FEBS Letters

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Lipid droplet-mediated lipid and protein homeostasis in budding yeast

Martin Graef[1,2]

1 Max Planck Institute for Biology of Ageing, Cologne, Germany
2 Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

Correspondence
M. Graef, Max Planck Institute for Biology of Ageing, Joseph-Stelzmann-Strasse 9b, 50931 Cologne, Germany
Fax: +49 221 3797088470
Tel: +49 221 37970470
E-mail: martin.graef@age.mpg.de

(Received 3 January 2018, revised 24 January 2018, accepted 25 January 2018, available online 16 February 2018)

doi:10.1002/1873-3468.12996

Edited by Wilhelm Just

Lipid droplets are conserved specialized organelles that store neutral lipids. Our view on this unique organelle has evolved from a simple fat deposit to a highly dynamic and functionally diverse hub—one that mediates the buffering of fatty acid stress and the adaptive reshaping of lipid metabolism to promote membrane and organelle homeostasis and the integrity of central proteostasis pathways, including autophagy, which ensure stress resistance and cell survival. This Review will summarize the recent developments in the budding yeast *Saccharomyces cerevisiae*, as this model organism has been instrumental in deciphering the fundamental mechanisms and principles of lipid droplet biology and interconnecting lipid droplets with many unanticipated cellular functions applicable to many other cell systems.

Keywords: autophagy; lipid droplets; membrane homeostasis

Lipid droplets are dedicated to the dynamic storage of triacylglycerols (TAG) and steryl esters (SE)—neutral lipids, which, enclosed by a single phospholipid leaflet, form the hydrophobic core of the organelle. Lipid droplets are virtually universally conserved organelles from bacteria to mammals [1], however, their number and size as well as their lipid and protein composition can vary considerably depending on cell type and metabolic conditions. Recent advances in the field have shed light on the protein machinery and the molecular mechanisms underlying their formation, regulation of number and size, and mobilization and turnover. Furthermore, the physiological relevance of the dynamic behavior of lipid droplets has emerged for the homeostasis of fatty acid and phospholipid metabolism, for cellular membrane and organellar integrity, and for proteostasis, stress response, and survival.

Lipid droplet biogenesis

The most common model for lipid droplet biogenesis proposes an origin from within the ER membrane, where newly synthesized neutral lipids coalesce in between the two membrane leaflets into lense-like structures [2] (Fig. 1A). Driven by additional neutral lipid synthesis, these lenses grow and nascent lipid droplets emerge from the ER toward the cytosol surrounded by a phospholipid monolayer connected to the ER by ER-lipid droplet junctions. Subsequently, mature lipid droplets may remain connected or bud off and physically separate from the ER [3,4]. ER-associated biogenesis of lipid droplets seems evolutionarily conserved. Interestingly, in budding yeast, lipid droplet formation appears to be spatially coordinated with ER-vacuole contact sites (nuclear-vacuolar junctions; NVJs), which physically expand in response to

Abbreviations
CDP-DAG, CDP-diacylglycerol; CoA, Coenzyme A; DAG, diacylglycerol; ERES, ER exit sites; FIT, fat-inducing transcript; Ire1, inositol-requiring enzyme 1; MAG, monoacylglycerol; MVB, multivesicular body; NPC, Niemann-Pick type C; NVJ, nucleus-vacuole junction; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SE, steryl esters; TAG, triacylglycerols; TORCI, target of rapamycin complex I; UPR, unfolded protein response; UPS, ubiquitin-proteasome system.

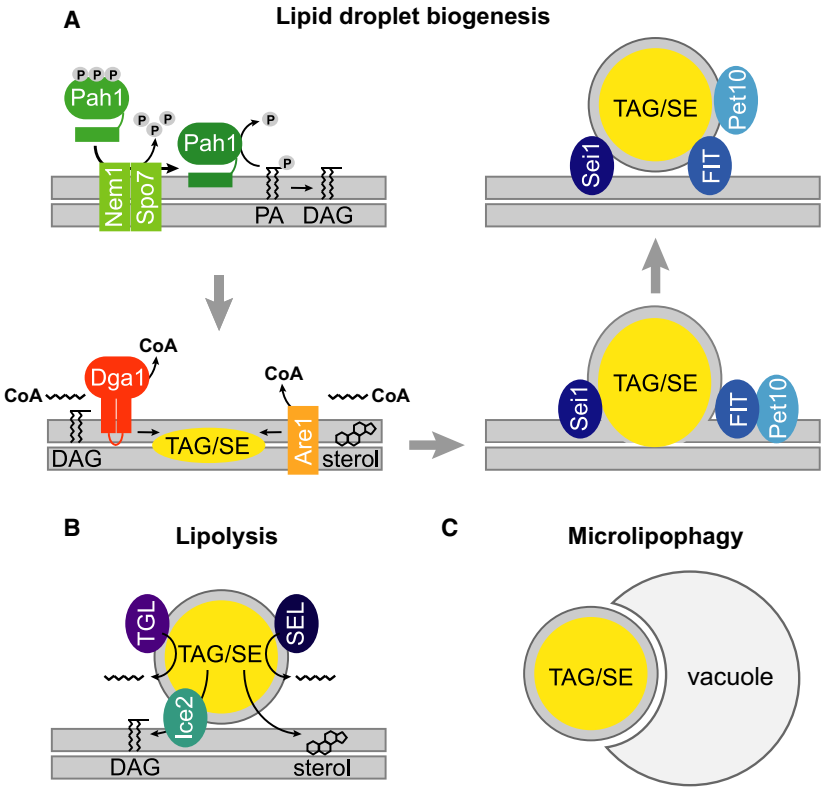

Fig. 1. Mechanisms of lipid droplet biogenesis and mobilization. (A) A simplified schematic for the machineries and mechanisms underlying lipid droplet biogenesis. For a detailed description see the main text. For simplification, Lro1 and Are2 were not included. Multiple phosphorylation sites in Pah1 are indicated by three phosphate groups (B,C) Mechanisms for lipid droplet mobilization. (A) Schematic for lipolysis driven by TAG (TGL) and SE (SEL) lipases. (C) Schematic of microlipophagy, the direct invagination of the vacuolar membrane to deliver lipid droplets for vacuolar degradation. CoA, Coenzyme A; DAG, diacylglycerol; TAG, triacylglycerol; SE, steryl ester; P, inorganic phosphate; PA, phosphatidic acid.

metabolic cues and organize membrane tether proteins and fatty acid activating machinery for neutral lipid synthesis and lipid droplet emergence [5]. Neutral lipid synthesis by itself, however, is insufficient to form neutral lipids into the native structure of lipid droplets, indicating that they arise in a nonrandom and organized fashion. Indeed, an evolutionarily conserved protein machinery of emerging complexity has been identified over recent years that organizes key aspects of lipid droplet biogenesis.

Lipins/Pah1

A pivotal regulatory step that decides whether cells channel lipid resources into glycerolphospholipid synthesis or into the generation of triacylglycerol (TAG) is the conversion of phosphatidic acid (PA) to either CDP-diacylglycerol (CDP-DAG) or diacylglycerol (DAG) respectively. CDP-DAG is generated by the essential CDP-DAG synthase, Cds1, and serves as a precursor for the synthesis of all major glycerolphospholipid species including phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) [6–9]. The competing conversion of PA to DAG constitutes the penultimate step of TAG synthesis and is catalyzed by the yeast lipin, Pah1, a Mg2 + -dependent phosphatidate phosphatase [10] (Fig. 1A). Thus, the competing activities of Cds1 and Pah1 determine whether resources are dedicated to membrane biogenesis or to neutral lipid storage [11], and it is therefore not surprising that they constitute a regulatory hub of lipid metabolism. A complex network of protein kinases including Pho85-Pho80, Cdc28-Cyclin B, protein kinase A, protein kinase C, and casein kinase II controls Pah1 by phosphorylation affecting its activity, localization, and protein stability [12–16]. One of the key regulatory steps is the dynamic association of the peripheral membrane protein Pah1 with membranes where its substrate PA resides [14,17]. Upon dephosphorylation by the conserved membrane-bound Nem1-Spo7 phosphatase complex, the otherwise mainly cytosolic Pah1 anchors onto perinuclear and ER membranes *via* a short amphipathic helix at its N-terminus [17–19]. The activity of the Nem1-Spo7 complex is in part inhibited by phosphorylation by the target of rapamycin complex I (TORCI) kinase [20]. Consistent with the central function of this module in regulating the balance between phospholipid and neutral lipid synthesis, both, Pah1- and Nem1-Spo7-deficient cells show defects in the biogenesis of lipid droplets with significantly reduced TAG levels and, instead, display aberrantly expanded nuclear/ER membranes likely caused

FEBS Letters **592** (2018) 1291–1303 © 2018 The Authors. *FEBS Letters* published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies.

by increased biosynthesis of phospholipids [10,19,21–23]. Interestingly, in Pah1-deficient cells during log-phase, the total amount of neutral lipids is maintained at a level similar to wild-type cells due to increased SE synthesis, but they predominantly reside within the ER membrane [23]. This suggests that Pah1 activity is required to coordinate neutral lipid synthesis with the local formation of lipid droplets. Consistent with this notion, focal Nem1-Spo7 complexes localize next to formed lipid droplets [23].

