a studies on sphingolipid metabolism [137–139] that successfully described the metabolic reactions in accordance with available

data about gene expression levels, kinetic properties of the enzymes and metabolite concentrations, being further implemented in genome-scale metabolic model [140].

The development of these models based on organisms like yeast is an advantageous strategy since the models are simpler and, once validated, can be translated into more complex systems, such as mammalian cells. The use of these models will help researchers to test different scenarios of perturbation in the system, leading to potentially interesting insights into points of regulation.

Finally, due to the availability of different high throughput datasets such as from transcriptome [141], proteome [142], phosphoproteome [143], metabolome [144] and interactome [145] analysis *S. cerevisiae* is the model organism with the most comprehensive experimental dataset available (Fig. 2).

In the future, yeast lipidomics will add a fundamental piece to this cell biology puzzle. The complexity of lipid metabolism involves several layers of regulation, and efforts need to be done in order to integrate different experimental data into single models that provide a detailed description of cellular processes. An overwhelming amount of information is available now about lipids and lipid metabolism. At this time, however, there is no yeast database that combines results from multiple lipidomics methods in a harmonious way, nor a database that systematically collects annotated information related to lipids, similar to existing databases for proteins and genes.

15. Final considerations

The current research on lipids is now shifting from molecular characterization of lipid structures to a global understanding of dynamic lipid changes in a cellular/organismal context in response to intracellular cues and extracellular challenges. And as for other areas of cell biology, studies in yeast have greatly contributed to the study of lipid homeostasis and function, yielding major insights into fundamental pathways that could be transferred to more complex organisms [4].

It is clear that lipidomic analysis is a powerful tool to be used not only as a characterization method, but also as a discovery method. By combining genetic screens, different environmental conditions and the assessment of lipid changes under multiple parallel conditions, we will be able to uncover many unsuspected and novel associations of genes, proteins and lipids.

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