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REVIEW

Lipid droplet dynamics in budding yeast

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Abstract Eukaryotic cells store excess fatty acids as neutral lipids, predominantly triacylglycerols and sterol esters, in organelles termed lipid droplets (LDs) that bulge out from the endoplasmic reticulum. LDs are highly dynamic and contribute to diverse cellular functions. The catabolism of the storage lipids within LDs is channeled to multiple metabolic pathways, providing molecules for energy production, membrane building blocks, and lipid signaling. LDs have been implicated in a number of protein degradation and pathogen infection processes. LDs may be linked to prevalent human metabolic diseases and have marked potential for biofuel production. The knowledge accumulated on LDs in recent years provides a foundation for diverse, and even unexpected, future research. This review focuses on recent advances in LD research, emphasizing the diverse physiological roles of LDs in the model system of budding yeast.

Keywords Endoplasmic reticulum · Triacylglycerol · Sterol ester · Phospholipid · Membrane · Metabolism

Abbreviations

ACAT	Acyl-CoA:cholesterol acyltransferase
ATGL	Adipose triacylglyceride lipase
CCT	CTP:phosphocholine cytidylyltransferase
CoA	Coenzyme A
CDP-DAG	Cytidine diphosphate-diacylglycerol
CTP	Cytidine triphosphate
DAG	Diacylglycerol
DGAT	Acyl-CoA:diacylglycerol acyltransferase
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
FA	Fatty acid
FIT	Fat-inducing transcript
GPAT	Glycerol-3-phosphate acyltransferase
LD	Lipid droplet
lyso-PA	1-acylglycerol-3-phosphate
MAG	Monoacylglycerol
PA	Phosphatidic acid
PAP	Phosphatidate phosphatase
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
SE	Sterol ester
TAG	Triacylglycerol

Introduction

Lipid droplets (LDs), also termed as lipid particles, lipid bodies, fat bodies, oil bodies, or adiposomes, are ubiquitously found in virtually all eukaryotes and even some bacteria. Although they were long thought to be inert fat storage depots, recent discoveries of LD-specific molecules have attracted much of the scientific interests. LDs are now perceived as metabolically active and highly dynamic organelles with a specialized function in regulating cellular lipid homeostasis (reviewed in [1–3]). The contents of LDs, predominantly triacylglycerols (TAGs) and sterol esters (SEs), provide cells with membrane and energy sources, and also protect cells against lipotoxicity. The accumulating evidence suggests that the surface of LDs may create an

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environment of temporal protein inactivation. Several pathogens hijack this energy-rich organelle for use in their life cycles. In addition, LDs are involved in prevalent lipid metabolic disorders and biofuel production, making LD-related research an attractive field of modern cell biology.

Unraveling the biogenesis, maintenance, and physiological roles of LDs among all systems are important for understanding the nature of this organelle. The budding yeast *Saccharomyces cerevisiae* is instrumental for biomedical research. This amenable model system offers powerful genetic, cell biology, and biochemical manipulation tools necessary for dissecting scientific questions at the cellular and molecular levels. This organism accumulates LDs, most prominently when cells enter the stationary phase or encounter environmental stresses. Consistent with observations in other eukaryotes, the yeast LDs appear to be well controlled for their size and number. The detailed knowledge of biosynthetic pathways and signaling networks provide the yeast system with essential information for current and future LD studies. Here, I review recent advances and highlight the dynamic roles of LDs in yeast.

LD morphology and dynamics

The basic structure of cellular LDs in eukaryotes is composed of a hydrophobic core and a phospholipid monolayer (Fig. 1a). The most prominent neutral lipids in the core are TAGs and SEs, but other hydrophobic lipids such as retinyl esters [4], wax, ether lipids [5], and squalene [6] are also possible constituents, depending on the cell types and growth conditions. For example, LDs in white adipocytes contain mostly TAGs, while those in macrophages contain mostly SEs. In wild-type yeast cells grown in normal laboratory conditions with glucose as the carbon source, TAGs and SEs accumulate equally and are typically stored within the same LDs. Differential scanning calorimetry and X-ray studies suggest that randomly packed fluid-state TAGs in the center of LDs are surrounded by several rigidly packed SE shells beneath the phospholipid monolayer (Fig. 1a) [7]. The composition of the LD phospholipid monolayer is distinct from its origin, the endoplasmic reticulum (ER), indicative of a selective partitioning event coordinated with LD biogenesis. In general, the LD monolayer is enriched in anionic phospholipids. Mammalian LDs contain mostly phosphatidylcholine (PC) and phosphatidylethanolamine (PE), but little phosphatidylserine (PS) and phosphatidic acid (PA) [5]. In yeast, LDs comprise mostly of PC, phosphatidylinositol (PI), and PE and are enriched in double-unsaturated fatty acid species [8–10]. Lyso-PE, lyso-PC, and PC, but not sphingomyelin, PS, and PA, are enriched in the yeast LD

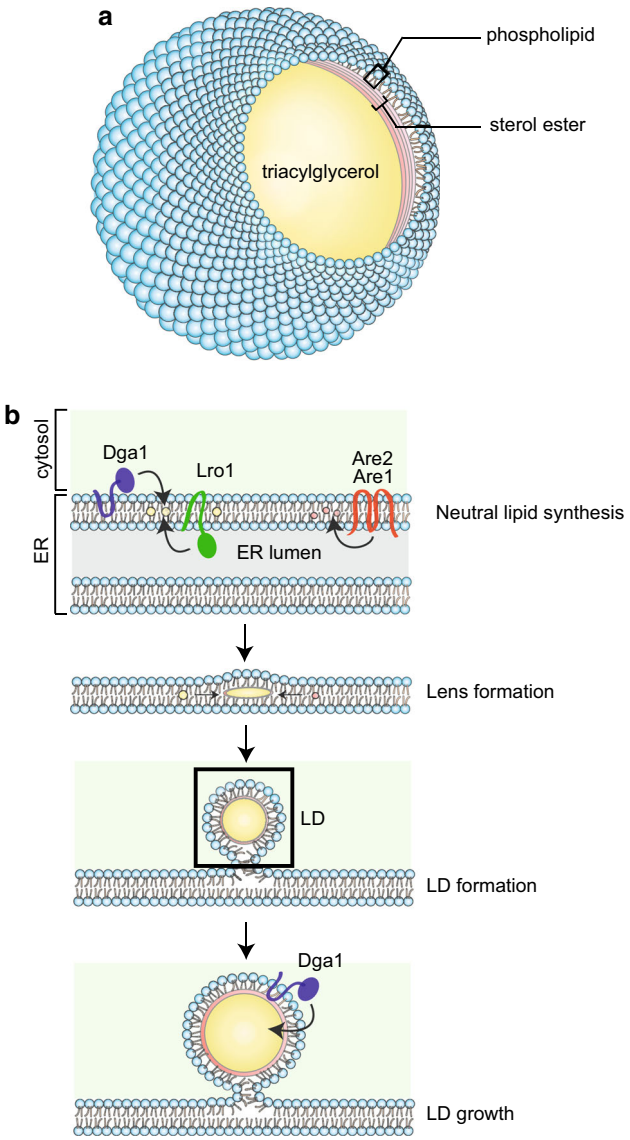

Fig. 1 The formation of LD. **a** LD composition. **b** A simple model of LD formation and growth. Yeast contains four neutral lipid synthesis enzymes. Are1 and Are2 catalyze the synthesis of sterol ester (SE), while Dga1 and Lro1 catalyze the synthesis of triacylglycerol (TAG). Neutral lipids accumulated within the ER bilayer on reaching a critical threshold form lens, and subsequently a nascent LD buds into the cytoplasm. LDs can grow by recruiting Dga1 to the surface of LDs for TAG synthesis

monolayers relative to the total membranes [5]. The biophysical properties and roles of these phospholipids during droplet emulsion were reviewed by Thiam et al. in 2013 [11].