The function of Pah1 is opposed by the CTP-dependent DAG kinase, Dgk1, that catalyzes the phosphorylation of DAG to generate PA at the nuclear membrane [24,25]. Consistently, additional deletion of *DGK1* can suppress the elevated PA level, the abnormal nuclear and ER membrane expansion, and the reduced number of lipid droplets of a single Pah1-deficient strain [10,24].

Neutral lipid synthesis

Lipin/Pah1 provides the precursor DAG as a substrate for two diacylglycerol-acyltransferases, Dga1 and Lro1, that catalyze the final step in TAG synthesis (Fig. 1A). Dga1 contains two transmembrane domains, which might adopt a hairpin-structure and mediates a dual localization to the ER and the phospholipid monolayer of lipid droplets, which is critical for the initiation as well as the growth of lipid droplets during early stationary phase [3,26]. Mainly during stationary phase, Dga1 esterifies DAG using diverse acyl-CoA species with a substrate preference of oleoyl-CoA (18 : 1), palmityl-CoA (16 : 0), myristyl-CoA (14 : 0), stearoyl-CoA (18 : 0), arachidonyl-CoA (20 : 4), and linoleoyl-CoA (18 : 2) [27,28]. The ER transmembrane protein Lro1 functions as an acyl-CoA-independent phospholipid:diacyl-glycerol acyltransferase and transfers acyl chains from the phospholipids PE or PC to DAG generating TAG and lyso-PE or lyso-PC, respectively, mainly during exponential growth [2,29–32]. Steryl ester synthesis is catalyzed by two acyl-coenzyme A:sterol acyltransferases, Are1 and Are2, which reside in the ER membrane and esterify sterol precursors using predominantly unsaturated acyl-CoA oleoyl-CoA (18 : 1) and palmitoleoyl-CoA (16 : 1) as substrate [33–35] (Fig. 1A). Both, Are1 and Are2, have been shown to generate a minor fraction of TAG in the absence of Dga1 and Lro1 [27,28,32,36,37]. The concomitant deletion of *DGA1*, *LRO1*, *ARE1,* and *ARE2* produces cells that are completely devoid of lipid droplets [27,37], indicating that this set of enzymes is composed of the only neutral lipid synthesizing enzymes in *S. cerevisiae*.

Seipins

The conserved yeast seipin, Sei1 (formerly known as Fld1), forms homooligomeric complexes consisting of nine subunits of the two-transmembrane protein in the ER, which physically interacts with the ER membrane protein Ldb16 [38–41]. The seipin complex promotes the formation of nascent lipid droplets required to suppress the accumulation of neutral lipids in the ER membrane and ensures vectorial budding of lipid droplets toward the cytosol of the ER membrane [42] (Fig. 1A). In response to alterations in the phospholipid composition, the absence of the seipin complex affects lipid droplet morphology and causes lipid droplets to accumulate either as "supersized" organelles or as irregular clusters that are entangled by aberrant ER in close proximity to the nuclear envelop [41,43–45]. Interestingly, Pah1 and Nem1-Spo7 are highly enriched at these sites resulting in locally elevated PA levels, which likely underlie the abnormal proliferation of nuclear ER and the clustering of entangled lipid droplets [44], suggesting the existence of coordinated functions of lipin and the seipin complex for lipid droplet biogenesis. In addition to its role during biogenesis, the seipin complex localizes to ER-lipid droplet contact sites and prevents the equilibrium of ER and lipid droplet surface components, which is important in order to establish and maintain the unique lipid droplet identity [39,40,46]. Recent data suggest that the seipin complex physically interacts and cooperates with two Ldo proteins, Ldo45 and Ldo16, which are generated by a unique splicing event of two overlapping genes. Interestingly, Ldo45 and Ldo16 display distinct protein expression profiles and seem to regulate different aspects of lipid droplet biology: Ldo45 overexpression promotes TAG accumulation by increased Dga1-mediated synthesis and by dampening lipolysis, whereas Ldo16 plays a role in lipophagy (see below) [47]. Nevertheless, both specify a subpopulation of lipid droplets marked by Pdr16/Sfh3 at the nucleus-vacuole junction (NVJ) contact site [47–49]. Pdr16/Sfh3 has been shown to function as a lipid-transfer protein at organellar contact sites and to inhibit neutral lipid mobilization on lipid droplets [48,50].

FIT proteins and perilipin

FIT (for fat-inducing transcript) proteins are conserved ER transmembrane proteins with two FIT2-homologues in yeast, Scs3 and Yft2 [51,52]. FIT proteins bind directly to TAG, which is critical to organize neutral lipids within the ER into nascent lipid droplets [53]. Thus, lipid droplets fail to emerge from the ER and

remain enwrapped by additional ER membrane in the absence of Scs3 or Yft2 [2]. In addition, both FIT proteins are required for normal ER membrane synthesis in response to perturbations in lipid metabolism and ER stress [51]. Perilipin, Pet10, defined by the presence of a PAT domain, is a lipid droplet resident protein and directly binds to TAG-containing lipid droplets. Perilipin functionally interacts with the seipin complex and the FIT proteins and binds to nascent lipid droplets at an early stage affecting their rate of formation and morphology [54] (Fig. 1A).

While we have made great progress in identifying factors contributing to the complex mechanisms of lipid droplet biogenesis, the next task will be to comprehensively describe the functional cooperation and temporal coordination between these above-mentioned machineries to refine our understanding and to explore the contextual modifications of common underlying principles in order to adapt lipid droplet biogenesis to the specific needs of cells in face of diverse metabolic conditions.

Lipid droplet mobilization

Lipid droplets are an integral part of a dynamic cellular network that not only controls lipid storage and buffering, but also critically depends on the biosynthesis and redistribution of lipids. Hence, the mechanisms underlying the mobilization of neutral lipids from lipid droplets are equally important to the processes leading to the formation and growth of lipid droplets.

