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REVIEWS

Lipid Droplet Metabolism Across Eukaryotes: Evidence from Yeast to Humans

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Abstract—The lipid droplet (LD) is a highly dynamic organelle that maintains cellular lipid homeostasis in addition to storing energy sources. Current research suggests LDs are responsible for the transportation, storage and lipolysis-driven mobilization of lipids within cells. Here, we review the landscape of evidence for LD involvement in regulating lipid homeostasis. LD interactions with other organelles, particularly the endoplasmic reticulum, mitochondria, lysosomes (or vacuoles in yeast), and peroxisomes, highlight their importance for lipid transfer and metabolism.

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INTRODUCTION

For many organisms, the most efficient form of energy storage is fat. At the cellular level, the ability to store neutral lipids in cytoplasmic lipid droplets (LD) is essential for cellular and organismal survival. LDs, also termed lipid bodies, oil bodies or adiposomes, are multi-functional organelle that consists of a core of neutral lipids, such as sterol esters (SE) and triacylglycerols (TAG), and a coating of a monolayer of phospholipids decorated by LD-associated proteins that are variable with organisms and tissues [1, 2].

LDs appear to be conserved, as they are found in nearly all organisms. The organelles have been isolated from many different organisms, including bacteria (*Rhodococcus opacus* and *Rhodococcus ruber*) [3], green algae (*Chlamydomonas reinhardtii*) [4], yeast (*Saccharomyces cerevisiae*) [5, 6], nematodes (*Caenorhabditis elegans*) [7], insects (*Drosophila melanogaster*) [8, 9], plants (*Arabidopsis thaliana, Sesamum indicum* L., *Brassica napus*) [10–12], fish (*Ctenopharyngodon idella*) [13], mammals such as mice, rat, macaque monkey and human cells [14–17]. LD-associated proteins have been investigated through the proteomic analysis of LDs isolated from the aforementioned species [3–17].

LDs are highly dynamic and are controlled by a constant cycle of recruitment and disassociation

