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Review article

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Heterogeneity, dynamics and organelle interactions of lipid droplets

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Abstract

Lipid droplets (LDs) are emerging as key factors in cellular physiology, with roles beyond energy storage, including metabolic homeostasis, signalling and development. Together with a growing list of functions, diverse LD populations are being identified in different tissue types as well as within the context of single cells. Here we summarize recent work highlighting LD diversity from three perspectives: their lipid and protein compositional heterogeneity; differences in abundance, size and spatial organization within cells; and the diverse contacts they form with other organelles, all of which contribute to LD function. We also discuss tools and approaches used to visualize LD heterogeneity, the role of LDs in pathophysiology and disease, and open questions in the field.

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Introduction

Organisms must adapt to changes in nutrient availability to survive in uncertain environments. To store currently available nutrients for the future, lipid droplets (LDs) have evolved in eukaryotes as specialized organelles to store energy in the form of highly concentrated neutral lipids. LDs exhibit unique natural variation in their protein and lipid contents, as well as their biophysical properties[1]. The neutral lipid core of LDs is hydrophobic and typically composed of triglyceride and steryl esters[2]. The droplet surface is coated by a phospholipid monolayer, rather than the bilayer that surrounds other eukaryotic compartments[2]. LDs are also defined by a specific proteome of more than 100 proteins that decorate their surface[3,4]. Like other organelles, LDs undergo a life cycle that includes biogenesis, dynamics and turnover[5–7] (Fig. 1). As new methods have been developed in recent years to study these organelles (Box 1), an emerging theme is that LDs exhibit remarkable structural and functional heterogeneity. This includes heterogeneity in LD lipid and protein composition, their spatial distribution within cells, and the physical contacts and functional communication they establish with other organelles at membrane contact sites (MCSs). This heterogeneity supports a diversity of functions that go beyond lipid storage, including the channelling of fatty acids into anabolic or catabolic metabolism, protection against lipotoxicity, lipid signalling, protein quality control, and cellular responses to stress and infection[8].

First and foremost, we want to highlight that despite their relatively small size, LDs store a remarkably large quantity of cellular chemical energy. Estimates based on an ~400 nm diameter LD suggest it can store ~21 million triglyceride molecules capable of generating ~7 billion ATP equivalents (Robert Farese Jr, personal communication). Triglyceride acyl chains also fuel membrane biosynthesis and other important signalling pathways. Given that cells typically contain dozens or more LDs, elucidating the impact of their structural and functional diversity on bioenergetics and beyond is crucial to understanding the important roles they have in development, physiology, metabolic diseases, cancer and neurodegeneration.

Extensive research cataloguing LD characteristics using established and emerging techniques has revealed that different cell and tissue types exhibit variation in LD size, organization, composition and function. Understanding this heterogeneity is increasingly important as we learn that LDs perform diverse roles in cell physiology and are linked to a myriad of genetic and metabolic diseases (Box 2). Here, we review LD structure, function and dynamics from the perspective of LD heterogeneity. We discuss heterogeneity in the lipid and protein composition of LDs, as well as in their spatial distribution, and how