TAG and SE lipases

In yeast, Tgl3, Tgl4, Tgl5, Ayr1, and Ldh1 function as TAG lipases, which contain a characteristic GXSXG motif and predominantly localize to lipid droplets, and catalyze the hydrolysis of TAG to DAG and free fatty acids [55–59] (Fig. 1B). Interestingly, in addition to their TAG lipase activity, Tgl3 and Tgl5 are bifunctional enzymes and contain also an acyltransferase motif, which catalyzes the acylation of lysoPE and lysoPA respectively [60]. Moreover, Tgl4, the functional orthologue of adipose TAG lipase, ATGL, exhibits TAG lipase, steryl ester hydrolase, phospholipase A(2), and acyl-CoA dependent acylation activity toward lysoPA, suggesting that Tgl4 might function not only in lipid degradation but also in fatty acid channeling and phospholipid remodeling [61]. The presence of these diverse activities raises the important question of how they are coordinately regulated. At least in part, protein localization and substrate availability seem to determine the activity of Tgl3, Tgl4, and Tgl5. In the absence of lipid droplets, Tgl3 localizes to the ER where it lacks lipolytic and acyltransferase activity, suggesting that these activities are restricted to lipid droplets. In addition, Tgl3 is strongly destabilized in the ER resulting in lowered protein steady-state level [62]. Protein instability and loss of catalytic activity can be partially explained by the role of a C-terminal stretch of seven amino acids including two aspartate residues, which face the inside of LDs and are crucial for lipase activity of Tgl3. In the ER, the C-terminus of Tgl3 is exposed to the cytosol resulting in protein instability [63]. Similarly, Tgl4 and Tgl5 localize to the ER and also lose their lipolytic activity in the absence of lipid droplets [64]. Interestingly, they do retain their activity as lysophospholipid acyltransferases in contrast with Tgl3 [64], however, the biological relevance of this activity in the ER remains to be determined. Yju3, Rog1, and Mgl2 are the monoacylglycerol (MAG) lipases, generating a free fatty acid and glycerol as the ultimate step in TAG hydrolysis, and are predominantly associated with membranes and lipid droplets [65–67]. The mobilization of SE is catalyzed by three partially functionally redundant lipases, Tgl1, Yeh1, and Yeh2 [68–70] (Fig. 1B). Tgl1 and Yeh1 localize exclusively to lipid droplets, whereas Yeh2 associates with the plasma membrane [68–70]. In addition to SE, Tgl1 can use TAG as substrate and function as a TAG lipase [70]. In line with the control of the activity of TAG lipases, Tgl1 and Yeh1 are strongly regulated by cellular localization, since both are retained in the ER in the absence of lipid droplets, but they become highly unstable and lose their enzymatic activity [71].

The mobilization of neutral lipids from lipid droplets generates free fatty acids and DAG, which can be channeled into the biosynthesis of phospholipids *via* the CDP-DAG pathway and the Kennedy pathway [9,25,72,73]. In order for DAG to become a precursor for phospholipid biogenesis, DAG needs to efficiently shuttle from lipid droplets to the ER membrane. Ice2, a predicted multispanning ER membrane protein with a single cytosolic domain mediates the physical contact between lipid droplets and the ER and thereby promotes DAG channeling [74]. The pivotal role for these physical association of lipid droplets and the ER for neutral lipid mobilization is highlighted by the observation that Ice2-deficient cells show an extended lag-phase after exiting stationary phase to a similar extent as can be observed for cells lacking lipid droplets altogether [75]. Additionally, when Ice2 is absent, the newly generated DAG catalyzed by the TAG lipases remains on lipid droplets and enters a potentially futile cycle of re-esterification to TAG [74].

Lipophagy – autophagic turnover of lipid droplets

While lipid droplet-resident lipases and hydrolases play a central role, neutral lipids can also be mobilized by autophagic turnover of whole lipid droplet organelles in the vacuole, a process termed lipophagy. Generally, dependent on the mechanisms involved, two forms of autophagy can be distinguished in yeast: macro- and microautophagy. Macroautophagy is characterized by the *de novo* formation of a unique double-membrane vesicle structure, the autophagosome, which nucleates, expands and engulfs parts of the cytoplasm during its *de novo* biogenesis driven by a hierarchically organized and functioning multicomponent autophagy core machinery [76,77]. Upon closure, the newly generated outer membrane of the autophagosome fuses with the vacuolar membrane and releases the inner vesicle and enclosed cargo into the vacuolar lumen for degradation by resident hydrolases [78,79]. Macroautophagy has been shown to target lipid droplets for autophagic turnover in mammalian systems [80]. In yeast, however, it appears that lipophagy proceeds mainly *via* the mechanisms of microautophagy [81–85] (Fig. 1C). During microautophagy, the vacuole membrane itself invaginates, thereby starting to enclose the associated cargo, and buds off as a vesicle into the vacuolar lumen fully encapsulating the cytoplasmic cargo, which is then degraded by vacuolar hydrolases [86]. When yeast cells enter stationary phase or specifically starve for glucose, lipid droplets transition from the perinuclear ER to the membrane of the vacuole, a process that depends on the integrity of the tubulin cytoskeleton suggesting an active transport mechanism [83,85]. The vacuolar membrane displays fascinating dynamics and partitions lipids and proteins into microdomains when cells enter the stationary phase or are exposed to stresses [87]. Lipid droplets, destined for microlipophagy, specifically associate with specialized sterol-enriched, liquid-ordered vacuolar microdomains, whose formation and integrity is a prerequisite for functional recruitment and vacuolar turnover of lipid droplets [81,82,85]. Vacuolar microdomains form and expand when sterols are transported by the multivesicular body (MVB) pathway to the vacuole and distributed to the vacuolar membrane by Niemann-Pick type C (NPC) proteins, Ncr1 and Npc2 [81]. Likely, these initially formed vacuolar microdomains facilitate microlipophagy, which, in turn, provides an additional source of sterols that have been generated in lipid droplets by Are1 and Are2, to accelerate further vacuolar microdomain formation in a feed-forward process [81,82]. Additionally, the regulatory subunit of the seipin complex, Ldo16, has been implicated in microdomain formation and microlipophagy [47]. During glucose starvation, Atg14 localizes from ER exit sites to the vacuolar membrane in an AMPK/Snf1-dependent manner and triggers the formation of vacuolar microdomains in cooperation with Atg6 to support microautophagic turnover of lipid droplets [85]. In order to encapsulate the associated lipid droplets, the ESCRT machinery localizes to and drives the invagination of the vacuolar membrane [84,88]. Microlipophagy displays a complex functional interaction with core macroautophagy machinery. As mentioned above, Atg14 and Atg6 as well as some other core macroautophagy components seem to be required for microlipophagy under some, but not under all conditions [82–85,88]. The requirement for intact macroautophagy might be explained by the fact that the Atg machinery affects NPC protein trafficking and might contribute to autophagosome-mediated transport of sterols to the vacuolar membrane and, consequently, the generation of vacuolar microdomains [81]. The conditional role of Atg proteins suggests the existence of alternative pathways and/or functionally redundant factors and pointing toward a remarkable mechanistic plasticity in response to diverse metabolic or stress-associated cues. However, it seems clear that the adaptor for receptor-mediated selective forms of macroautophagy, Atg11, is consistently dispensable for selective microlipophagy [82,83,85]. Thus, how lipid droplets are selectively targeted for vacuolar turnover and how the rate of microlipophagy is regulated will be an important question to address in the future.

The role of lipid droplets in maintenance of cellular homeostasis

The generation of a lipid droplet-deficient yeast strain, which lacks the neutral lipid synthesizing enzymes *DGA1*, *LRO1*, *ARE1,* and *ARE2*, has been instrumental for our insights into the physiological roles of lipid droplets for cellular function that go far beyond that of a passive fat deposit simply storing chemical energy [27,37]. The synthesis of neutral lipids is not essential for cell viability under nonstress conditions, and lipid droplet-deficient cells only show a delay in cell growth after stationary phase, which is likely caused by the reduced availability of lipid precursors for the synthesis of phospholipids. However, logarithmically growing cells without lipid droplets display signs of chronic ER stress, as their unfolded protein response is constitutively activated, show alterations in the phospholipid composition of their membranes, and present vacuoles with an aberrant and highly fragmented membrane morphology [75,89,90], indicating that the maintenance of cellular homeostasis is intimately linked to lipid

droplet biology. Indeed, when cells are challenged by changes in their nutrient environment, by alterations in their lipid metabolism, or by protein folding stress, lipid droplets provide essential functions in buffering excess fatty acids, in adaptive responses to lipid imbalances, in upholding proteostatic mechanisms including autophagy and inclusion body clearance, and in maintaining stress resistance and cell viability [21,75,84,90–95]. Clearly, lipid droplets have emerged as a central hub for a wide variety of previously unanticipated cellular functions.

Lipid droplet-mediated membrane homeostasis

Biological membranes are essential for cellular life. The establishment and maintenance of the specific physicochemical properties of individual membranes within a cell is a major challenge. In addition to the head groups that define the major phospholipid classes, the fatty acid composition of membrane lipids determines key physical features of biological membranes such as lipid packing and membrane fluidity. On one hand, cells have evolved sophisticated sensing and regulatory mechanisms to dynamically adapt the composition of their membranes in response to disturbances. On the other hand, lipid droplets have emerged as integral organelles in order to provide cells with a capacity for buffering environmental or intracellular challenges to their lipid metabolism that prevents functionally compromising alterations in their biological membranes. Collectively, these mechanisms allow cells to tolerate ever-changing environments and provide them with an adaptive plasticity fundamental to stress resistance and cell survival.