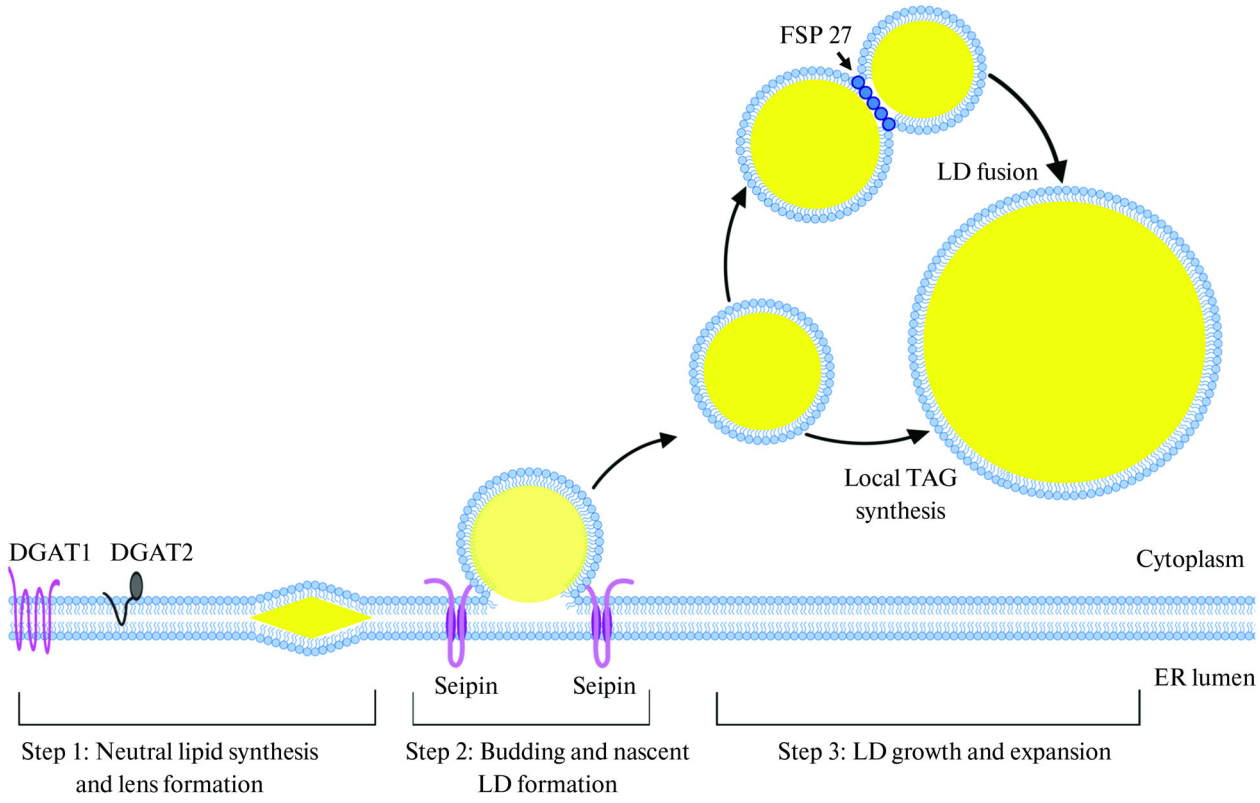

Fig. 1. Steps in lipid droplet formation. Lipid droplets (LDs) emerge from the endoplasmic reticulum (ER). Step 1: Neutral lipids are synthesized and accumulate within the ER bilayer. Beyond a certain concentration, the neutral lipids demix and coalesce into a lens. Step 2: As the lens accumulates additional neutral lipids, the bilayer deforms and a nascent LD buds into the cytoplasm. Seipins, as an important LD biogenesis factor, are recruited to the lens structure and facilitate the growth of the nascent LD. The nascent LD may remain attached to the ER or separate completely. Step 3: LDs bud from the ER and grow through fusion or local lipid synthesis. In adipocytes, FSP27 is involved during fusion.

of proteins from the LD surface. As key players in lipid metabolism, LDs frequently communicate with other organelles via direct membrane contact sites or vesicle transport in response to stress responses and cellular signaling. In order to manage lipid storage and lipid homeostasis, LDs interact with organelles including endoplasmic reticulum (ER), mitochondria, lysosome, and peroxidases [18–20]. A large subset of the research into LD biogenesis and metabolism has been conducted in yeast. Research into LDs in human cells generally is focused on the role of LDs in human lipid-accumulation diseases. In this article, we review recent findings on the formation of LDs from the ER, followed by LD growth and breakdown. In particular, we focus on how LDs regulate lipid transport and metabolism in yeast and human cells.

LIPID DROPLET BIOGENESIS

Most models suggest that LDs originate from the ER bilayer and then either remain connected to the ER, or bud off to become independent organelles [21]. ER membranes are often found close to LDs [22]. Enzymes (e.g. diacylglycerol O-acyltransferase [DGAT] and sterol O-acyltransferase [ACAT]) that synthesize neutral lipids for the cores of LDs are localized primarily in the ER [22]. Electron microscopy data reveal close apposition between LDs and the ER [23–25], further supporting the ER model of LD biogenesis. After budding off, LDs continue to maintain a relationship with the ER throughout their lifespan (Fig. 1).

The first step of ER biogenesis involves synthesis of neutral lipids. Neutral lipids are initially

generated by enzymes in the ER lumen, and then disperse into the leaflets of the ER bilayer. As their concentration increases, neutral lipids eventually coalesce and form lens-like structures. The growing droplet progressively distends the ER membrane and eventually buds off into the cytoplasm to form a nascent LD [26–28].

Nascent LDs, which may be connected to the ER or separated completely from it, reengage with the ER during expansion. In yeast, the lipid monolayer of LDs remains contiguous with the ER membrane [29]. The existence of ER–LD membranous bridges was supported by high-pressure freezing electron microscopy and tomography analyses, which showed direct connections between the LD phospholipid monolayer and the adjacent ER [24, 30]. Such bridges permit the exchange of the membrane proteins and lipids between the two compartments [29]. At the contact sites and membrane extensions, neutral lipids synthesized in the ER are transported to LDs [27, 31]. TG synthesis enzymes [e.g. glycerol-3-phosphate acyltransferase (GPAT4) and diacylglycerol O-acyltransferase 2 (DGAT2)] also partially or fully migrate from the ER to LDs via membrane bridges [24]. In higher order eukaryotes, a population of LDs eventually detaches from the ER, but may reattach later.

Re-attachment of LDs to the ER requires the COPI coatomer complex. The COPI machinery promotes the formation of membrane bridges between LDs and ER [32, 33]. The COPI complex is recruited to LDs by the small GTPase ARF1 and its guanine nucleotide exchange factor (GEF) GBF1, which promotes the budding of very small droplets called "nano-droplets" from the existing LDs. Nano-droplets remove phospholipids from the LD surface, which in turn increases LD surface tension and facilitates LD fusion with the ER bilayer. The mechanisms behind COPI facilitation of LD fusion to the ER remain under investigation. Upon fusion, integral membrane proteins such as DGAT2 and GPAT4 are able to diffuse from the ER to LDs, indicating the re-establishment of membrane bridges between the two organelles.