LDs in many organisms, including yeast, are visible structures by light, particularly with differential interference contrast, microscopy. The size, number, and distribution of LDs vary under different physiological states, implying that they are dynamically regulated in response to physiological cues. Several cells, such as

adipocytes, accumulate LDs up to 100 μm in diameter, while in most cells LDs are small and often less than 1 μm wide. Yeast cells normally form LDs of less than 250 nm, but supersized LDs of more than 1 μm are formed in some mutants [12–14]. LDs in yeast are highly dynamic. In the exponential growth phase, small and highly mobile LDs disperse and are connected to the ER, including the perinulcear ER and cortical ER [15]. When cells reach the diauxic shift, LDs typically surround the perinuclear ER and expand. LDs continue to grow and become even more prominent during the stationary phase. The size and contents of LDs can change rapidly. Yeast cells treated with oleic acids had larger LDs, with only TAGs, not SEs, being synthesized and loaded into the LD core [16]. It is important to note that the quality and quantity of the varying LD components may influence the LDs' stability and behavior. Thus, experimental parameters, such as growth media and conditions, might readily impact the LD analyses.

Neutral lipid synthesis and the underlying lipid metabolic pathways

LD formation is solely dependent on the synthesis of neutral lipids, TAGs, and SEs [17] (Fig. 2). In mammalian cells, the acyl-coenzyme A (acyl-CoA):diacylglycerol acyltransferase (DGAT) enzymes DGAT1 and DGAT2 catalyze TAG synthesis by transferring a fatty acyl chain to diacylglycerol (DAG). The major DGAT enzyme in yeast is Dga1 (the mammalian DGAT2 ortholog) [18]. In addition to Dga1, yeast contains Lro1, a mammalian lecithin-cholesterol acyltransferase (LCAT) homolog, responsible for the synthesis of the other TAG pool [19, 20]. The preferred acyl donors for Lro1 are phospholipids, particularly PC and PE, suggesting that the major role of Lro1 is in remodeling membrane phospholipids [21, 22]. The two TAG synthesis enzymes vary in their functions under different growth conditions. Lro1 activity is more prominent in the exponential growth phase, whereas Dga1 significantly contributes to TAG synthesis during the stationary phase [23]. The mammalian SE is synthesized by two major acyl-CoA:cholesterol acyltransferase (ACAT) enzymes, ACAT1 and ACAT2. In yeast, Are1 and Are2 are the ACAT homologs that catalyze the esterification of sterol intermediates [21, 24]. The preferred substrate for Are2 is ergosterol, while Are1 largely synthesizes the lanosterol esters [25]. Are2 catalyzes most of the SE synthesis in yeast grown under normal laboratory conditions, whereas Are1 appears to be activated under anaerobic conditions. These two enzymes may also contribute to the residual TAG synthesis in cells lacking Dga1 and Lro1, as Are2 is able to perform DGAT activity in vitro [23]. Importantly, the synthesis of neutral lipids in cells is tightly connected to, and thus is affected by, the metabolism of fatty acids (FAs), glycerolipids, and sterols (Fig. 2). These lipid metabolic pathways are discussed below.

Cells can synthesize FAs de novo. This pathway is important because no exogenous FAs are added in typical yeast growth media. The first step of FA synthesis requires the activity of acetyl-CoA carboxylase Acc1 to convert acetyl-CoA to malonyl-CoA (Fig. 2). The fatty acid synthases (Fas1 and Fas2) subsequently elongate the acyl chain step by step to form medium-chain fatty acids, mostly C16 and C18 in yeast. Acetyl-CoA carboxylase is the rate-limiting enzyme for FA synthesis, and it is regulated by complex transcriptional controls in conjunction with phospholipid synthesis [27] and post-translational controls via Snf1/mammalian AMP-activated protein kinase [28]. In addition to the de novo pathway, free FAs may be derived from an exogenous supply or from lipolysis. The activation of FAs in yeast is mediated through five acyl-CoA synthetases that differ in their localization and substrate specificities. Three of these enzymes, Faa1, Faa4, and Fat1, localize to the LDs and likely function there to activate the FAs derived from TAG and SE breakdown. In the exponential phase, the catabolism of TAG in yeast cells allows for the rapid synthesis of membrane lipids for use in cell growth and division [29, 30].

Excess FAs are toxic to cells and, thus, the metabolic flux of FAs needs to be incorporated either into phospholipids or the biologically inert TAGs. TAG synthesis depends on two major precursors, PA and DAG, and both are central intermediates for phospholipid synthesis [22, 31] (Fig. 2). The PA precursor 1-acylglycerol-3-phosphate (lyso-PA) is synthesized either from the acylation of glycerol-3-phosphate by the glycerol-3-phosphate acyltransferases (GPATs), Gpt2/Gat1 and Sct1/Gat2, or from the acylation of dihydroxyacetone phosphate by a two-step reaction involving Gpt2/Gat1 and Sct1/Gat2 followed by Ayr1 [32]. The two GPAT enzymes exhibit substrate specificity, thus contributing differentially to the synthesis of TAG species [33, 34]. Lyso-PA is then acylated at the sn-2 position by the two lyso-PA acyltransferases, Slc1 and Ale1/Slc4, to yield PA. The two enzymes display different substrate specificities and may also be involved in the FA exchange at the sn-2-position of mature glycerophospholipids [35]. In addition to Slc1 and Ale1/Slc4, the LD-localized protein Loa1/Vps66 also functions as a lyso-PA acyltransferase with a specificity for oleoyl-CoA [36].The dephosphorylation of PA in yeast is performed by the phosphatidate phosphatase (PAP), Pah1, an ortholog of mammalian lipin, to give rise to DAG production [37–39]. Finally, the esterification of DAG by Dga1 and Lro1 generates TAGs that are stored within LDs.