Lipid droplet buffering of unsaturated fatty acids

Wild-type yeast cells tolerate a wide range of external saturated and unsaturated fatty acids. The induction of neutral lipid synthesis and the concomitant biogenesis of lipid droplets are critically important in order to prevent the lipotoxic consequences, in particular, of excess unsaturated fatty acids. For example, as cells accumulate higher levels of phospholipids and free fatty acids and lower levels of TAG in the absence of the lipin Pah1, they show an increased sensitivity toward external palmitic acid, palmitoleic acid, and oleic acid [21]. Importantly, excess palmitoleic acid induces the enzymatic activity of Pah1 and accelerates TAG synthesis in wild-type cells, suggesting that partitioning of fatty acids into neutral lipid synthesis is not passive, but is rather actively sensed and regulated by thus far unknown mechanisms [21]. Cells that are completely deficient in lipid droplet biogenesis are uniquely sensitive to external unsaturated fatty acids and, while the generation of SE shows only a minor impact, it is the synthesis of TAG by Dga1 and Lro1 that is indispensable for the detoxification of unsaturated fatty acids, with shorter chain length and increasing degree of unsaturation exacerbating their toxicity [75,91,92]. Strikingly, the viability of lipid droplet-deficient cells exposed to a lethal concentration of unsaturated fatty acids can be rescued by the cosupplementation of saturated fatty acids, demonstrating that the ratio between saturated and unsaturated fatty acids determines toxicity independently of the absolute concentration of the unsaturated fatty acid [75]. In line with active regulation, unsaturated fatty acids are preferentially channeled into neutral lipids, which is critical to maintain the balanced composition of saturated and unsaturated fatty acid residues in phospholipids and the function of biological membranes [75]. When cells lack the capacity to synthesize neutral lipids, unsaturated fatty acids are incorporated into phospholipids, which leads to a significant shift toward unsaturation in the membrane lipids resulting in the induction of ER stress signaling, massive expansion of ER membranes organized in so-called ER whorls, and, ultimately, lipoapoptosis [75,92]. It is interesting to note that an enhanced synthesis of phospholipids can partially compensate for defects in lipid droplet-mediated fatty acid buffering. For example, the deletion of the DAG-kinase *DGK1* suppresses the induction of phospholipid synthesis and the expansion of the ER membrane in cells lacking Pah1, but at the cost of rendering these double mutant cells even more sensitive toward unsaturated fatty acids [21]. Furthermore, genetic uncoupling of phospholipid synthesis by the deletion of the lipid sensor and transcription inhibitor *OPI1* in lipid droplet-deficient cells improves their resistance against external unsaturated fatty acids [90]. However, the latter case also exemplifies that cells have not evolved to exhaust their full potential of phospholipid synthesis to buffer excess fatty acids when lipid droplet-mediated buffering capacity is exceeded. Thus, cells mainly depend on the buffering and detoxification function of lipid droplets in order to counteract the potentially disastrous consequences of an excess of unsaturated fatty acids.

While genome-wide screens for changes in lipid droplet morphology have provided important insight into the mechanisms controlling lipid droplet biogenesis, the shape and number of lipid droplets by itself, however, is a poor predictor of a cell's sensitivity toward unsaturated fatty acids [41,43,96]. For example, an increased sensitivity toward fatty acids may arise not

only from a failure to partition excess fatty acids into neutral lipids, but also from an overactivation of neutral lipid synthesis and formation of lipid droplets at the expense of essential phospholipid synthesis [96]. Thus, it will be of critical importance in future research to comprehensively characterize the sensing and regulatory mechanisms underlying selective and measured partitioning of unsaturated fatty acids into lipid droplets in order to maintain membrane integrity and cellular function.

Mechanisms to counterbalance an excess of saturated fatty acids

Whereas cells with deficient biogenesis of lipid droplets are highly sensitive toward lipotoxicity induced by an excess of unsaturated fatty acids, they show a great tolerance toward the presence of high concentrations of saturated fatty acids similar to wild-type cells [21,75,92]. In contrast with unsaturated fatty acids, which cells have to detoxify by channeling them into neutral lipid synthesis, incorporated saturated fatty acids undergo substantial metabolic modifications by elongation and desaturation, which balances and fine-tunes the chain length and the degree of fatty acid desaturation within lipids and maintains the function of diverse organellar membranes [75]. At the center of this adaptive response is the single and essential oxygen-dependent Δ9-fatty acid desaturase, Ole1 (human SCD1), a ER transmembrane protein consisting of an N-terminal desaturase domain and a C-terminal cytochrome b5 domain, which introduces C9-C10 double bonds in saturated acyl-CoA substrates [97–99]. As the degree of fatty acid desaturation determines key physical parameters of biological membranes, cells evolved sophisticated sensing and regulatory mechanisms, including the so-called OLE pathway, to coordinate Ole1 expression with the demand for unsaturated fatty acids or to counterbalance membrane stress with the induction of the unfolded protein response (UPR) [100,101]. Ole1 is an intrinsically unstable protein allowing cells to dynamically adjust protein level by tightly regulating its expression by a system of two transcription factors, Spt23 and Mga2, that are produced as 120 kDa precursor proteins bound to the ER membrane *via* their C-terminal transmembrane helices. Both, Spt23 and Mga2 precursor forms are cleaved by the cytosolic ubiquitin-proteasome system (UPS) in response to alterations in the physicochemical properties of the ER membrane resulting in the release of 90 kDa fragments from the ER, which induce expression of *OLE1* as transcriptional coactivators [102–104]. As recently shown, Mga2 itself constitutes a lipid-packing sensor that is responsive to changes in phospholipid saturation in the ER membrane: C-terminal transmembrane helices of Mga2 protein homodimers display a dynamic interplay of helix-helix and helix-membrane interactions, which transduce altered membrane-protein interactions into conformational changes that facilitate the UPS-mediated cleavage of the 120 kDa form [105]. Consistent with central and nonredundant functions for the regulation of the OLE pathway, deletion of *MGA2* results in significantly reduced levels of Ole1 and in severe alterations of the membrane lipid composition together with induction of stress and morphological changes in the ER [106]. Similarly, employing a genetic system that conditionally suppresses Ole1 function, Pineau *et al.* showed that an excess of saturated fatty acids causes severe ER stress and dramatic morphological changes in the ER including a detachment of the cortical ER from the plasma membrane and the swelling of ER tubules before cells undergo cell death [107].

An aberrant lipid composition in the ER membrane can also be directly sensed by the highly conserved sensor of protein unfolding stress in the ER membrane and sole UPR activator in yeast, the inositol-requiring enzyme 1 (Ire1) [108]. A juxta-membrane amphipathic helix of Ire1 responds to the physicochemical properties of the membrane and controls Ire1 dimerization and oligomerization, which leads to the activation of the UPR [108]. The activation of UPR signaling has been shown to drive ER membrane expansion and constitutes an integral part of the ER response to overcome ER stress [109]. However, increased UPR signaling in lipid droplet-deficient cells does not necessarily lead to an expanded ER and, furthermore, while modifying the shape of the expanded ER in response to fatty acid stress, is also not a prerequisite for ER expansion and fatty acid buffering [75,90].

Lipid droplets within adaptive responses to phospholipid imbalances

Yeast cells have evolved a remarkable plasticity to adapt to disturbances in their phospholipid metabolism. Recent work revealed that lipid droplets are an integral part of an adaptive response to chronically defective PC biosynthesis [84]. Specifically, upon acute lipid imbalance, yeast cells initially suffer from severe changes in their lipid composition concomitant with drastic defects in morphology and distribution of the ER and mitochondria together with growth impairment. However, during chronic defects in PC synthesis, cells recover and display clear signs of adaptation. Significantly, synthesis of TAG and induction of lipid

droplet biogenesis with subsequent microlipophagic turnover of excess lipids and associated damaged protein aggregates are essential for cell adaptation [84], indicating that the lipid droplets act as critical buffers and as hubs for lipid redistribution to reshape and adapt cellular lipids to regain organellar integrity and cellular function.