The second step of LD biogenesis is the recruitment of biogenesis factors such as seipin to the lens structure in order to facilitate the growth of the nascent LD. Seipin, an integral ER membrane protein, was originally identified in congenital generalized lipodystrophy [34] and has been increasingly identified as a major player in LD growth. Seipin is localized to ER–LD contact sites and may directly transfer neutral lipids and/or modulate phospholipid transfer at the contact site [35, 36]. Seipin may also act as a diffusion barrier for proteins between the ER and LDs [36]. In accordance with its proposed gatekeeper function, seipin deletion mutations lead to the abnormal relocalization of GPAT4 to LDs instead of the ER [35, 37]. Seipin is also necessary to maintain a population of appropriately sized LDs. Experimental depletion of seipin causes defects in LD morphology, ranging from the accumulation of small LDs with both heterogeneous size and shape, to the creation of supersized LDs [27, 38]. However, the exact cause of this LD size heterogeneity remains elusive. Another study suggested seipin could control the sites at which LDs develop, facilitate continuous delivery of triglycerides from the ER to LDs, and prevent their shrinkage by ripening [39]. Another important regulator of LD growth and maturation is Rab18, a Rab guanosine triphosphatase. Recent discoveries revealed the Rab18-NRZ-SNARE complex is critical for tethering ER–LD and establishing the ER–LD contact site to promote LD growth [40]. Finally, the ER-localized protein DFCP1 acts as a Rab18 effector for LD localization, and mediates LD expansion by interacting with the Rab18-ZW10 complex and thus controlling ER–LD contact formation [41]. Through the interactions of these proteins, the LD grows.

LDs may continue to grow by local synthesis of neutral lipids or via fusion with other LDs [42, 43]. The translocation of TAG synthetic enzymes GPAT4, AGPAT3 (1-acyl-sn-glycerol-3-phosphate acyltransferase 3), and DGAT2 to the LD is one way that LDs may grow in size [24]. Phospholipids are required to stabilize the LD neutral lipid core, which are replenished by CTP:phosphocholine cytidylyltransferase (CCT), a rate-limiting enzyme in the phospholipid synthesis pathway. Newly synthesized phosphatidylcholine (PC) may be transferred from ER to LDs by LD-ER conduits, but other pathways remain to be discovered. LD growth can also result from fusion of

LDs, which rarely occurs under normal conditions [44]. However, LD fusion frequently occurs under conditions of PC deficiency or accumulation of phosphatidic acid (PA) [45, 46], which permit the exchange of lipids between LDs.

An additional pathway is found in adipocytes, where cell death-inducing DNA fragmentation factor-alpha-like effector (CIDE) proteins can induce droplet fusion [47]. LD–LD contact sites are established by CIDE proteins (e.g. CIDEC/Fsp27) [48, 49]. Gong et al. show that Fsp27 localizes to membrane contact sites between adjacent LDs, where the protein appears to form stable "contacts" between the LD and mediates net unidirectional transfer of neutral lipids to the larger LD [50]. Another possibility is that SNARE proteins mediate LD fusion [50], similar to their roles in the fusion of vesicle bilayers. Triacylglycerol transfer invariably occurs from the smaller LD to the larger LD, which is hypothesized to be driven by differences in internal pressure and by surface tension [48].

LIPID DROPLET BREAKDOWN

The breakdown of LDs is mediated by the degradation of neutral lipids under energetically demanding conditions. During nutrient stress, cells shift their metabolism from reliance on glucose metabolism to mitochondrial fatty acids (FAs) oxidation [51]. FAs are important sources of cellular energy after being released from neutral lipids. FAs transported intracellularly to mitochondria for oxidation and ATP generation. Cells use two primary mechanisms for mobilizing FAs: lipolysis of LDs, and autophagy of membrane-bound organelles (e.g. the ER) or LDs [52–56].

When cells are starved, lipolysis can mobilize the lipids inside the LD through LD-associated neutral lipases. Adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoglyceride lipase (MGL) directly hydrolyze triacylglycerols on the LD surface in response to the cellular metabolic status and thus liberate fatty acids (FA) for the mitochondria [51, 57]. ATGL catalyzes the first step of lipolysis by hydrolyzing TAG and generating free FA and DAG. HSL then hydrolyzes DAG, which creates a free FA and monoacylglycerol (MAG). Finally, MGL converts MAG to glycerol and a free FA. This pathway can be regulated at the level of lipase activity, fine-tuned by the cell to its current energetic requirements [58, 59].