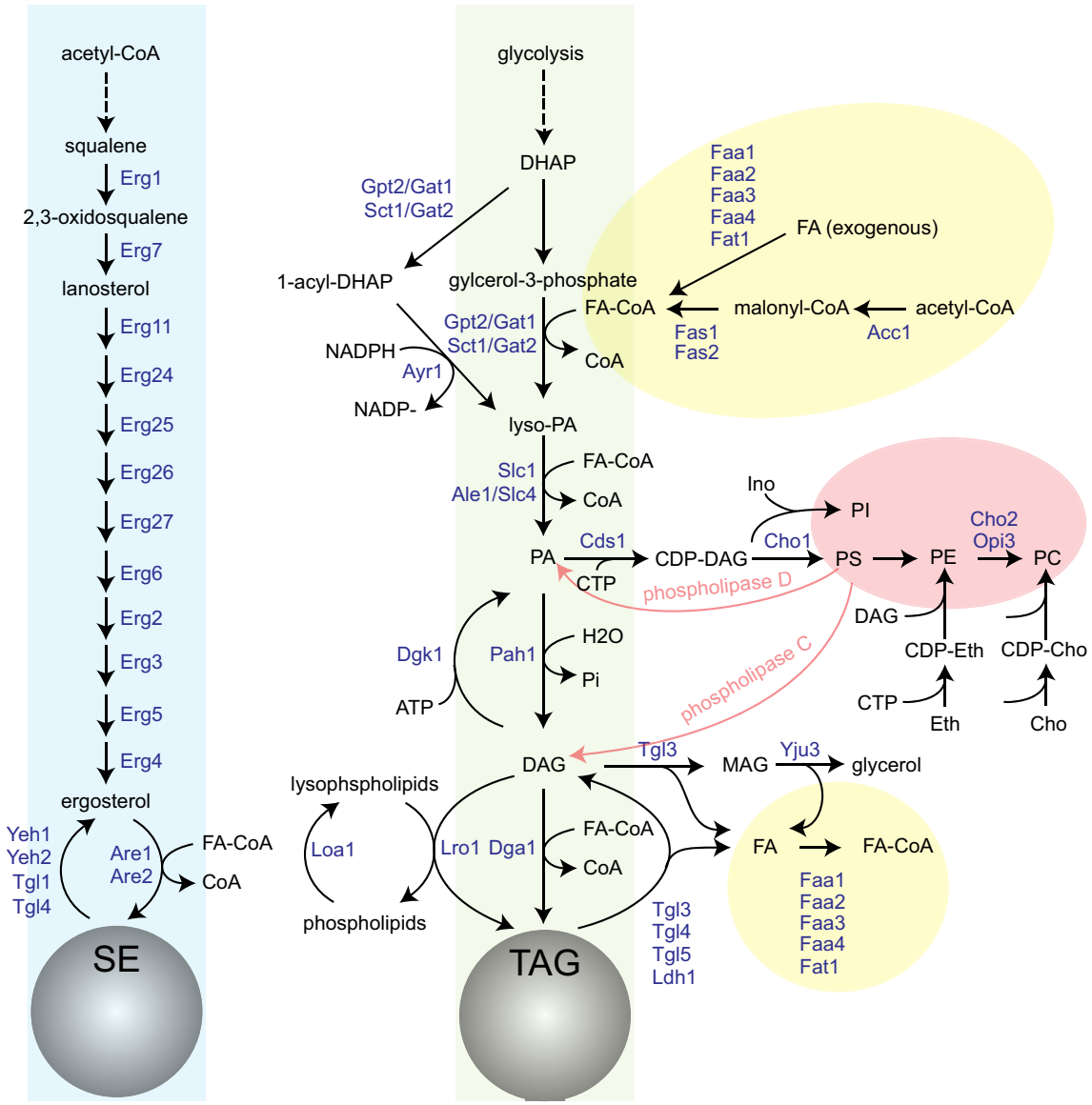

Fig. 2 The metabolic pathways of SE and TAG in *S. cerevisiae* (adopted from Rajakumari et al. [26] and Currie et al. [105]). Enzymes are indicated in *blue*. *Cyan zone* formation of SE. *Green zone* formation of TAG. *Yellow zone* formation of FA-CoA. *Red zone* formation of phospholipids. *FA* fatty acid, *CoA* coenzyme A, *FA-CoA* fatty acyl-CoA, *DHAP* dihydroxyacetone phosphate, *lyso-PA* 1-acyl-glycerol-3-phosphate, *PA* phosphatidic acid, *DAG* diacylglycerol, *CDP-DAG* cytidine diphosphate-diacylglycerol, *Ino* inositol, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PE* phosphatidylethanolamine, *PC* phosphatidylcholine, *CDP-Eth* cytidine diphosphate-ethanolamine, *CDP-Cho* cytidine diphosphate-choline, *Eth* ethanolamine, *Cho* choline, *MAG* monoacylglycerol, *CTP* cytidine triphosphate, *Pi* inorganic phosphate

In addition to synthesizing TAG, PA also supplies cells with phospholipids via the activation of CTP by Cds1 to produce CDP-DAG, a precursor for PI, PS, PE, phosphatidylglycerol, cardiolipin, and PC (reviewed in [40, 41]) (Fig. 2). The PA level in the ER is monitored by Scs2 and Opi1. When the PA level in the membrane elevates, the *INO1* repressor Opi1 is trapped by Scs2 in the ER and the synthesis of *INO1* activates PI synthesis by reducing PA and CDP-DAG levels [42, 43]. PA can also be derived from phospholipids by the action of phospholipase D or by the phosphorylation of DAG. The DAG kinase Dgk1, which counteracts Pah1 activity, is thought to be particularly important for consuming storage lipids during growth resumption [44, 45]. In addition to the CDP-DAG pathway, in the Kennedy pathway choline or ethanolamine is first activated with CTP and ultimately attached to DAG to form PC or PE, providing an alternative route for membrane lipid biosynthesis derived from DAG [46–48]. In general, the core precursors of phospholipid and TAG synthesis are PA and DAG.

Yeast cells, like mammalian cells, utilize most of the sterols (ergosterols in yeast and cholesterols in mammals) in the plasma membrane [49]. Sterol biosynthesis is strictly aerobic and requires heme in yeast. The major precursor for sterol synthesis is squalene, which is modified by a sequential reaction from acetyl-CoA (Fig. 2). Squalene is converted to 2,3-oxidosqualene by the squalene epoxidase Erg1 [50, 51]. The next step in sterol biosynthesis involves the oxidosqualene cyclase Erg7 to synthesize lanosterols [52, 53], followed by several steps to generate ergosterols. Eukaryotic cells have evolved sophisticated mechanisms at the transcriptional, translational, and post-translational levels to fine-tune the cellular sterol levels. One such example is that the enzymatic activity of Erg1 is regulated by its dual localization between the ER and LDs [51] and that the sterol abundance is sensed and subjects Erg1 to degradation by a branch of the ER-associated protein degradation (ERAD) pathway [54]. Similar to TAGs that play an important role in buffering excess FAs, the conversion of sterols to biologically inert SEs potentially prevents the accumulation of excess sterols, which is harmful to the cells.

Biogenesis of LDs

In eukaryotic cells, LDs form de novo from the outer leaflet of the ER (Fig. 1b). Inducible LD biogenesis has been studied using yeast cells genetically modified to express a sole neutral lipid synthesis enzyme under an inducible promoter [55]. In this system, LDs arise from the perinuclear ER upon induction of neutral lipid synthesis and once formed associate with the ER for most of the time. Many LD proteins partition freely between ER and LDs and re-localize from the ER to nascent LDs during LD biogenesis. Several models have been proposed to explain LD biogenesis from the ER (reviewed in [2, 56]). The most popular model hypothesizes that the neutral lipids between two leaflets of the ER membrane are organized into a lens that bulges out toward the cytosolic face of the ER to form LDs (Fig. 1b). However, a pure physical model proposes that TAGs and the phospholipid surfactants accumulate until they reach levels sufficient for spontaneous LD emulsification and budding [57]. It remains unclear how the orientation of LD budding is determined, as chylomicrons, TAG-containing lipoprotein particles in the absorptive cells of small intestines, are packaged and bud toward the luminal site of the ER [58]. Although the exact mechanisms of LD biogenesis remain largely unknown, the process is likely driven by the availability of lipids, enzymes, and structural proteins.

In yeast, neutral lipids are synthesized by the four acyltransferases Dga1, Lro1, Are1, and Are2 (Fig. 2). Mutants lacking all four of these enzymes have a complete loss of LD formation, indicating that the synthesis of neutral lipids is a prerequisite for LD formation [59]. Wild-type yeast cells store equal amount of TAGs and SEs in their LDs. In *dga1Δ lro1Δ* cells, LDs contain mostly SEs but their numbers are drastically reduced [18].In contrast, the *are1Δ are2Δ* cells produce LDs mostly containing TAGs, but the number of LDs was not significantly affected [17, 23]. Thus, LD formation is correlated with the activity of these acyltransferases, particularly Dga1 and Lro1, which synthesize TAGs. Many neutral lipid synthesis enzymes in eukaryotic cells are found in the ER. For example, Lro1, Are1, and Are2 are exclusively localized to the ER, and not to the LDs, in yeast. Fluorescence microscopy studies indicate that the phospholipid-remodeling acyltransferase Lro1 in yeast is associated with a highly dynamic subdomain that moves along the ER and may transiently interact with the LDs [60]. Intriguingly, topological studies on Lro1 suggest that its active site is localized within the ER lumen [61]. Similarly, the conserved active sites of Are1 and Are2 are exposed to the luminal side of the ER [62]. How exactly the neutral lipids are organized into nascent LDs is currently unknown. The mammalian DGAT2 and its ortholog yeast Dga1 can be recruited to, and function on, the LD surface, indicating that TAG synthesis can also occur directly on the LD surface [55, 63] (Fig. 1b). It has been proposed that the LDs' recruitment of Dga1 may use a stretch of its hydrophobic residues. Moreover, LD formation by Dga1 in yeast is independent of temperature and energy, thus ruling out vesicle formation [55].

In addition to TAGs, DAGs and PAP, an enzyme required for DAG synthesis, are critical factors for LD biogenesis. The disruption of the yeast PAP, Pah1, largely impairs LD formation, concomitant with the accumulation of neutral lipids in the ER [64]. Pah1 is crucial for LD formation even in cells synthesizing only SEs. Interestingly, the LD biogenesis defect caused by *pah1Δ* can be bypassed by the absence of the Dgk1 activity that converts DAGs to PA. Thus, the DAGs generated by Pah1 is important for LD biogenesis. DAGs displaying a negative curvature in the membrane may either offer a structural advantage or serve as a protein-binding platform during LD formation. The binding of DAGs to LD proteins, such as perilipin 3 [65] and CTP:phosphocholine cytidylyltransferase (CCT) [66], has been reported in other eukaryotes.