Lipid droplets fulfil critical functions for proteostasis

When cells starve for nitrogen, namely amino acids and ammonium, and cease to divide, external glucose is channeled into glycolysis and fatty acid synthesis and the resulting fatty acids are stored as neutral lipids in lipid droplets. Notably, in cells lacking the capacity to form lipid droplets, the ER undergoes drastic morphological changes: the initially wild-type-like, interconnected network of tubular and sheet-like cortical ER under nonstarvation conditions is transformed into a simplified network of continuous and dilated tubules and locally expanded ER with whorl-like appearances [90,93]. Reminiscent of the changes seen in the ER in the presence of excess external fatty acids, remarkably, during starvation, the intracellular fatty acids generated by *de novo* fatty acid synthesis cause ER deformation in the absence of the fatty acid buffering provided by lipid droplets [90]. It is currently unexplored how unbuffered fatty acid synthesis specifically interferes with the homeostasis of ER membranes, but the observed morphological changes are consistent with an imbalance in the fatty acid composition of membrane lipids suggesting that the sensing and regulatory mechanisms such as the OLE pathway are insufficient to counterbalance the disturbances caused by compromised lipid droplet buffering. Whatever the mechanisms will be, it is remarkable that the regulatory circuits controlling fatty acid synthesis and membrane stress sensing evolved in way that they inherently depend on the fatty acid buffer capacity of lipid droplets [110]. The ER and in particular specialized subregions of the ER, so-called ER exit sites (ERES) dedicated to the formation of COPII-transport vesicle, play an essential role for the *de novo* formation of autophagosomes when starving cells induce macroautophagy [111,112]. The failure of lipid droplet-deficient cells to maintain homeostasis of ER membranes severely compromises their ability to generate autophagosomes for a stress-appropriate autophagy response [90,93,94], and, as a physiological consequence, renders them highly sensitive to nutrient stress with a drastically reduced viability upon prolonged periods of starvation [90]. Lipid droplets have been proposed to function as one of the membrane sources for forming autophagosome [93,113]. While the accumulation of aberrant autophagic precursors that are spatially linked to ERES, but seem to fail to grow into mature autophagosomes in lipid droplet-deficient cells is generally consistent with a lack of sufficient membrane material, it turns out that the primary defects are a lack of fatty acid buffering and alterations in the phospholipid composition of cellular membranes that impinge upon the regulation of autophagy [90]. Specifically, inhibition of fatty acid synthesis or genetically increasing the buffering capacity of lipid droplet-deficient cells partially restores autophagy [90]. In addition, a defect in lipid droplet biogenesis is also associated with changes in the phospholipid composition of cellular membranes with an increase in PI and a decrease in PG and PA [90]. Strikingly, metabolically correcting the phospholipid composition to wild-type level in combination with reduced fatty acid stress is sufficient to cure the autophagy defects in lipid droplet-less cells [90]. Thus, these observations provide compelling evidence that lipid droplets are dispensable as general membrane sources for autophagosome biogenesis and fulfil fundamental functions in maintaining ER homeostasis to uphold nutrient stress resistance. However, this does not exclude the possibility that lipid droplets may contribute lipid precursors to autophagic membranes under some conditions. For example, when neutral lipid mobilization is compromised in the absence of the two TAG lipases, Ayr1 and ldh1, or the SE lipase Yeh1, cells show a reduction in their autophagy response [93]. Similarly, defects in Ice2, functioning in establishing and maintaining the physical contact of lipid droplets with the ER and in channeling of DAG to the ER, or Ldb16, a physical interactor of the seipin complex, also partially compromise starvation-induced autophagy suggesting that, in the presence of lipid droplet biogenesis, neutral lipid mobilization might directly support autophagy [93]. While the underlying mechanistic details await further analysis, the currently available evidence clearly supports the notion of an intimate relationship of lipid droplets biology and the regulation of autophagy.

In addition to autophagy, recent work has assigned a direct role for lipid droplets in the clearance of inclusion bodies [95]. Lipid droplets spatially and physically associate with formed inclusion bodies. The inclusion body-localized form of Iml2 is required for efficient clearance of inclusion bodies and physically interacts with the lipid droplet proteins perilipin/Pet10 and Pdr16, which specifies a subpopulation of lipid droplets at nucleus-vacuole junction contact sites [49,54,95]. Consistent with a functional role, lipid

droplet-deficient cells display a similar impairment in inclusion body clearance as cells lacking Iml2 [95]. In contrast with starvation-induced autophagy, removal of inclusion bodies specifically depended on SE synthesis, but occurred independently of TAG synthesis [90,95]. Fascinatingly, lipid droplets seem to provide a sterol-based metabolite that might function as a chemical chaperone, as supplementation of 25-hydroxycholesterol can rescue the clearance defects in the absence of SE synthesis [95].

Perspective

We have made substantial progress in identifying the mechanisms and factors involved in mediating the astonishing dynamics of lipid droplets in face of diverse metabolic conditions. It will be important to deepen our insights into how these multifactorial machineries cooperate in a functional as well as temporal manner to induce the biogenesis or the mobilization of lipid droplets according to cellular needs. A fundamental question remains in how the number, size, and, most importantly, the absolute lipid droplet capacity of a given cell under diverse metabolic conditions is determined. Unraveling these mechanisms will likely provide us with novel targets for therapeutic intervention in the many diseases linked to altered lipid metabolism. Additionally, given the central role for lipid droplets in the homeostasis of lipid metabolism and of cellular membranes, a major challenge will be to identify the detailed mechanisms that cells employ to sense subtle changes and how they fine-tune counterbalancing synthesis and, in particular, the selective, moderate, and potentially locally restricted buffering responses centering on neutral lipid synthesis and mobilization. This is particularly important, as it has become clear that the regulatory mechanisms that cells evolved are inherently limited in their capacity to compensate for exceeded lipid droplet buffering. Finally, since lipid droplets have emerged as being deeply involved in stress resistance and survival, we will need a more mechanistic and comprehensive understanding of the complex interrelationship of lipid droplets with diverse cellular functions including proteostasis pathways such as autophagy. Here, the budding yeast system will likely continue to be extremely informative in providing the basis for system-wide analyses.

Acknowledgments

This work was supported by the Max Planck society and the Deutsche Forschungsgemeinschaft (SFB 1218/TP A04).

References

1 Murphy DJ (2012) The dynamic roles of intracellular lipid droplets: from archaea to mammals. *Protoplasma* **249**, 541–585.
2 Choudhary V, Ojha N, Golden A and Prinz WA (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J Cell Biol* **211**, 261–271.
3 Jacquier N, Choudhary V, Mari M, Toulmay A, Reggiori F and Schneiter R (2011) Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. *J Cell Sci* **124**, 2424–2437.
4 Mishra S, Khaddaj R, Cottier S, Stradalova V, Jacob C and Schneiter R (2016) Mature lipid droplets are accessible to ER luminal proteins. *J Cell Sci* **129**, 3803–3815.
5 Hariri H, Rogers S, Ugrankar R, Liu YL, Feathers JR and Henne WM (2017) Lipid droplet biogenesis is spatially coordinated at ER-vacuole contacts under nutritional stress. *EMBO Rep* **19**, 57–72.
6 Carter JR and Kennedy EP (1966) Enzymatic synthesis of cytidine diphosphate diglyceride. *J Lipid Res* **7**, 678–683.
7 Kelley MJ and Carman GM (1987) Purification and characterization of CDP-diacylglycerol synthase from Saccharomyces cerevisiae. *J Biol Chem* **262**, 14563–14570.
8 Shen H, Heacock PN, Clancey CJ and Dowhan W (1996) The CDS1 gene encoding CDP-diacylglycerol synthase in Saccharomyces cerevisiae is essential for cell growth. *J Biol Chem* **271**, 789–795.
9 Henry SA, Kohlwein SD and Carman GM (2012) Metabolism and regulation of glycerolipids in the yeast Saccharomyces cerevisiae. *Genetics* **190**, 317–349.
10 Han GS, Wu WI and Carman GM (2006) The Saccharomyces cerevisiae Lipin homolog is a Mg2 + -dependent phosphatidate phosphatase enzyme. *J Biol Chem* **281**, 9210–9218.
11 He Y, Yam C, Pomraning K, Chin JS, Yew JY, Freitag M and Oliferenko S (2014) Increase in cellular triacylglycerol content and emergence of large ER-associated lipid droplets in the absence of CDP-DG synthase function. *Mol Biol Cell* **25**, 4083–4095.
12 Su WM, Han GS and Carman GM (2014) Yeast Nem1-Spo7 protein phosphatase activity on Pah1 phosphatidate phosphatase is specific for the Pho85-Pho80 protein kinase phosphorylation sites. *J Biol Chem* **289**, 34699–34708.
13 Hsieh LS, Su WM, Han GS and Carman GM (2016) Phosphorylation of yeast Pah1 phosphatidate phosphatase by casein kinase II regulates its function in lipid metabolism. *J Biol Chem* **291**, 9974–9990.
14 Choi HS, Su WM, Morgan JM, Han GS, Xu Z, Karanasios E, Siniossoglou S and Carman GM

(2011) Phosphorylation of phosphatidate phosphatase regulates its membrane association and physiological functions in Saccharomyces cerevisiae: identification of SER(602), THR(723), AND SER(744) as the sites phosphorylated by CDC28 (CDK1)-encoded cyclin-dependent kinase. *J Biol Chem* **286**, 1486–1498.