Lipolysis is modulated by the interaction of lipases present at the surface of the LD, with the structural proteins that surround LD and with inhibitory proteins in the cytosol [57]. Perilipins, for example, were proposed to slow lipolysis by shielding the triacylglycerol core of LDs from lipases [60]. Perilipin 1 was first identified as a LD-coating protein in mammalian cells [22], and its phosphorylation controls lipase access and anchorage to LDs [61]. Its relative, perilipin 5, normally serves as a barrier to lipolysis by regulating oxidative LD hydrolysis and controlling local FA flux to protect mitochondria against excessive exposure to FA during physiological stress. Perilipin 5 also regulates lipolysis through its interactions with ATGL and its activator ABHD5 (1-acylglycerol-3-phosphate O-acyltransferase) [62].

A second mechanism for mobilizing FAs during starvation is autophagy. Autophagy is an evolutionarily conserved physiological process for the degradation of proteins and organelles in the lysosome or vacuole of the cell to maintain homeostasis [63]. LDs are either sequestered into the autophagosome (macrolipohagy), or directly engulfed into the lysosome/vacuole lumen (microlipophagy) [64]. Both prolonged and brief kiss-and-run interactions between LDs and lysosomes have been observed in mammalian cells [65–67]. Recent data implicate these contacts as sites for the degradation of perilipin 2 and perilipin 3 via chaperone-mediated autophagy (CMA) [66]. In addition to the activity of cytosolic lipases, FAs can be mobilized from LDs through macroautophagy in mammalian cells. During macroautophagy, also known as macrolipophagy, a LD is engulfed into an autophagosome that subsequently fuses with a lysosome, which will digest the LD and release free FAs that move into the cytoplasm [55]. Microlipophagy is the engulfment of a LD in an invaginated vacuole or lysosome. In yeast, the vacuole is major catabolic organelle equivalent to the metazoan lysosome. Recent studies highlight a complex interplay between autophagy and LDs: LDs are degraded by autoph-

agy via lipophagy, but LDs have also been implicated in regulation of the autophagic process in mammals.

Although it may seem contradictory, the number of LDs increases during prolonged periods of nutrient deprivation. The raw lipid metabolites generated in autolysosomes appear to directly contribute to droplet production [51, 68]. Master growth regulator mTOR complex 1 (mTORC1) was reported to control autophagy-dependent LD biogenesis during starvation [51]. mTORC1-regulated autophagy degrades membranous organelles, releasing FAs that are selectively channeled by DGAT1 into new LDs during nutrient deprivation, which in turn protects mitochondria from lipotoxic disruption [51]. Under these conditions, the biogenesis of LDs is necessary to sequester FAs in TAG-rich LDs, preventing accumulation of acyl-carnitine and subsequent mitochondrial dysfunction. However, autophagy-dependent LD biogenesis may not be limited to nutrient-limited conditions, acting instead as a general protective response to high levels of autophagy. These findings underscore the high degree of crosstalk between autophagy, the ER, LDs, and mitochondria that maintains lipid and energy homeostasis. Recent evidence suggests that, upon sensing a decline in nutrients, yeast exhibit a "bloom" of LDs organized at the periphery of the nuclear ER–vacuole junction (NVJ) [69, 70]. The NVJ-tethering protein Mdm1p appears to play a critical role in this LD accumulation. Analogous ER–autolysosomal contacts may be important for lipid droplet biogenesis during nutrient deprivation in mammalian cells.

In order to avoid FA toxicity, LD autophagy is regulated and works in parallel with other pathways, such as the coordinate of FA influx into mitochondria with storage of excess FAs in LDs [51]. FAs liberated from digestion of LDs can be used for energy production via mitochondrial (metazoans) or peroxisomal (yeasts and plants) beta-oxidation [71]. LDs provide an "on demand" source of FAs that can be mobilized in response to fluctuations in nutrient abundance. The autophagy-dependent LDs clustered in close proximity to mitochondria and LD-mitochondrial contacts have been proposed to function as sites for FA transfer [51, 72]. Such spatial positioning of LDs and mitochondria ensures oxidative tissues that require high FA influx have sufficient supply for their energy demands. Data from transmission electron microscopy, fluorescence imaging, and reconstitution assays in yeast demonstrated that LDs physically contact mitochondria in vivo and in vitro, and other experiments have described protein interactions between LDs and mitochondria or peroxisomes [73]. While exploring how FAs move into mitochondria in starved cells, Rambold et al. found that FAs used LDs as their conduit for delivery to organelles [51], instead of lingering in the cytoplasm.