A critical issue for LD biogenesis is how cells couple the expansion of the LD phospholipid monolayer to the growth of the neutral lipid core. In mammalian cells, the major phospholipid in the LD monolayer is PC, which serves as a surfactant and prevents LDs from coalescing [11]. Additional PC loading onto the LD surface is mediated through the Kennedy pathway [66]. In mice and *Drosophila*, the

rate-limiting enzyme CCT in the Kennedy pathway is targeted to the growing LDs for LD expansion during the FA-induced LD formation. CCT can be activated by DAG and PA, and the protein prefers binding with membranes deficient in PC. Thus, de novo PC synthesis might also modulate the PC level on the LD surface. An alternative pathway that contributes to PC synthesis on the LD surface is the Lands cycle that forms PC from lyso-PC and fatty acyl-CoA. Lyso-PC transferase 1 and 2 are recruited to, and function on, LDs [67]. In *Drosophila* cells, the enzyme glycerol-3-phosphate acyltransferase 4 in the TAG synthesis pathway is relocalized from the ER to the population of large, but not small, LDs [63], supporting the hypothesis that PC and TAG synthesis are coordinated with the LD growth. Although a similar mechanism has not been reported in yeast, many enzymes involved in TAG synthesis localize at least partially to LDs [34, 68].

In addition to the requirements of specific lipids and enzymes, structural proteins might also be involved in LD biogenesis. Although yeast genome-wide genetic screens did not identify a single gene essential for LD formation [12, 69, 70], studies did uncover Sei1/Fld1 and Ldb16, which are molecules targeted specifically to the ER/LD contact sites [14, 69]. Sei1/Fld1 is an ortholog of the human lipodystrophy protein seipin, also known as Berardinelli–Seip congenital lipodystrophy type 2 [71]. Seipin homologs have been identified widely in eukaryotes and their deficiencies result in an irregular LD morphology. In yeast cells lacking Sei1/Fld1, LDs become either small clusters tangled with the ER or supersized [12, 69], suggesting an assembly defect. The mutant cells accumulate supersized LDs under inositol starvation, a condition that suppresses phospholipid synthesis [48], and accumulate small clustered LDs when exogenous inositol is supplied [13], indicating that the phospholipid metabolism is altered in the absence of seipin. Whether Sei1/Fld1 provides a structural or lipid regulatory role for LD assembly is currently under debate; however, the protein is assembled into a higher-ordered complex with Ldb16 at the ER/LD contact sites [14, 72]. Additionally, an Ldb16 deficiency also results in aberrant LDs in yeast. Topological studies have indicated that the large domain of seipin, which houses most lipodystrophy mutations, is exposed to the luminal side of the ER, but its function is still unclear [71]. In an inducible LD formation system, an Sei1/Fld1 deficiency and an Sei1/Fld1 mutant lacking an N-terminal peptide delay LD formation with a concomitant accumulation of neutral lipids in the ER [73], suggesting a role for Sei1/Fld1 in LD biogenesis. Whether Sei1/Fld1 controls the partitioning of enzymes and/or lipids during LD biogenesis requires further investigation. However, the localization of acyltransferase Dga1 to the LD surface is not affected by the lack of Sei1/Fld1 [55]. Sei1/Fld1 and human seipin may also control enzyme activity levels. Recent findings in mammalian cells indicate that seipin physically interacts with 1-acylglycerol-3-phosphate O-acyltransferase 2 and lipin [74]. Thus, seipin may act as a docking site for multiple enzymes or even regulate enzyme activity during LD biogenesis. In another line of evidence, seipin has been shown to interact with sarcoplasmic/endoplasmic reticulum calcium ATPase, an ER-localized calcium pump, and thus is proposed to function in moving calcium from the cytosol to the ER [75]. It is currently unclear whether Sei1/Fld1 acts similarly in yeast.

The fat-inducing transcript (FIT) proteins, Fit1 and Fit2, may have roles in LD biogenesis [76, 77]. The overexpression of these proteins in mouse liver results in LD accumulation, whereas fewer LDs and TAGs are seen in Fit2-deficient cells. FIT proteins are transmembrane proteins residing in the ER and are thought to mediate the filling of LDs with TAGs, rather than regulating TAG synthesis. Yeast cells contain two FIT protein homologs, Scs3 and Yft2. Cells lacking Scs3 exhibit inositol auxotrophy, indicative of an altered phospholipid metabolism, a phenotype that can be complemented by expressing human Fit2. Scs3 and Yft2 have both shared and unique functions in lipid metabolism, vesicular trafficking, and membrane biogenesis [78]. However, it remains unclear whether the two molecules affect TAG metabolism or LD biogenesis in yeast.

Most cells, including yeast, maintain a discrete LD size for yet unknown reasons. Fusion is a common phenomenon for most organelles, but not for LDs. However, LD fusion can occur under specific conditions, such as the supersized LDs formed in cells lacking Sei1/Fld1, which may be a result of LD fusion [12]. The level of PC on the LD surface is important for LD maintenance, as LDs are prone to fuse in cells containing a knockdown of the PC biosynthesis enzyme CCT [79]. Consistently, compromised PC synthesis in yeast also resulted in supersized LDs [13]. The most well-known protein participating in LD growth is the fat-specific protein Fsp27 (also known as CIDEC), which is involved in adipocyte differentiation. The protein forms a stable pore between the LD/LD contact sites to facilitate the transfer of TAGs from the small to the large LDs [80], a process known as LD ripening. The LD protein perilipin 1 interacts with Fsp27 to promote large LD formation [81]. However, there is no perilipins or Fsp27 homologsin yeast.

Regression of LDs

TAGs and SEs are degraded by TAG lipases and SE hydrolases, respectively. Lipolysis, the process that mobilizes TAGs to generate energy and membrane lipids, is best studied in 3T3-L1 adipocytes. During the first step of adipocyte lipolysis, activated adipose triacylglyceride

lipase (ATGL) on LDs hydrolyzes TAGs to generate DAGs and FAs, and several LD-localized proteins, including perilipins and CGI-58, modulate the ATGL activity [82–85]. Hormone-sensitive lipase, regulated by hormones and PKA phosphorylation, catalyzes the second step of lipolysis on LDs. Its products, monoacylglycerols (MAGs), undergo subsequent breakdown by the cytosolic MAG lipase to produce glycerol and FAs [86, 87]. Lipolysis occurs at the expense of LD volume, but the fates of the LD membrane monolayer and proteins have not been well studied.

In yeast, Yju3 is the MAG lipase ortholog, and its deletion results in marked MAGs accumulation [88]. Unlike the cytosolic MAG lipase in humans, Yju3 predominately associates with membranes and LDs. The LD-localized enzymes Tgl3, Tgl4, and Tgl5, containing a GXSXG motif at their active sites, are characterized as members of the conserved patatin domain-containing hydrolases [89–91]. Tgl3 and Tgl4, but not Tgl5, deletions lead to increases in LD size, thereby serving as major TAG lipases in yeast [89, 90]. Tgl3 activity is relatively unspecific and it hydrolyzes not only TAGs, but also DAGs. The carboxyl terminus of Tgl3 that faces the inside of the LDs is important for its stability [92]. Tgl4 has been characterized as the yeast ATGL [93]. It shows a preference for TAG species with C14 and C16 acyl chains and may also function as an SE hydrolase or phospholipase A2 [90]. In addition, the protein may have acyltransferase activity that catalyzes the acyl-CoA-dependent acylation of lyso-PA to PA [94]. Similarly, lyso-PA and lyso-PE acyltransferase activities have also been reported for Tgl3 and Tgl5. Cells deficient in Tgl5 accumulate TAGs with C26 acyl-chain, indicating that the function of this lipase is specific to the breakdown of very long-chain FAs [90]. A residual TAG lipase activity exists in cells lacking all three of these lipases when grown under oleic acid conditions, which have led to the discovery of Ayr1 as another TAG lipase in yeast [95]. Moreover, Ldh1 localizes to yeast LDs and also functions as a TAG lipase. In the absence of Ldh1, oleate-treated cells form giant LDs and accumulate neutral lipids and phospholipids [96, 97]. Overall, yeast cells have multiple TAG lipases that exhibit substrate specificity for different TAG species. These properties may lead to the production of various types of phospholipids with specific lengths or types of acyl chains and, thus, may be used by cells for different purposes. Deciphering the timing and the signaling pathways that activate these lipases should advance our knowledge of LD physiology.