15 Choi HS, Su WM, Han GS, Plote D, Xu Z and Carman GM (2012) Pho85p-Pho80p phosphorylation of yeast Pah1p phosphatidate phosphatase regulates its activity, location, abundance, and function in lipid metabolism. *J Biol Chem* **287**, 11290–11301.

16 Su WM, Han GS, Casciano J and Carman GM (2012) Protein kinase A-mediated phosphorylation of Pah1p phosphatidate phosphatase functions in conjunction with the Pho85p-Pho80p and Cdc28p-cyclin B kinases to regulate lipid synthesis in yeast. *J Biol Chem* **287**, 33364–33376.

17 Karanasios E, Han GS, Xu Z, Carman GM and Siniossoglou S (2010) A phosphorylation-regulated amphipathic helix controls the membrane translocation and function of the yeast phosphatidate phosphatase. *Proc Natl Acad Sci USA* **107**, 17539–17544.

18 Siniossoglou S, Santos-Rosa H, Rappsilber J, Mann M and Hurt E (1998) A novel complex of membrane proteins required for formation of a spherical nucleus. *EMBO J* **17**, 6449–6464.

19 Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S and Siniossoglou S (2005) The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. *EMBO J* **24**, 1931–1941.

20 Dubots E, Cottier S, Peli-Gulli MP, Jaquenoud M, Bontron S, Schneiter R and De Virgilio C (2014) TORC1 regulates Pah1 phosphatidate phosphatase activity via the Nem1/Spo7 protein phosphatase complex. *PLoS One* **9**, e104194.

21 Fakas S, Qiu Y, Dixon JL, Han GS, Ruggles KV, Garbarino J, Sturley SL and Carman GM (2011) Phosphatidate phosphatase activity plays key role in protection against fatty acid-induced toxicity in yeast. *J Biol Chem* **286**, 29074–29085.

22 Barbosa AD, Sembongi H, Su WM, Abreu S, Reggiori F, Carman GM and Siniossoglou S (2015) Lipid partitioning at the nuclear envelope controls membrane biogenesis. *Mol Biol Cell* **26**, 3641–3657.

23 Adeyo O, Horn PJ, Lee S, Binns DD, Chandrahas A, Chapman KD and Goodman JM (2011) The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets. *J Cell Biol* **192**, 1043–1055.

24 Han GS, O'Hara L, Carman GM and Siniossoglou S (2008) An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth. *J Biol Chem* **283**, 20433–20442.

25 Han GS, O'Hara L, Siniossoglou S and Carman GM (2008) Characterization of the yeast DGK1-encoded CTP-dependent diacylglycerol kinase. *J Biol Chem* **283**, 20443–20453.

26 Stone SJ, Levin MC and Farese RV Jr (2006) Membrane topology and identification of key functional amino acid residues of murine acyl-CoA: diacylglycerol acyltransferase-2. *J Biol Chem* **281**, 40273–40282.

27 Oelkers P, Cromley D, Padamsee M, Billheimer JT and Sturley SL (2002) The DGA1 gene determines a second triglyceride synthetic pathway in yeast. *J Biol Chem* **277**, 8877–8881.

28 Sorger D and Daum G (2002) Synthesis of triacylglycerols by the acyl-coenzyme A:diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast Saccharomyces cerevisiae. *J Bacteriol* **184**, 519–524.

29 Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H and Stymne S (2000) Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. *Proc Natl Acad Sci USA* **97**, 6487–6492.

30 Horvath SE, Wagner A, Steyrer E and Daum G (2011) Metabolic link between phosphatidylethanolamine and triacylglycerol metabolism in the yeast Saccharomyces cerevisiae. *Biochem Biophys Acta* **1811**, 1030–1037.

31 Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein SD and Daum G (1999) Identification and characterization of major lipid particle proteins of the yeast Saccharomyces cerevisiae. *J Bacteriol* **181**, 6441–6448.

32 Oelkers P, Tinkelenberg A, Erdeniz N, Cromley D, Billheimer JT and Sturley SL (2000) A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. *J Biol Chem* **275**, 15609–15612.

33 Zweytick D, Leitner E, Kohlwein SD, Yu C, Rothblatt J and Daum G (2000) Contribution of Are1p and Are2p to steryl ester synthesis in the yeast Saccharomyces cerevisiae. *Eur J Biochem* **267**, 1075–1082.

34 Yang H, Bard M, Bruner DA, Gleeson A, Deckelbaum RJ, Aljinovic G, Pohl TM, Rothstein R and Sturley SL (1996) Sterol esterification in yeast: a two-gene process. *Science* **272**, 1353–1356.

35 Yu C, Kennedy NJ, Chang CC and Rothblatt JA (1996) Molecular cloning and characterization of two isoforms of Saccharomyces cerevisiae acyl-CoA:sterol acyltransferase. *J Biol Chem* **271**, 24157–24163.

36 Sandager L, Dahlqvist A, Banas A, Stahl U, Lenman M, Gustavsson M and Stymne S (2000) An acyl-CoA: cholesterol acyltransferase (ACAT)-related gene is involved in the accumulation of triacylglycerols in Saccharomyces cerevisiae. *Biochem Soc Trans* **28**, 700–702.

