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 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...
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
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), palmitoyl-CoA (16 : 0), myristoyl-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 [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 [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 Yfi2 [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 GXXSG 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 multspanning 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 **OP11** 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 microautophagic 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 fulfill 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 fulfill 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 [90,95]. Fascinatingly, lipid droplets seem to provide a sterol-based metabolite that might function as a chemical chaperone, as supplementation of 25-hydroxycholesterol-based metabolite that might function as a chemi-

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.

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Lipid droplet-mediated homeostasis

M. Graef

1300

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Lipid droplet-mediated homeostasis

M. Graef

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