SE hydrolysis in yeast involves three enzymes, Tgl1, Yeh1, and Yeh2, which are homologs of mammalian acid lipases with the conserved GXSXG motif at the active sites [98]. Yeh2 is a plasma membrane-localized enzyme, whereas Tgl1 and Yeh1 are localized to the LDs. The highest SE hydrolase activities were found in the plasma membrane and secretory vesicle fractions [49]. However, the bulk SE mobilization rate in the *yeh2Δ* strain is similar to that of the wild type; thus other enzymes likely have overlapping functions [99]. Mutant analysis results indicate that *tgl1Δ* causes a marked accumulation of SEs, while *yeh1Δ* and *yeh2Δ* cells show no effects. However, Yeh1 appears to be the most important SE hydrolase in *hem1Δ* cells that mimic anaerobic growth condition [100]. The three SE hydrolases exhibit preferences for certain SE substrates. Yeh1, similar to Yeh2, has a preference for zymosterol, lanosterol, and ergosterol esters [99, 101], while Tgl1 seems to prefer ergosterol and zymosterol esters [101]. Defects in the SE hydrolases, however, do not appear to affect the morphology or the number of cellular LDs.

How LD regression is regulated at the cellular level has just begun to be understood. Lipolysis can be activated upon growth resumption, as the LD size shrinks when the stationary phase yeast cells are switched back to fresh medium [29, 30]. The Dga1 targeted to LD surface synthesizes most of the TAGs during the stationary phase [23, 55]. Therefore, substrate DAGs for Dga1 must be supplied from the ER to the LDs during that stage. Conversely, DAGs derived from TAG lipolysis by lipases on LDs must be returned to the ER for membrane synthesis during growth resumption. A multi-spanning ER membrane protein, Ice2, is important for the efficient utilization of LDs during growth resumption [102]. Ice2 dispersed in the ER during the exponential phase accumulates into punctate structures adjacent to LDs during the stationary phase. During growth resumption, Ice2 quickly leaves the punctate structures and returns to the ER, preceding Dga1 relocalization. By binding to LDs through a cytosolic loop at the carboxyl terminus, Ice2 facilitates efficient DAG shuttling between the ER and LDs, thus promoting the phospholipid synthesis necessary for growth resumption. Additionally, the protein suppresses a futile cycle of TAG synthesis and degradation on the LDs by promoting Dga1 movement from the LDs to the ER [102].

Targeting of proteins to LDs

LDs can be purified by simple flotation methods, and the LD proteomes have been studied in various species. In higher eukaryotic cells, the perilipins belong to a family of conserved LD proteins [103]. However, no perilipin-like proteins exist in yeast. The LD proteins found in yeast proteomes are mostly enzymes involved in lipid metabolism [9, 46, 104–106] (Table 1). LDs contain the enzymes Ayr1, Slc1, Gpt2/Gat1, and Dga1, which are involved in the stepwise synthesis of glycerolipids. The SE hydrolases Yeh1 and Tgl1, the TAG lipases Tgl3, Tgl4, and Tgl5, and

Table 1 LD-associated proteins in *S. cerevisiae*

Gene	Systemic name	A	B	G	C	N	Localization (SGD)	Function (SGD)
ACH1	YBL015W			V			C/M	Protein with CoA transferase activity
ACS1	YAL054C		V				C/M	Acetyl-coA synthetase
ACT1	YFL039C		V				Actin	Actin
ADH1	YOL086C			V			C/PM	Alcohol dehydrogenase
ADH2	YMR303C		V				C	Glucose-repressible alcohol dehydrogenase II
ADY2	YCR010C		V				M/PM	Acetate transporter
ALD4	YOR374W		V				M	Mitochondrial aldehyde dehydrogenase
ALG9	YNL219C			V			ER	Mannosyltransferase
ANR2	YKL047W				V		C/LP	Unknown
ARF1	YDL192W		V				G	ADP-ribosylation factor
ATF1	YOR377W			V			LP	Alcohol acetyltransferase
ATP1	YBL099W		V				M	Alpha subunit of the F1 sector of mitochondrial F1F0 ATP synthase
ATP2	YJR121W		V	V			M	Beta subunit of the F1 sector of mitochondrial F1F0 ATP synthase
ATP4	YPL078C		V				M	Subunit b of the stator stalk of mitochondrial F1F0 ATP synthase
ATP6	Q0085		V				M	Subunit a of the F0 sector of mitochondrial F1F0 ATP synthase
ATP7	YKL016C		V				M	Subunit d of the stator stalk of mitochondrial F1F0 ATP synthase
AYR1	YIL124W	V	V	V	V	V	ER/C/LP/M	Bifunctional triacylglycerol lipase and 1-acyl DHAP reductase
BAT1	YHR208W		V				M	Mitochondrial branched-chain amino acid (BCAA) aminotransferase
BMH2	YDR099W		V				C/N/PM	14-3-3 protein
BSC2	YDR275W			V			LP	Unknown
CAB5	YDR196C				V		ER/M/LP	Subunit of the CoA-synthesizing protein complex (CoA-SPC)
CAT2	YML042W		V				M/P	Carnitine acetyl-CoA transferase
CIT1	YNR001C		V				M	Citrate synthase
COR1	YBL045C		V				M	Core subunit of the ubiquinol cytochrome-c reductase complex
COY1	YKL179C			V			G	Golgi membrane protein with similarity to mammalian CASP
CPR5/CYP5	YDR304C			V			ER/C	Peptidyl-prolylcis-trans isomerase (cyclophilin) of the ER
CRM1	YGR218W		V				N	Major karyopherin
CSR1/SFH2	YLR380W			V			C/E/M/LP	Phosphatidylinositol transfer protein
CST26/PSI1	YBR042C			V		V	LP	Putative transferase involved in phospholipid biosynthesis
CTA1	YDR256C		V				M/P	Catalase A
CWH43	YCR017C			V			PM	Putative sensor/transporter protein involved in cell wall biogenesis
DFM1	YDR411C			V			ER	ERAD
DGA1	YOR245C			V	V	V	ER/LP	Diacylglycerol acyltransferase
DLD1	YDL174C		V				M	D-lactate dehydrogenase
DPL1	YDR294C			V			ER	Dihydrosphingosine phosphate lyase
DPM1	YPR183W		V	V			ER/M	Dolichol phosphate mannose (Dol-P-Man) synthase
ECM29	YHL030W				V		C/N	Scaffold protein
EFT1/2	YOR133W/ YDR385W		V				ribosome	Elongation factor 1/2
EHT1	YBR177C	V	V	V	V	V	LP/M	Acyl-coenzymeA:ethanol O-acyltransferase
ENO2	YHR174W			V			PM/M/V	Enolase II
ENV9	YOR246C			V			M/LP	Protein proposed to be involved in vacuolar functions
ERG1	YGR175C	V	V	V	V	V	ER/LP	Squalene epoxidase
ERG2	YMR202W		V				ER	C-8 sterol isomerase
ERG27	YLR100W		V	V	V	V	ER/LP/M	3-keto sterol reductase
ERG6	YML008C	V	V	V	V	V	ER/LP/M	Delta(24)-sterol C-methyltransferase
ERG7	YHR072W	V	V	V	V	V	LP	Lanosterol synthase
ERV14	YGL054C		V				ER/vesicles	COPII-coated vesicle protein