37 Sandager L, Gustavsson MH, Stahl U, Dahlqvist A, Wiberg E, Banas A, Lenman M, Ronne H and Stymne S (2002) Storage lipid synthesis is non-essential in yeast. *J Biol Chem* **277**, 6478–6482.
38 Binns D, Lee S, Hilton CL, Jiang QX and Goodman JM (2010) Seipin is a discrete homooligomer. *Biochemistry* **49**, 10747–10755.
39 Wang CW, Miao YH and Chang YS (2014) Control of lipid droplet size in budding yeast requires the collaboration between Fld1 and Ldb16. *J Cell Sci* **127**, 1214–1228.
40 Szymanski KM, Binns D, Bartz R, Grishin NV, Li WP, Agarwal AK, Garg A, Anderson RG and Goodman JM (2007) The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc Natl Acad Sci USA* **104**, 20890–20895.
41 Fei W, Shui G, Gaeta B, Du X, Kuerschner L, Li P, Brown AJ, Wenk MR, Parton RG and Yang H (2008) Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *J Cell Biol* **180**, 473–482.
42 Cartwright BR, Binns DD, Hilton CL, Han S, Gao Q and Goodman JM (2015) Seipin performs dissectible functions in promoting lipid droplet biogenesis and regulating droplet morphology. *Mol Biol Cell* **26**, 726–739.
43 Fei W, Shui G, Zhang Y, Krahmer N, Ferguson C, Kapterian TS, Lin RC, Dawes IW, Brown AJ, Li P *et al*. (2011) A role for phosphatidic acid in the formation of "supersized" lipid droplets. *PLoS Genet* **7**, e1002201.
44 Wolinski H, Hofbauer HF, Hellauer K, Cristobal-Sarramian A, Kolb D, Radulovic M, Knittelfelder OL, Rechberger GN and Kohlwein SD (2015) Seipin is involved in the regulation of phosphatidic acid metabolism at a subdomain of the nuclear envelope in yeast. *Biochem Biophys Acta* **1851**, 1450–1464.
45 Wolinski H, Kolb D, Hermann S, Koning RI and Kohlwein SD (2011) A role for seipin in lipid droplet dynamics and inheritance in yeast. *J Cell Sci* **124**, 3894–3904.
46 Grippa A, Buxo L, Mora G, Funaya C, Idrissi FZ, Mancuso F, Gomez R, Muntanya J, Sabido E and Carvalho P (2015) The seipin complex Fld1/Ldb16 stabilizes ER-lipid droplet contact sites. *J Cell Biol* **211**, 829–844.
47 Teixeira V, Johnsen L, Martinez-Montanes F, Grippa A, Buxo L, Idrissi FZ, Ejsing CS and Carvalho P (2017) Regulation of lipid droplets by metabolically controlled Ldo isoforms. *J Cell Biol* **217**, 127–138.
48 Ren J, Pei-Chen Lin C, Pathak MC, Temple BR, Nile AH, Mousley CJ, Duncan MC, Eckert DM, Leiker TJ, Ivanova PT *et al*. (2014) A phosphatidylinositol transfer protein integrates phosphoinositide signaling with lipid droplet metabolism to regulate a developmental program of nutrient stress-induced membrane biogenesis. *Mol Biol Cell* **25**, 712–727.
49 Eisenberg-Bord M, Mari M, Weill U, Rosenfeld-Gur E, Moldavski O, Castro IG, Soni KG, Harpaz N, Levine TP, Futerman AH *et al*. (2017) Identification of seipin-linked factors that act as determinants of a lipid droplet subpopulation. *J Cell Biol* **217**, 269–282.
50 Selitrennik M and Lev S (2016) The role of phosphatidylinositol-transfer proteins at membrane contact sites. *Biochem Soc Trans* **44**, 419–424.
51 Moir RD, Gross DA, Silver DL and Willis IM (2012) SCS3 and YFT2 link transcription of phospholipid biosynthetic genes to ER stress and the UPR. *PLoS Genet* **8**, e1002890.
52 Kadereit B, Kumar P, Wang WJ, Miranda D, Snapp EL, Severina N, Torregroza I, Evans T and Silver DL (2008) Evolutionarily conserved gene family important for fat storage. *Proc Natl Acad Sci USA* **105**, 94–99.
53 Gross DA, Zhan C and Silver DL (2011) Direct binding of triglyceride to fat storage-inducing transmembrane proteins 1 and 2 is important for lipid droplet formation. *Proc Natl Acad Sci USA* **108**, 19581–19586.
54 Gao Q, Binns DD, Kinch LN, Grishin NV, Ortiz N, Chen X and Goodman JM (2017) Pet10p is a yeast perilipin that stabilizes lipid droplets and promotes their assembly. *J Cell Biol* **216**, 3199–3217.
55 Athenstaedt K and Daum G (2003) YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of Saccharomyces cerevisiae. *J Biol Chem* **278**, 23317–23323.
56 Athenstaedt K and Daum G (2005) Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast Saccharomyces cerevisiae are localized to lipid particles. *J Biol Chem* **280**, 37301–37309.
57 Ploier B, Scharwey M, Koch B, Schmidt C, Schatte J, Rechberger G, Kollroser M, Hermetter A and Daum G (2013) Screening for hydrolytic enzymes reveals Ayr1p as a novel triacylglycerol lipase in Saccharomyces cerevisiae. *J Biol Chem* **288**, 36061–36072.
58 Debelyy MO, Thoms S, Connerth M, Daum G and Erdmann R (2011) Involvement of the Saccharomyces cerevisiae hydrolase Ldh1p in lipid homeostasis. *Eukaryot Cell* **10**, 776–781.
59 Thoms S, Debelyy MO, Connerth M, Daum G and Erdmann R (2011) The putative Saccharomyces cerevisiae hydrolase Ldh1p is localized to lipid droplets. *Eukaryot Cell* **10**, 770–775.
60 Rajakumari S and Daum G (2010) Janus-faced enzymes yeast Tgl3p and Tgl5p catalyze lipase and acyltransferase reactions. *Mol Biol Cell* **21**, 501–510.
61 Rajakumari S and Daum G (2010) Multiple functions as lipase, steryl ester hydrolase, phospholipase, and

acyltransferase of Tgl4p from the yeast Saccharomyces cerevisiae. *J Biol Chem* **285**, 15769–15776.
62 Schmidt C, Athenstaedt K, Koch B, Ploier B and Daum G (2013) Regulation of the yeast triacylglycerol lipase TGl3p by formation of nonpolar lipids. *J Biol Chem* **288**, 19939–19948.
63 Koch B, Schmidt C, Ploier B and Daum G (2014) Modifications of the C terminus affect functionality and stability of yeast triacylglycerol lipase Tgl3p. *J Biol Chem* **289**, 19306–19316.
64 Klein I, Klug L, Schmidt C, Zandl M, Korber M, Daum G and Athenstaedt K (2016) Regulation of the yeast triacylglycerol lipases Tgl4p and Tgl5p by the presence/absence of nonpolar lipids. *Mol Biol Cell* **27**, 2014–2024.
65 Heier C, Taschler U, Rengachari S, Oberer M, Wolinski H, Natter K, Kohlwein SD, Leber R and Zimmermann R (2010) Identification of Yju3p as functional orthologue of mammalian monoglyceride lipase in the yeast Saccharomycescerevisiae. *Biochem Biophys Acta* **1801**, 1063–1071.
66 Vishnu Varthini L, Selvaraju K, Srinivasan M and Nachiappan V (2015) ROG1 encodes a monoacylglycerol lipase in Saccharomyces cerevisiae. *FEBS Lett* **589**, 23–30.
67 Selvaraju K, Gowsalya R, Vijayakumar R and Nachiappan V (2016) MGL2/YMR210w encodes a monoacylglycerol lipase in Saccharomyces cerevisiae. *FEBS Lett* **590**, 1174–1186.
68 Koffel R, Tiwari R, Falquet L and Schneiter R (2005) The Saccharomyces cerevisiae YLL012/YEH1, YLR020/YEH2, and TGL1 genes encode a novel family of membrane-anchored lipases that are required for steryl ester hydrolysis. *Mol Cell Biol* **25**, 1655–1668.
69 Mullner H, Deutsch G, Leitner E, Ingolic E and Daum G (2005) YEH2/YLR020c encodes a novel steryl ester hydrolase of the yeast Saccharomyces cerevisiae. *J Biol Chem* **280**, 13321–13328.
70 Jandrositz A, Petschnigg J, Zimmermann R, Natter K, Scholze H, Hermetter A, Kohlwein SD and Leber R (2005) The lipid droplet enzyme Tgl1p hydrolyzes both steryl esters and triglycerides in the yeast, Saccharomyces cerevisiae. *Biochem Biophys Acta* **1735**, 50–58.
71 Klein I, Korber M, Athenstaedt K and Daum G (2017) The impact of nonpolar lipids on the regulation of the steryl ester hydrolases Tgl1p and Yeh1p in the yeast Saccharomyces cerevisiae. *Biochem Biophys Acta* **1862**, 1491–1501.
72 Fakas S, Konstantinou C and Carman GM (2011) DGK1-encoded diacylglycerol kinase activity is required for phospholipid synthesis during growth resumption from stationary phase in Saccharomyces cerevisiae. *J Biol Chem* **286**, 1464–1474.
73 Kuchler K, Daum G and Paltauf F (1986) Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in Saccharomyces cerevisiae. *J Bacteriol* **165**, 901–910.
74 Markgraf DF, Klemm RW, Junker M, Hannibal-Bach HK, Ejsing CS and Rapoport TA (2014) An ER protein functionally couples neutral lipid metabolism on lipid droplets to membrane lipid synthesis in the ER. *Cell Rep* **6**, 44–55.
75 Petschnigg J, Wolinski H, Kolb D, Zellnig G, Kurat CF, Natter K and Kohlwein SD (2009) Good fat, essential cellular requirements for triacylglycerol synthesis to maintain membrane homeostasis in yeast. *J Biol Chem* **284**, 30981–30993.
76 Suzuki K, Kubota Y, Sekito T and Ohsumi Y (2007) Hierarchy of Atg proteins in pre-autophagosomal structure organization. *Genes Cells* **12**, 209–218.
77 Feng Y, He D, Yao Z and Klionsky DJ (2014) The machinery of macroautophagy. *Cell Res* **24**, 24–41.
78 Kraft C and Martens S (2012) Mechanisms and regulation of autophagosome formation. *Curr Opin Cell Biol* **24**, 496–501.
79 Lamb CA, Yoshimori T and Tooze SA (2013) The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* **14**, 759–774.
80 Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM and Czaja MJ (2009) Autophagy regulates lipid metabolism. *Nature* **458**, 1131–1135.
81 Tsuji T, Fujimoto M, Tatematsu T, Cheng J, Orii M, Takatori S & Fujimoto T. (2017) Niemann-Pick type C proteins promote microautophagy by expanding raft-like membrane domains in the yeast vacuole. *eLife* **6**, e25960.
82 Wang CW, Miao YH and Chang YS (2014) A sterol-enriched vacuolar microdomain mediates stationary phase lipophagy in budding yeast. *J Cell Biol* **206**, 357–366.
83 van Zutphen T, Todde V, de Boer R, Kreim M, Hofbauer HF, Wolinski H, Veenhuis M, van der Klei IJ and Kohlwein SD (2014) Lipid droplet autophagy in the yeast Saccharomyces cerevisiae. *Mol Biol Cell* **25**, 290–301.
84 Vevea JD, Garcia EJ, Chan RB, Zhou B, Schultz M, Di Paolo G, McCaffery JM and Pon LA (2015) Role for Lipid Droplet Biogenesis and Microlipophagy in Adaptation to Lipid Imbalance in Yeast. *Dev Cell* **35**, 584–599.
85 Seo AY, Lau PW, Feliciano D, Sengupta P, Gros MAL, Cinquin B, Larabell CA and Lippincott-Schwartz J (2017) AMPK and vacuole-associated Atg14p orchestrate mu-lipophagy for energy production and long-term survival under glucose starvation. *eLife* **6**, e21690.
86 Uttenweiler A and Mayer A (2008) Microautophagy in the yeast Saccharomyces cerevisiae. *Methods Mol Biol* **445**, 245–259.
87 Toulmay A and Prinz WA (2013) Direct imaging reveals stable, micrometer-scale lipid domains that segregate proteins in live cells. *J Cell Biol* **202**, 35–44.