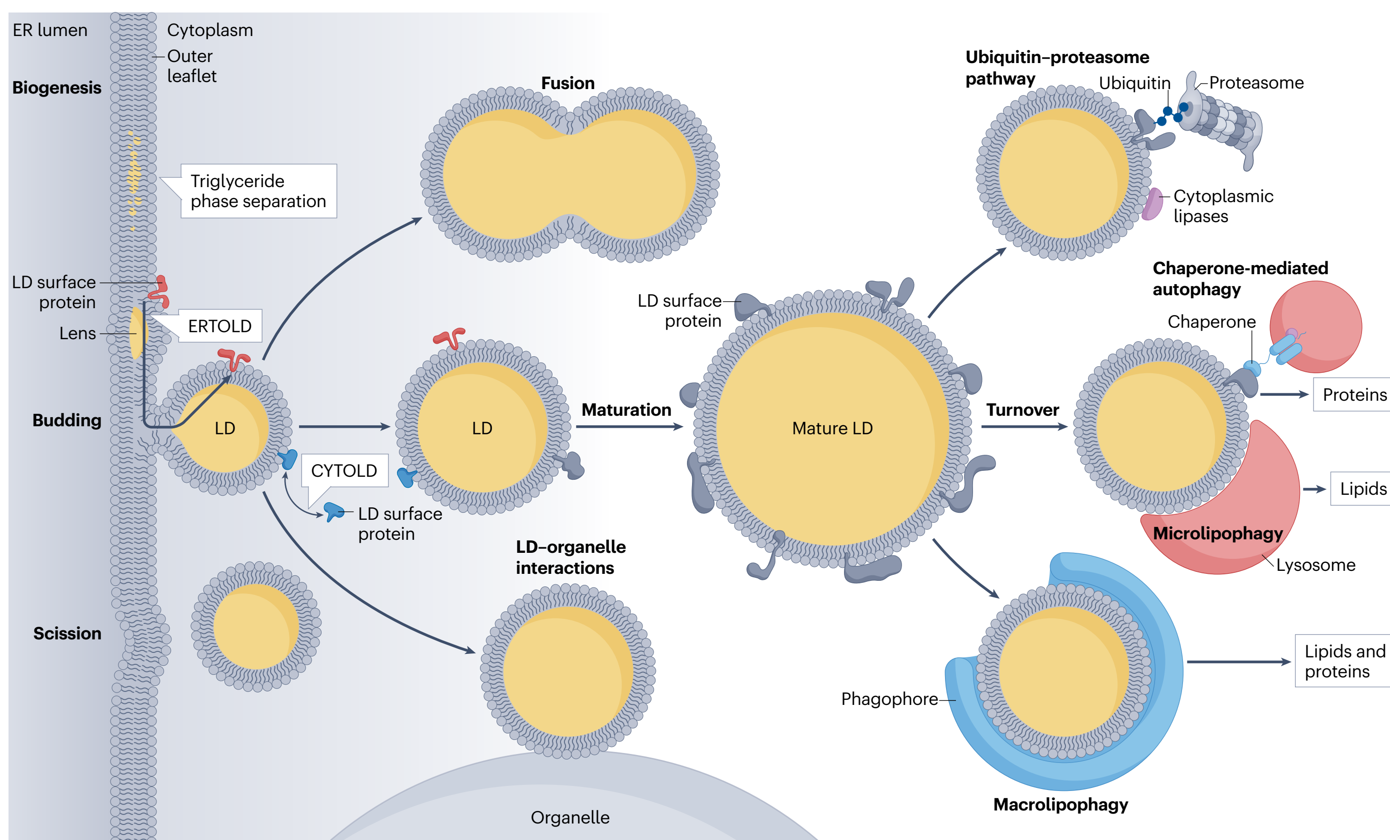


Fig. 1 | The life cycle of an LD. Lipid droplet (LD) biogenesis occurs at the endoplasmic reticulum (ER), and is initiated by triglyceride phase separation and accumulation of a neutral lipid lens between the leaflets of the ER bilayer. In mammalian cells, the LD then buds towards the cytoplasm and undergoes scission, although most LDs later re-associate with the ER to exchange lipids and proteins. For simplicity, some LDs in this figure are shown detached from the ER. LDs undergo dynamic changes including maturation through the recruitment of LD surface proteins, LD–LD fusion and interactions with other organelles such as mitochondria and peroxisomes. LD turnover occurs by hydrolysis of lipids by cytoplasmic lipases and proteolysis of LD surface proteins via the proteasome and chaperone-mediated autophagy. Additional forms of autophagy, microlipophagy and macrolipophagy break down both lipids and proteins via lysosomal lipases and proteases. The figure also denotes protein targeting to LDs via cytoplasm-to-LD targeting (CYTOLD) and ER-to-LD targeting (ERTOLD).