Table 1 continued

Gene	Systemic name	A	B	G	C	N	Localization (SGD)	Function (SGD)
FAA1	YOR317W	V	V	V	V		ER/LP/M/PM	Long-chain fatty acyl-CoA synthetase
FAA2	YER015W		V				P/M	Medium-chain fatty acyl-CoA synthetase
FAA3	YIL009W			V			Unknown	Long-chain fatty acyl-CoA synthetase
FAA4	YMR246W	V	V	V	V	V	LP/C	Long-chain fatty acyl-CoA synthetase
FAS1	YKL182W			V			C/LP/M	Beta subunit of fatty acid synthetase
FAT1	YBR041W	V	V	V	V	V	ER/LP/P	Very long-chain fatty acyl-CoA synthetase and fatty acid transporter
FBA1	YKL060C		V				C/M	Fructose 1,6-bisphosphate aldolase
FMP52	YER004W		V	V			ER/M	Unknown
FOX2	YKR009C		V				P	3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase
GPI8	YDR331W		V				ER	ER membrane glycoprotein subunit of the GPI transamidase complex
GPT2/ GAT1	YKR067W			V			ER/LP/C	Glycerol-3-phosphate/dihydroxyacetone phosphate sn-1 acyltransferase
GTT1	YIR038C		V	V	V		ER/M/PM	ER-associated glutathione S-transferase
GUT2	YIL155C		V				M	Mitochondrial glycerol-3-phosphate dehydrogenase
GVP36	YIL041W		V	V			G/C	BAR domain protein that localizes to early and late Golgi vesicles
HFD1	YMR110C		V	V	V		LP/E/M	Hexadecenal dehydrogenase
HHF1/2	YBR009C/ YNL030W		V				N	Histone H4
HOM3	YER052C		V				C	Aspartate kinase
HSC82	YMR186W		V				C/M/PM	Cytoplasmic chaperone of the Hsp90 family
HSP104	YLL026W		V				C/N	Disaggregase
HSP12	YFL014W			V			C/E/PM/N	Plasma membrane protein involved in maintaining membrane organization
HSP60	YLR259C		V				M	Tetradecameric mitochondrial chaperonin
HTB1/2	YDR224C/ YBL002 W		V				N	Histone H2B
HXT6/7	YDR343C/ YDR342C		V				PM/M	High-affinity glucose transporter
IDP3	YNL009W		V				C/P/M	Peroxisomal NADP-dependent isocitrate dehydrogenase
ILV5	YLR355C		V				M	Acetohydroxyacid reductoisomerase and mtDNA binding protein
KAR2	YJL034W		V	V			ER	ATPase involved in protein import into the ER
KES1/OSH4	YPL145C			V			C/G	oxysterol binding protein
KGD1	YIL125W		V				M	Subunit of the mitochondrial alpha-ketoglutarate dehydrogenase complex
LAP4	YKL103C			V			C/V	Vacuolar aminopeptidase
LDB16	YCL005W			V			LP/M	Unknown
LDH1	YBR204C			V	V	V	LP	Serine hydrolase
LDS1*	YAL018C						LP	Protein involved in spore wall assembly
LDS2*	YOL047C						C/LP	Protein involved in spore wall assembly
LOA1/ VPS66	YPR139C			V			ER/LP/C	Lysophosphatidic acid acyltransferase
LPL1	YOR059C	V		V	V	V	LP	Phospholipase
MIR1	YJR077C		V				M	Mitochondrial phosphate carrier
MSC1	YML128C		V	V			M/ER/PM	Unknown
NCE2	YPR149W		V				C/ER/M/PM	Unknown
NDE1	YMR145C		V				M	Mitochondrial external NADH dehydrogenase
NDE2	YDL085W		V				M	Mitochondrial external NADH dehydrogenase
NDI1	YML120C		V				M	NADH:ubiquinone oxidoreductase
NTE1	YML059C				V		ER	Serine esterase

Table 1 continued

Gene	Systemic name	A	B	G	C	N	Localization (SGD)	Function (SGD)
NUS1	YDL193W	V		V	V	V	ER/LP	Putative prenyltransferase
OM45	YIL136W		V				M	Mitochondrial outer membrane protein
OSW5	YMR148W			V			Unknown	Unknown
PCS60	YBR222C		V				P/C	Oxalyl-CoA synthetase
PDC1	YLR044C		V				C/N	Major of three pyruvate decarboxylase isozymes
PDI1	YCL043C		V	V			ER	Protein disulfide isomerase
PDR16/ SFH3	YNL231C		V	V		V	LP/C/PM	Phosphatidylinositol transfer protein (PITP)
PET10	YKR046C	V	V	V	V	V	LP	Unknown
PET9	YBL030C		V				M	Major ADP/ATP carrier of the mitochondrial inner membrane
PGC1	YPL206C			V	V		LP/M	Phosphatidyl glycerol phospholipase C
PGK1	YCR012W		V				M/C/PM	3-phosphoglycerate kinase
PHB1	YGR132C		V				M/P	Subunit of the prohibitin complex
PHO81	YGR233C				V		N/C	Cyclin-dependent kinase (CDK) inhibitor
PIL1	YGR086C			V			C/M/PM	Eisosome core component
PMA1	YGL008C			V			PM/M	Plasma membrane P2-type H+-ATPase
PMA2	YPL036W		V				PM/M	Plasma membrane H+-ATPase
PMT1	YDL095W		V	V			ER	Protein O-mannosyltransferase
PMT2	YAL023C			V			ER	Protein O-mannosyltransferase
POR1	YNL055C		V	V			M/C	Mitochondrial porin
POX1	YGL205W		V	V			P	Fatty-acyl-coenzyme A oxidase
QCR2	YPR191W		V				M	Subunit 7 of ubiquinol cytochrome-c reductase (Complex III)
QCR7	YDR529C		V				M	Subunit 7 of ubiquinol cytochrome-c reductase (complex III)
RER2	YBR002C				V		ER/LP	Cis-prenyltransferase involved in dolichol synthesis
RHO1	YPR165W		V	V			M/P/G/PM	GTP-binding protein of the rho subfamily of Ras-like proteins
RIB1	YBL033C		V				C/N	GTP cyclohydrolase II
RPL10	YLR075W			V			Ribosome	Ribosomal 60S subunit protein L10
RPL4A	YBR031W		V				Ribosome	Ribosomal 60S subunit protein L4A
RPL5	YPL131W		V	V			Ribosome	Ribosomal 60S subunit protein L5
RPL7A	YGL076C		V				Ribosome	Ribosomal 60S subunit protein L7A
RPS19B	YNL302C			V			Ribosome	Protein component of the small (40S) ribosomal subunit
RPS1B	YML063W			V			Ribosome	Ribosomal protein 10 (rp10) of the small (40S) subunit
RPS3	YNL178W			V			Ribosome	Protein component of the small (40S) ribosomal subunit
RPS31	YLR167W			V			Ribosome	Fusion protein cleaved to yield ribosomal protein S31 and ubiquitin
RPS5	YJR123W		V				Ribosome	Protein component of the small (40S) ribosomal subunit
RPS7A	YOR096W		V				Ribosome	Protein component of the small (40S) ribosomal subunit
RRT8	YOL048C			V			PM/LP	Protein involved in spore wall assembly
RTN1	YDR233C		V				ER/G/M	Reticulon
RTN2	YDL204W			V			ER/C/N	Reticulon
SAR1	YPL218W		V				ER/vesicles	GTP-binding protein of the ARF family
SAY1	YGR263C				V		ER/LP	Sterol deacetylase
SDH1	YKL148C		V				M	Flavoprotein subunit of succinate dehydrogenase
SEC21	YNL287W		V				COPI vesicles	Gamma subunit of coatomer
SEC61	YLR378C			V			ER	Conserved ER protein translocation channel
SEC63	YOR254C		V	V			ER/M	Essential subunit of Sec63 complex
SHE10	YGL228W			V			Unknown	Protein involved in outer spore wall assembly
SLC1	YDL052C	V	V	V	V	V	LP	1-acyl-sn-glycerol-3-phosphate acyltransferase
SNA2	YDR525W-A			V			C/V	Unknown
SNX41	YDR425W			V			E	Sorting nexin