88 Oku M, Maeda Y, Kagohashi Y, Kondo T, Yamada M, Fujimoto T and Sakai Y (2017) Evidence for ESCRT- and clathrin-dependent microautophagy. *J Cell Biol* **216**, 3263–3274.
89 Olzmann JA and Kopito RR (2011) Lipid droplet formation is dispensable for endoplasmic reticulum-associated degradation. *J Biol Chem* **286**, 27872–27874.
90 Velazquez AP, Tatsuta T, Ghillebert R, Drescher I and Graef M (2016) Lipid droplet-mediated ER homeostasis regulates autophagy and cell survival during starvation. *J Cell Biol* **212**, 621–631.
91 Connerth M, Czabany T, Wagner A, Zellnig G, Leitner E, Steyrer E and Daum G (2010) Oleate inhibits steryl ester synthesis and causes liposensitivity in yeast. *J Biol Chem* **285**, 26832–26841.
92 Garbarino J, Padamsee M, Wilcox L, Oelkers PM, D'Ambrosio D, Ruggles KV, Ramsey N, Jabado O, Turkish A and Sturley SL (2009) Sterol and diacylglycerol acyltransferase deficiency triggers fatty acid-mediated cell death. *J Biol Chem* **284**, 30994–31005.
93 Shpilka T, Welter E, Borovsky N, Amar N, Mari M, Reggiori F and Elazar Z (2015) Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis. *EMBO J* **34**, 2117–2131.
94 Li D, Song JZ, Li H, Shan MH, Liang Y, Zhu J and Xie Z (2015) Storage lipid synthesis is necessary for autophagy induced by nitrogen starvation. *FEBS Lett* **589**, 269–276.
95 Moldavski O, Amen T, Levin-Zaidman S, Eisenstein M, Rogachev I, Brandis A, Kaganovich D and Schuldiner M (2015) Lipid droplets are essential for efficient clearance of cytosolic inclusion bodies. *Dev Cell* **33**, 603–610.
96 Ruggles KV, Garbarino J, Liu Y, Moon J, Schneider K, Henneberry A, Billheimer J, Millar JS, Marchadier D, Valasek MA *et al*. (2014) A functional, genome-wide evaluation of liposensitive yeast identifies the "ARE2 required for viability" (ARV1) gene product as a major component of eukaryotic fatty acid resistance. *J Biol Chem* **289**, 4417–4431.
97 Stukey JE, McDonough VM and Martin CE (1989) Isolation and characterization of OLE1, a gene affecting fatty acid desaturation from Saccharomyces cerevisiae. *J Biol Chem* **264**, 16537–16544.
98 Stukey JE, McDonough VM and Martin CE (1990) The OLE1 gene of Saccharomyces cerevisiae encodes the delta 9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. *J Biol Chem* **265**, 20144–20149.
99 Mitchell AG and Martin CE (1995) A novel cytochrome b5-like domain is linked to the carboxyl terminus of the Saccharomyces cerevisiae delta-9 fatty acid desaturase. *J Biol Chem* **270**, 29766–29772.
100 Ballweg S and Ernst R (2017) Control of membrane fluidity: the OLE pathway in focus. *Biol Chem* **398**, 215–228.
101 Walter P and Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081–1086.
102 Zhang S, Burkett TJ, Yamashita I and Garfinkel DJ (1997) Genetic redundancy between SPT23 and MGA2: regulators of Ty-induced mutations and Ty1 transcription in Saccharomyces cerevisiae. *Mol Cell Biol* **17**, 4718–4729.
103 Zhang S, Skalsky Y and Garfinkel DJ (1999) MGA2 or SPT23 is required for transcription of the delta9 fatty acid desaturase gene, OLE1, and nuclear membrane integrity in Saccharomyces cerevisiae. *Genetics* **151**, 473–483.
104 Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD and Jentsch S (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell* **102**, 577–586.
105 Covino R, Ballweg S, Stordeur C, Michaelis JB, Puth K, Wernig F, Bahrami A, Ernst AM, Hummer G and Ernst R (2016) A eukaryotic sensor for membrane lipid saturation. *Mol Cell* **63**, 49–59.
106 Surma MA, Klose C, Peng D, Shales M, Mrejen C, Stefanko A, Braberg H, Gordon DE, Vorkel D, Ejsing CS *et al*. (2013) A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. *Mol Cell* **51**, 519–530.
107 Pineau L, Colas J, Dupont S, Beney L, Fleurat-Lessard P, Berjeaud JM, Berges T and Ferreira T (2009) Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. *Traffic* **10**, 673–690.
108 Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, Hanelt I, Hummer G and Ernst R (2017) Activation of the unfolded protein response by lipid bilayer stress. *Mol Cell* **67**, 673–684. e8
109 Schuck S, Prinz WA, Thorn KS, Voss C and Walter P (2009) Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. *J Cell Biol* **187**, 525–536.
110 Tehlivets O, Scheuringer K and Kohlwein SD (2007) Fatty acid synthesis and elongation in yeast. *Biochem Biophys Acta* **1771**, 255–270.
111 Suzuki K, Akioka M, Kondo-Kakuta C, Yamamoto H and Ohsumi Y (2013) Fine mapping of autophagy-related proteins during autophagosome formation in Saccharomyces cerevisiae. *J Cell Sci* **126**, 2534–2544.
112 Graef M, Friedman JR, Graham C, Babu M and Nunnari J (2013) ER exit sites are physical and functional core autophagosome biogenesis components. *Mol Biol Cell* **24**, 2918–2931.
113 Dupont N, Chauhan S, Arko-Mensah J, Castillo EF, Masedunskas A, Weigert R, Robenek H, Proikas-Cezanne T and Deretic V (2014) Neutral lipid stores and lipase PNPLA5 contribute to autophagosome biogenesis. *Curr Biol* **24**, 609–620.