Box 1 | Methods for studying LD heterogeneity

Emerging methods have been instrumental in revealing the diversity of lipid droplets (LDs). LDs can be biochemically isolated from cells or tissues by organelle fractionation and flotation protocols[202], which rely on the intrinsic buoyancy of LDs in aqueous buffers that enables them to float, whereas multistep fractionation protocols enable the separation of LD subsets based on various factors. For example, LDs have been fractionated based on their size or interaction with other organelles such as mitochondria, revealing structurally and functionally distinct LD subpopulations[142,203]. Microscopy provides information about the ultrastructure and spatial distribution of LD subpopulations within cells and tissues. Optical immunofluorescence microscopy has been used to label proteins that decorate specific LDs. For example, in mammals perilipins decorate specific LD subsets[78,204], whereas in yeast several proteins including lipid droplet organizing (Ldo) proteins, perilipin 1 (Pln1) (also known as Pet10) and triglyceride lipid droplet protein 1 (Tld1) decorate LD subsets based on their proximity to the yeast vacuole, their lipid composition or other factors[92,93,205]. Polarized light microscopy is an optical microscopy technique that uses living or fixed cells and a microscope equipped with a polarizer to specifically image liquid–crystalline-phase LDs, which exhibit birefringence[56,206]. This method can be coupled with vital dyes such as BODIPY staining.

Cryo-electron microscopy uses in situ frozen samples and 3D electron tomography to image LDs at high spatial resolutions. This approach has provided the necessary molecular resolution to detect the onion-like layers of smectic liquid–crystalline steryl ester lattices within the LD hydrophobic core[56,57]. Finally, organelle contact sensors allow visualization of LD–organelle contact sites when the LD membrane comes into close enough proximity with another organelle to reconstitute a split fluorescent protein or enzymatic activity[207,208]. Split yellow fluorescent protein (YFP) has been used to visualize LD contacts with the endoplasmic reticulum, mitochondria, peroxisome, vacuole and plasma membrane in yeast[100]. Contact-FP is a toolkit of dimerization-dependent fluorescent proteins targeted to organelle membranes that has been used to visualize dynamic LD contacts with endoplasmic reticulum, mitochondria, peroxisomes, lysosomes, plasma membrane and caveolae in mammalian cells[102]. FABCON uses fluorogen-activated bimolecular complementation to visualize contact sites upon addition of an organic fluorophore. It has been used to visualize LD contacts with endoplasmic reticulum, mitochondria and peroxisomes in mammalian cells[103]. Together, these methods facilitate the investigation of heterogeneity in LD composition, structure, distribution and interactions with other organelles.

this contributes to LD function. Next, we focus on the heterogeneity of LD interactions with other organelles, including endoplasmic reticulum (ER), Golgi, plasma membrane, mitochondria, peroxisomes and lysosomes. These contacts have important roles in the exchange of lipids and proteins, contributing to LD biogenesis, function and turnover. Finally, we discuss open questions and future directions for LD research.

Overview of the LD life cycle and functions

The purpose of this Review is to focus on the current state of knowledge of LD heterogeneity. LD biogenesis[9–12] and protein targeting to LDs[13–15] have recently been reviewed elsewhere, and we encourage our readers to examine those reviews. Therefore, we discuss LD biogenesis and LD functions only briefly to provide sufficient background information for the reader to follow subsequent sections.

LD biogenesis

LD biogenesis occurs at the ER, the site of many lipid synthesis reactions. In response to excess lipids (exogenous or de novo synthesized) or various stimuli or stresses, neutral lipids accumulate between the leaflets of the ER bilayer, forming a lens that buds outwards towards the cytoplasm[16] (Fig. 1). In yeast, LDs may remain permanently attached to the ER through thin membrane stalks that serve both as connections for lipid flow and protein enrichment[17–19]. In mammalian cells, LDs appear capable of detachment and re-attachment through poorly understood mechanisms, and live-cell imaging in monkey fibroblasts (COS-7 cell line) suggested that at any given time, >80% of LDs are associated with the ER[20]. Numerous proteins contribute to LD biogenesis by organizing specialized subdomains in the ER, modulating local membrane curvature and funneling lipids into nascent LDs, as well as influencing the directionality of LD budding[9–12]. Chief among these is seipin, which can influence the nucleation of LDs and supports LD biogenesis[21–26]. The unique proteome of LDs is achieved by two primary mechanisms: targeting of proteins from the cytoplasm using the cytoplasm-to-LD (CYTOLD) pathway, and from the ER based on the ER-to-LD pathway (ERTOLD)[13–15]. As discussed in more depth below, protein targeting to LDs via the CYTOLD or ERTOLD pathways depends primarily on their architecture, with soluble cytoplasmic proteins recruited to LDs by insertion of hydrophobic moieties, whereas ER-anchored proteins transition from the ER to LDs through the lipidic bridges connecting them. LDs undergo a maturation process, with recruitment of surface proteins occurring sequentially[27,28]. After their biogenesis, LDs undergo a variety of dynamic processes, including motor-driven movement, fusion, and interactions with other organelles[5,29–32] (Fig. 1), contributing to LD heterogeneity in both space and time. Finally, LDs undergo turnover, which releases lipids that can drive bioenergetics, support membrane biogenesis or function in cell signalling. Multiple catabolic processes contribute to the turnover of LD protein and lipid components, or of the entire organelle. These processes include proteolysis by the proteasome, chaperone-mediated autophagy (CMA), cytoplasmic lipases, and microautophagy and macroautophagy[33–35], which are discussed below in more detail.