Table 1 continued

Gene	Systemic name	A	B	G	C	N	Localization (SGD)	Function (SGD)
SPS19	YNL202W		V				P	Peroxisomal 2,4-dienoyl-CoA reductase
SRT1	YMR101C			V			LP	Cis-prenyltransferase
SSA1	YAL005C			V			N/C/PM/V	ATPase involved in protein folding and NLS-directed nuclear transport
SSA3	YBL075C		V				C	ATPase involved in protein folding and the response to stress
SSB1	YDL229W		V				C/PM	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone
SSO1	YPL232W			V			V/PM	Plasma membrane t-SNARE
TAZ1	YPR140W				V		M	Lyso-phosphatidylcholine acyltransferase
TDH1	YJL052W	V		V			C/LP/M/PM	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
TDH2	YJR009C	V		V			C/LP/M/PM	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
TDH3	YGR192C	V		V			C/LP/M/PM	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
TEF1/2	YPR080W/ YBR118W		V	V			C/M/ribosome	Translational elongation factor EF-1 alpha
TES1	YJR019C		V				P/M	Peroxisomal acyl-CoA thioesterase
TGL1	YKL140W	V	V	V	V	V	LP	Steryl ester hydrolase
TGL3	YMR313C	V	V	V	V	V	LP	Bifunctional triacylglycerol lipase and LPE acyltransferase
TGL4	YKR089C			V	V	V	LP	Multifunctional lipase/hydrolase/phospholipase
TGL5	YOR081C			V	V	V	LP	Bifunctional triacylglycerol lipase and LPA acyltransferase
TIF1/2	YKR059W/ YJL138C		V				C/ribosome	Translation initiation factor eIF4A
TOM40	YMR203W		V				M	Component of the TOM complex
TSC10	YBR265W			V	V		LP/C/ER/M	3-ketosphinganine reductase
TUB2	YFL037W			V			microtubules	Beta-tubulin
UBX2	YML013W			V	V		ER/M/LP	Bridging factor involved in ER-associated protein degradation
USE1	YGL098W			V			ER	Essential SNARE protein localized to the ER
WBP1	YEL002C			V			ER	Beta subunit of the oligosaccharyltransferase glycoprotein complex
YDR018C	YDR018C			V			N	Unknown
YEH1	YLL012W			V			LP	Steryl ester hydrolase
YGR038C-B	YGR038C-B			V			N	Retrotransposon TYA Gag and TYB Pol genes
YHB1	YGR234W		V				C/M/N	Nitric oxide oxidoreductase
YIM1	YMR152W	V		V	V	V	LP/C/M	Unknown
YJU3	YKL094W	V		V	V	V	LP/M/C/PM	Monoglyceride lipase
YNL134C	YNL134C			V			C/N	Unknown
YNL208 W	YNL208W			V			M/ribosome	Unknown
YPR127 W	YPR127W		V				C/N	Putative pyridoxine 4-dehydrogenase
YPR147C	YPR147C				V		LP/C	Unknown
YPT7	YML001W			V			V/M	Rab family GTPase
ZEO1	YOL109W			V			PM/M	Peripheral membrane protein of the plasma membrane

C cytoplasm, *N* nucleus, *M* mitochondria, *P* peroxisome, *PM* plasma membrane, *LP* lipid particle, *ER* endoplasmic reticulum, *V* vacuole, *E* endosome

A Athenstaedt et al. [107], *B* Binns et al. [104], *G* Grillitsch et al. [9], *C* Currie et al. [105], *N* Natter et al. [46]

* Lin et al. [140]

the MAG lipase Yju3, used for the breakdown of SE and TAG, are found in LDs. The FA-activation enzymes Faa1, Faa4, and Fat1, and several sterol biosynthesis enzymes, namely Erg1, Erg6, Erg7, and Erg27, are abundant in LD proteins. The presence of these enzymes in LDs suggests that LDs are the sites of action. However, LD might also

serve as places for protein inactivation. For example, Erg1 displays dual localization pattern, while it is active only in the ER and not in the LDs [51].

LD proteomes also revealed proteins without known enzymatic functions. Ubx2 sorts into LDs upon LD formation [60]. It is needed for LD maintenance, as removing or forcing an Ubx2 association with LDs results in aberrant LDs. Ubx2 has been postulated to modulate TAG synthesis by regulating Lro1. However, the protein has other functions, acting as the Cdc48 adaptor for ERAD and for the processing of the transcription factors Spt23 and Mga2 to control the expression of the Δ9-desaturase Ole1 [108–110]. Whether these pathways involve LDs awaits further investigation, but LD formation is dispensable for ERAD [111]. Ldb16, the molecule assembled together with Sei1/Fld1 at the ER/LD contact site, also appears in the LD proteome. LDs lacking Ldb16, like Sei1/Fld1, become irregular [14]. In addition to supersized LDs, both mutants form small LDs entangled with the ER, probably resulting from inefficient segregation during cytokinesis [112]. The LD morphologies in the mutants are hypersensitive to the phospholipid precursor supplements, such as inositol, suggesting that the ratio of phospholipids to neutral lipids is important for LD maintenance [13]. Ldb16 requires Sei1/Fld1 for stability; otherwise it is targeted for degradation by a branch of ERAD, termed ERAD-C [14]. Pdr16/Sft3 is a PI-binding protein that localizes to LDs. Cells lacking Pdr16/Sft3 are sensitive to azole-based antifungals, suggesting a role for the protein in sterol synthesis and/or mobilization [113]. Pdr16/Sfh3 associates with and modulates the mobilization of LDs during yeast sporulation [114]. Pet10 is found in all yeast LD proteomes. The protein interacts with many proteins in LDs, mitochondria, and peroxisomes [115], but no functional insight is currently available. Many other enzymes and proteins involved in diverse cellular activities in the cytosol, ER, Golgi, peroxisomes, endosomes, vacuoles, and plasma membrane have been reported in the yeast LD proteome (Table 1). Their association with and importance for LDs await future investigations. It should be noted that LDs often interact with other organelles, such that contamination from other organelles during LD purification needs to be carefully ruled out. In addition, protein expression and stability might be regulated differentially during various physiological stages. For example, Lds1 and Lds2 are induced and localized to LDs during yeast sporulation [116]. A list of LD proteins identified in yeast is summarized in Table 1.

LDs are distinct from other organelles in that their surface consists of a phospholipid monolayer. Therefore, it is conceivable that proteins stabilized on the LD surface require special determinants. This concept has attracted many studies focusing on protein targeting to LDs in various eukaryotic systems. These mechanisms may include (a) binding to intrinsic amphipathic helices within the protein, such as CCT [66] and the hepatitis C virus core protein [117]; (b) binding to internal protein domain containing a hairpin, such as the oleosins [118] and caveolins [119–121]; (c) binding to short hydrophobic stretches of the protein, such as in AAM-B [122] and perilipin 1 [123]; (d) lipid conjugation of the protein, such as for Rab18 [124]; and (e) binding through interactions with other LD proteins, such as hormone-sensitive lipase binding to LDs through perilipin 1 [125]. Given the expanding knowledge on LDs, other mechanisms are likely to emerge in the future.

Physiological roles of LDs

LDs protect cells against lipotoxicity

The major function of LDs is to prevent lipotoxicity by converting excess FAs into neutral lipids (TAGs and SEs). Cells lacking SEs increase the sensitivity to sterol synthesis inhibitors, such as terbinafine, and grow poorly after several generations [25]. TAG formation is particularly important in buffering the excess FAs in cells. Cells lacking TAGs have reduced LD numbers and show a prolonged lag phase [18, 23]. In addition, genes involved in phospholipid metabolism are repressed in the mutants, which indicates that FA homeostasis is crucial for the control of membrane lipid synthesis and proliferation [126]. When the PA phosphatase *PAH1* is deleted, cells accumulate elevated levels of PA and FAs and reduced levels of DAG and TAG [127]. The mutants are sensitive to exogenous supplies of unsaturated FAs [45]. Similarly, *are1Δ are2Δ dga1Δ lro1Δ* LD-deficient cells accumulate a 2.5-fold higher amount of FAs than normal [17]. The quadruple mutant is sick, especially under nutrient-limited or stress conditions, while the cell loses viability under nitrogen starvation conditions [17]. An exogenous supply of oleic acids to the quadruple mutant causes a rapid block of the secretory pathway, an up-regulated unfolded protein response, and ultimately necrotic cell death with elevated levels of reactive oxygen species [128, 129]. The oleate-induced cell death of the quadruple mutant can be rescued by adaptation after the lag phase [16], and the suppression appears to involve mitochondrial DNA mutations [129]. As in yeast, TAG synthesis also plays a protective role against lipotoxicity in mammalian cells [128].