Perilipin 5 is a key player in establishing the lipid droplet-mitochondrial junction, and is highly expressed in oxidative tissues (liver, skeletal muscle, heart and brown adipose tissue) [74, 75]. Overexpression of perilipin 5 is sufficient to induce a dramatic recruitment of mitochondria to the periphery of LDs [74, 75]. Other experiments have suggested perilipin 5 recruits mitochondria to the LD surface through a C-terminal region and induces the physical contact between LD and mitochondria [75]. In addition to perilipin 5, the SNARE protein synaptosome associated protein 23 (SNAP23) was reported to mediate the interaction between LDs and mitochondria [76]. Knockdown of SNAP23 interrupts the interaction between mitochondria and LD [76].

Similar to LDs, peroxisomes are key to lipid and energy metabolism. In humans, most beta-oxidation of FAs occurs in mitochondria, but beta-oxidation of very-long-chain FAs and branched FA occurs in peroxisomes. Mice lacking peroxisomes accumulate enlarged LDs in the liver [77]. In yeast, peroxisomes are the sole sites of beta-oxidation. Delivery of lipases from peroxisomal extensions to LDs has been observed in *Arabidopsis* [78]. In yeast, peroxisome–droplet contacts are sites of FA transfer. Clear droplet–peroxisome contact sites have been observed in yeast undergoing stationary-phase growth [79]. In yeast exposed to FAs as their sole carbon source, LDs form prominent and stable associations with peroxisomes [79]. The LD membrane fuses with the outer phospholipid leaflet of the peroxisome bilayer, which may allow luminal peroxisome enzymes to directly access LD stored triacylglyc-

erol or facilitate protein transfer [79]. In wild type cells, LD-peroxisome contacts may facilitate the coupling of lipolysis on the LD to FA oxidation in the peroxisome. LDs also sequester lipotoxic FAs through their incorporation into TAGs [68]. However, a portion of autophagy-released lipids is immediately re-esterified to form triacylglycerol, which is then packaged into new pools of LDs [51, 68].

The mechanism by which FAs are transported out of autolysosomes and trafficked to the ER for triacylglycerol synthesis is not known. Membrane continuity between ER and LDs could also account for the reverse process i.e. the back transport of lipids from LDs to the ER during lipolysis. Under these conditions, LD-localized lipases hydrolyze TAG and steryl esters to liberate free FA, DAG and free sterols. These lipid intermediates are water insoluble, but could be released from LDs into the connecting ER membrane and thus enter the pool of lipids that are synthesized de novo by the ER-localized lipid biosynthetic enzymes. Lipolysis of LDs also induces ER–LD contacts for back transport of phospholipids and droplet proteins to the ER [80]. During lipolysis, for example, the diacylglycerols and FAs generated are directly transferred to the ER for phospholipid synthesis [81]. Similarly, a sterol transfer protein that was identified on the surface of LDs mediated the transport of released sterols back to the ER [81]. Early biochemical studies in yeast demonstrated the transport of membrane-bound LD marker proteins from the ER membrane to the newly made LDs. The transport is reversible and under lipolytic conditions, LD-localized membrane proteins will relocate to the ER [29]. Moreover, bidirectional transport of lipids between the ER and LDs must occur under lipogenic conditions, when LDs form and grow, and during lipolysis, when LDs regress [29].

Lipid droplet–nuclear envelope contacts also exist. LDs have been observed inside the nucleus in mammalian cells [82] and in yeast [83]. In yeast, LDs have been shown to bud directly from the inner nuclear membrane [83], as the lipid monolayer of the nuclear LDs remained contiguous with the inner nuclear membrane. Furthermore, seipin localizes to the inner nuclear membrane and is known to be necessary for the proper formation of the membrane bridge. Nuclear LDs may provide lipids for use in nuclear envelope expansion, store specific proteins, or sequester transcriptional factors [84, 85].

CONCLUSION

After budding off from the ER, LDs can associate with most cellular organelles through membrane contact sites. These contacts between LDs and other organelles are highly dynamic and coupled to the cycles of LD expansion and shrinkage. LD biogenesis and degradation, as well as their interactions with other organelles, are critical to cellular metabolism and to buffer the levels of toxic lipid species. LDs facilitate the coordination and communication between different organelles and act as vital hubs of cellular metabolism. Multiple mechanisms are involved in LD-mediated intracellular lipid trafficking. In view of evolution, these interactions appear less complex in yeast and become multifaceted, reflecting in protein–protein and lipid–protein interactions to accomplish diverging cellular functions in human cells. Further studies are needed to show detailed mechanisms that LDs are involved in various aspects of cellular physiology and pathology in human lipid-accumulated diseases.

The authors declare that they have no conflict of interest.

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