Role of LDs in physiology

Owing to their diverse architecture and spatial distribution within cells, LDs have many roles in cell and tissue physiology. In their most basic function, LDs are lipid reservoirs that can be collected following metabolic cues to fuel membrane synthesis or bioenergetics. In a common response to nutrient deprivation, yeast cells accumulate LDs to store surplus lipids not needed for membrane biosynthesis owing to slowed growth, which serve as a reservoir for later use in oxidative metabolism or membrane synthesis when nutrients are available again.

Box 2 | LDs in pathophysiology and disease

Lipid droplets (LDs) are a ubiquitous lipid-storage organelle, yet their disruption in different cell types can cause remarkably different disease pathologies[11]. One of the best examples are mutations in the *BSCL2* gene encoding seipin, which give rise to Berardinelli–Seip congenital lipodystrophy (BSCL) type 2 (ref. 86). Although seipin is highly expressed in adipose tissue and its loss leads to body fat loss, patients exhibit other phenotypes including muscle weakness, intellectual disability and neuromuscular dysfunction. With these heterogeneous disease symptoms, a pervasive question is how LD perturbation impacts different tissues. Recent work hypothesized that seipin loss or defective LD biogenesis results in increased levels of lipotoxicity across all tissues, and this may differentially impact cells specialized for lipid storage (adipose tissue), bioenergetics (muscle), or long-lived cells such as neurons that rely on lipid quality control for sustained function[209,210]. LDs also accumulate in metabolic dysfunction-associated fatty liver disease (MAFLD), chronic kidney disease, pancreatic steatosis and cardiovascular disease[11]. In MAFLD, hepatic LD accumulation can progress to fibrosis, cirrhosis, liver failure and hepatocellular carcinoma[211]. Similar to MAFLD, lipid accumulation in chronic kidney disease drives inflammation and fibrosis[212]. Pancreatic steatosis occurs due to either adipocyte infiltration of the exocrine pancreas or abnormal LD accumulation in the endocrine pancreas, and is detrimental for both its endocrine and exocrine roles[213]. In the heart, LD accumulation is generally protective, reducing lipotoxicity by sequestering long-chain fatty acids; however, accumulation of LDs in heart cells may contribute to cardiomyopathy and heart failure in severe obesity and diabetes mellitus[214]. In addition to these metabolic diseases, LDs are increasingly understood to have important roles in cancer and neurodegenerative disease[181,215]. LDs can promote cancer cell proliferation, migration and survival through multiple mechanisms, including providing building blocks for membrane lipid synthesis and substrates for ATP production by β-oxidation, and alleviating endoplasmic reticulum stress[11,215]. LD accumulation in glial cells was one of the initial observations in Alzheimer disease, along with amyloid-β plaques and tau tangles[216]. Recent work suggested that amyloid-β induces diacylglycerol *O*-acyltransferase 2 (DGAT2)-dependent LD accumulation in microglia, causing microglia to become dysfunctional and contributing to tau pathology in neurons and ultimately to neurodegeneration[217,218]. Finally, LDs also have roles in viral and bacterial infections. LDs support the replication of many viruses, including flaviviruses, poliovirus, coronavirus, rotavirus and rabies virus[219]. In the best-studied example of hepatitis C virus, replication organelle formation, virion assembly and virion maturation all occur in close proximity to LDs, with LDs providing an important lipid source for these processes[219]. In addition, LDs have important roles in the innate immunity response to bacterial infection, serving as platforms for host defence proteins such as interferon-inducible guanosine triphosphatases and the antimicrobial calthecidin[49]. Taken together, LDs are key factors in a wide variety of pathophysiological contexts. How LD heterogeneity and dynamics contribute to each of these conditions remains an open question.