Regulations of LDs during the cell cycle

Both TAG synthesis and lipolysis are subjected to cell cycle control. Pah1 and Tgl4 are lipid enzymes known to

be substrates for Cdc28, the main cyclin-dependent kinase governing the cell cycle in yeast. Pah1 is the key enzyme in TAG and phospholipid synthesis. The phosphorylation of Pah1 by Cdc28 occurs at the G2/M phase to inactivate Pah1 activity [130]. The phosphorylated form of Pah1 in the cytosol can be recruited to the ER membrane upon dephosphorylation by the ER-localized phosphatase complex Spo7–Nem1 [131, 132]. In addition to Cdc28, the cyclin-dependent kinase Pho85 also phosphorylates Pah1 to inhibit Pah1 phosphatase activity [133]. On the other hand, Cdc28 phosphorylates the major TAG lipase Tgl4 at the G1/S phase [93]. Cells lacking TAG lipolysis delay cell cycle progression at the stage of bud emergence. It remains unclear how the temporal control of lipolysis is monitored and how the lipolysis products actually contribute to the cell cycle. Overall, the catabolism of the LDs is likely coupled to the FAs and/or phospholipids supply for cell growth and division.

Delivery of LDs to the vacuoles by autophagy

Autophagy is a recycling pathway known to target damaged or excess organelles for breakdown in the lytic compartments, yeast vacuoles/mammalian lysosomes. Lysosomal targeting of LDs by autophagy was first discovered in hepatocytes [134], providing an alternative route for the mobilization of the LDs' contents. In yeast, two microautophagy pathways that involve the direct vacuolar engulfment of LDs have been reported [15, 135]. Yeast cells incubated with oleate [135], a condition that increases TAG synthesis, triggers LD uptake by the vacuole [16]. This LD autophagy pathway uses a unique subset of the autophagy machinery, including Atg15, the vacuolar lipase needed for TAG breakdown inside the vacuole [135]. A distinct type of LD autophagy pathway, termed stationary phase lipophagy, exists in the quiescent yeast cells [15]. When glucose becomes limited in the culture medium, yeast LDs gradually move from the ER toward the vacuole. LDs in the quiescent cells are engulfed by the vacuole and accumulated inside the vacuole lumen, a process that requires core autophagy components and a subset of selective autophagy proteins. This pathway is connected to a lipid phase partitioning event that segregates vacuolar proteins to either one of the forming vacuolar micro domains during the stationary phase [136]. LDs are specifically taken up by the sterol-enriched, termed liquid-ordered, vacuolar microdomain, which in turn provides the vacuolar membranes with sterols to sustain the lipid phase partitioning [15]. Thus, yeast cells may use the feedforward loop to recycle sterols and LDs by autophagy during quiescence.

Dynamics of LDs during yeast sporulation

During sporulation, de novo biogenesis of the prospore membranes defines a robust program in which a flux of FAs and membrane lipids are remodeled toward the ordered sequestration of the haploid genome produced by meiosis [137]. However, little is known about the molecular mechanisms that govern the prospore membrane nucleation, elongation, and completion. Several lines of evidence have implied that LDs may be crucial for sporulation. The deletion of Are2 alone, or in combination with Are1, reduces the sporulation efficiency [21]. The diploid yeast strains lacking the TAG lipase Tgl3 or Tgl4 and Tgl5 also bear sporulation defects [90]. However, the enzymatic function of Tgl3 needed for efficient sporulation is its acyltransferase, rather than its lipase activity [138]. It is not clear which steps in sporulation require these enzymes. Intriguingly, at the cellular level, a discrete population of LDs is found to associate with the ascal side (outer, in contrast to the inner, which engulfs the nuclei) of the prospore membrane, and these LDs recruit a unique set of protein components [139, 140]. The deletion of these LD proteins results in spore wall assembly defect by an unknown mechanism [140]. Tgl3 and the PI-binding protein Pdr16/Sfh3 selectively localize to the pool of LDs associated with the prospore membrane [114]. Pdr16/Sfh3 is thought to reduce the rate of LD lipid mobilization and thus tune the formation of the prospore membrane. However, the functional connection between the prospore membrane and the pool of prospore membrane-associated LDs remains to be determined. It is important to decipher whether LDs provide fuel, are directly involved in prospore membrane morphogenesis, or even play an active role during spore wall assembly.

Interplay between LDs and other organelles

LDs are often found in close proximity with other organelles. In differentiating 3T3-L1 cells, ER, LDs, mitochondria, and peroxisomes form constellations, suggesting a coupling of these organelles for efficient lipid metabolism [141]. In yeast, physical associations between LDs and peroxisomes have been observed [104]. Peroxisomes can form stable structures termed "pexopodia" that penetrate LDs in oleate-cultured cells, a phenomenon that likely also occurs transiently under normal yeast growth conditions. The mechanisms and importance of these interactions are currently unknown, but probably involve lipid exchange. Presumably, LDs can transfer lipids into peroxisomes and mitochondria for oxidation, and the establishment of organelle contact sites may be dependent

on specific protein–protein and/or protein–lipid interactions to mediate the binding. These notions await further supporting molecular evidence.

Potential contribution of LD studies in yeast to human metabolic diseases

LDs store fats. Excesses or deficiencies in fat storage lead to many different types of human diseases (reviewed in [142]). Pathological evidence has shown that patients suffering from obesity and other metabolic syndromes, such as hepatic steatosis and cardiovascular diseases, accumulate excess TAGs and LDs. A rare autosomal disorder, neutral lipid storage disease, is characterized by the accumulation of excess neutral lipids in multiple tissues and is associated with mutations in ATGL and CGI-58 genes that are involved in lipolysis [143]. In addition, several forms of human lipodystrophy, characterized by a loss of body fat storage, have been connected to mutations in the genes of 1-acylglycerol-3-phosphate O-acyltransferase 2 for TAG synthesis and Berardinelli–Seip congenital lipodystrophy type 2 for LD maintenance (reviewed in [144]). While it remains unclear how mutations in these genes with roles in LD formation and regulation cause lipodystrophy, it is possible that lipid perturbation inhibits adipocyte differentiation by affecting peroxisome proliferator-activated receptor γ signaling (reviewed in [145]).

Lipid-related metabolic disorders in humans involve various types of differentiated cells and organs. Nevertheless, the fundamental processes of TAG metabolism and LD formation and maintenance are evolutionarily conserved, and studies in yeast can further improve our knowledge, which can be applied to human diseases. Moreover, most metabolic syndromes in humans are caused by complex genetic and environmental factors that are difficult to resolve. Parallel studies of LD biology in yeast will contribute to the better understanding of the major regulatory pathways that govern feedback controls and signaling in TAG metabolism and lipid homeostasis, thus advancing our knowledge of the disease etiology in humans.

Conclusion

Tremendous progress has been made in the past several years in establishing the roles of LDs as versatile organelles rather than inert fat storage depots. However, many fundamental questions regarding the nature of LDs remain unanswered. In fact, we have learned that the biogenesis and regression of LDs are driven by complex mechanisms that are coupled to lipid homeostasis. Meanwhile, more knowledge is required to understand the mechanism behind lipid packaging into nascent LDs, the organization and the gating mechanism at the ER/LD contact sites, the actual parameters of LD size and number necessary for maintenance, and even the spatial and temporal cues that execute LD dynamics. From the physiological perspective, we have learned that LDs are metabolically active and can exchange lipids with other organelles to facilitate timely and efficient lipid metabolism. However, most of these interactions at the molecular levels and their significance have not yet been determined. FAs, phospholipids, and many other lipid metabolites are found to play diverse roles in various cellular activities, and it is conceivable that some might be coupled to the catabolism of storage lipids within LDs. Overall, it is evident that the field of LD biology will be driven by insights derived from fundamental LD research to increase our knowledge of disease pathology and to benefit growing bioindustries.

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