Mammalian cells also exhibit LD accumulation during starvation, storing de novo synthesized lipids, those derived from absorption, or lipids released by autophagy of membranes[36–38]. In addition, LDs serve a second major role as a metabolic buffer that prevents lipotoxic stress. One example is LD-mediated storage of excess sterols: cholesterol cannot be easily degraded and is relatively insoluble in the water-rich cytoplasm, forming crystalline deposits that can drive tissue inflammation and necrosis if not solubilized by LDs[39]. Similarly, fatty acids themselves are detergents that dissolve cellular membranes, but their esterification into triglyceride renders them inert and stable for extended time periods. Such lipid storage enables LDs to temporarily store fatty acids during nutrient deprivation, providing metabolic buffering[40]. The function of LDs as lipotoxic protectants was more recently highlighted by the finding that LDs can protect cells from ferroptosis by sequestering oxidation-prone polyunsaturated fatty acids in response to stresses such as cell cycle arrest[41–43]. In *Caenorhabditis elegans*, accumulation of unsaturated fatty acids in LDs reduces lipid oxidation during ageing, implying protection from ferroptosis[44]. These observations point to LDs as protective sinks for oxidation-prone lipids, and highlight their function as lipid quality control organelles that inhibit lipid peroxidation.

In addition to metabolic or lipotoxicity buffering, LDs can be platforms that sequester proteins for storage until they are used at a later stage in organismal development. *Drosophila* embryos store histones on the LD surface during development, and encode a specific adaptor protein called Jabba to recruit histones to the LD surface[45]. In mammals, the transcription factor Max-like protein X (Mlx) docks onto LDs when inactive and is released to drive metabolic transcriptional responses[46]. In a remarkable adaptation, LDs known as retinosomes found in retinal pigmented epithelial cells in the eye function as storage depots for retinyl esters, and serve to replenish the retinal chromophore 11-*cis*-retinal to aid in light sensing[47]. Another striking role for LDs has been described in the chicken eye, where a single LD was proposed to act as an optical lens within the cone cells aiding in the capturing of light for photo-sensing[48]. Finally, LDs serve as hubs of innate immune and inflammatory lipid signalling: in response to bacterial lipopolysaccharide, innate immune proteins become enriched on the surface of LDs, whereas perilipin 5 protein targeting to LDs is reduced. This correlates with LD decoupling from mitochondria, thereby shifting metabolism from oxidative phosphorylation to glycolysis[49]. Eicosanoids are important signalling lipids that regulate the initiation and resolution of inflammation. Recent evidence indicates that LDs are a source of arachidonic acid, the precursor for eicosanoid synthesis, and that enzymes including phospholipases, cyclooxygenases and lipoxygenases that contribute to eicosanoid synthesis localize to the surface of LDs[50]. No doubt additional roles for LDs in different physiological processes will continue to be uncovered.

LD compositional heterogeneity and spatial organization

Despite their initial characterization as inert fat globules within adipose tissue, LDs can be observed in almost all cell types and eukaryotic species across the kingdoms of life. Matching this broad evolutionary and tissue distribution is a remarkable diversity in LD composition, organizational patterning and function within different cell types. These variations enable LDs to serve distinct functional niches in

many contexts, providing metabolic versatility to this remarkable lipid-storing organelle. Here, we discuss LD heterogeneity from three distinct perspectives: LD lipid composition, LD protein composition and cellular spatial organization. Each of these types of heterogeneity contributes to LD function.

LD lipid heterogeneity

Architecturally, LDs are composed of a neutral lipid core surrounded by a phospholipid monolayer. The predominant neutral core lipids are triglycerides and steryl esters, whereas the monolayer is primarily composed of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEth) and phosphatidylinositol (PtdIns)[2,51]. The most abundant LD lipid is typically triglyceride, composed of three fatty acyl chains esterified to glycerol, which serves as a high-energy storage lipid that can be mobilized to supply fatty acids for membrane biosynthesis or cellular energetics. LDs can also store excess sterols, such as cholesterol in mammals or ergosterol in yeasts, in the form of steryl esters following fatty acid esterification. Both triglyceride and steryl ester synthesis occur within the ER membrane facilitated by the integral membrane proteins DGAT and sterol *O*-acyltransferase (SOAT), respectively, highlighting the close functional pairing between ER and LD[51]. In line with this, recent atomic structures of DGAT1 identified a cavity within the enzyme that enables lipid substrates such as diacylglycerol (DAG) to move laterally from the hydrophobic acyl chains of the ER membrane into the enzyme for catalysis[52,53]. These cavities indicate that neutral lipids such as triglycerides can flow into the hydrophobic region between the ER leaflets following their enzymatic synthesis, where they will accumulate until they phase separate into a nascent lens, initiating LD biogenesis. Mathematical modelling indicates that the threshold for this phase separation is ~3 mol% local triglyceride concentration in the ER bilayer[54].

Mammalian LDs typically contain both triglyceride and steryl esters, so a key question is how these neutral lipids influence each other during LD formation. For example, are pools of steryl esters in the ER membrane alone sufficient to nucleate an LD lens and, if so, how are steryl ester pools influenced by triglycerides? In vitro reconstitution as well as molecular modelling approaches suggest that steryl ester loading into LDs is promoted by the presence of triglycerides, indicating that triglycerides support the transition of steryl esters into LDs[55]. This may reflect the ability of triglycerides to maintain steryl esters in a disordered phase conducive to LD loading, although this requires further investigation. Following their incorporation into the LD core, these lipids continue to influence each other's biophysical properties. Typically, the LD core contains an amorphous mixture of triglyceride and steryl ester, and biochemical data indicate that the molar ratio of these neutral lipids is important to maintain the lipid structural organization within the LD core. Strikingly, conditions that alter the triglyceride to steryl ester ratio of an LD beyond a critical threshold, resulting in an excess of steryl esters, lead to the spontaneous demixing of the neutral lipids, and the phase transition of the steryl esters from an amorphous phase into a smectic liquid–crystalline phase[55–57]. These liquid–crystalline phase steryl esters can be observed by cryo-electron tomography as distinct 'onion-like' layers within the LD core, or by polarized light microscopy where such LDs exhibit a Maltese cross pattern (Fig. 2a,b and Box 1). It should be noted that the lattice-like arrangement of steryl esters alters the biophysical properties of the neutral lipid core, as well as the spatial arrangement of both steryl esters and triglyceride. Molecular dynamics simulations indicate that the steryl ester forms layers in the outer edges of the hydrophobic core, whereas the triglycerides concentrate in the LD centre[56,57]. Importantly, the physical arrangement of the lipids within the LD core may influence the LD surface proteome, as biochemical isolation of triglyceride-rich and steryl ester-rich LDs revealed differences in protein composition[56,58]. Liquid–crystalline deposits have been observed in disease states such as in atherosclerosis plaques in the cardiovascular system[59], but whether they exhibit specific functions or are simply the consequence of underlying pathologies is unclear. However, they may be highly relevant in adrenergic cell types such as the adrenal gland, as these store excess cholesterol as cholesteryl ester, and use cholesterol for steroidogenesis[60]. Of note, recent work revealed that cholesteryl ester-rich LDs accumulate during inflammatory signalling and are associated with the cell nuclear envelope[61]. Despite these insights, the physiological role of liquid–crystalline LDs remains unclear. They may influence accessibility, and thereby interaction, of core lipids to LD surface proteins such as lipases, but this remains to be tested.

Surrounding the LD neutral lipid core is a phospholipid monolayer that compositionally resembles the ER bilayer from which it originates. Glycerophospholipids including PtdCho and PtdEth constitute over 90% of this monolayer, which can also contain low amounts of electrostatically charged lipids such as phosphatidylserine (PtdSer) and PtdIns phospholipids including PtdIns(4)P (ref. 62). Each phospholipid class influences different aspects of LD homeostasis. For example, local PtdCho biosynthesis in flies has been proposed to support LD expansion through the local recruitment of the PtdCho-biosynthesis enzyme CTP:phosphocholine cytidylyltransferase (CCT)[63], although whether LD recruitment of CCT is required remains debated[64,65]. PtdEth is a conical-shaped lipid, and its addition can alter the local membrane tension of the LD monolayer and influence how nascent LDs emerge from the ER bilayer[66]. In line with this, the PtdEth-synthesis enzyme phosphatidylserine decarboxylase proenzyme 1 (PSD1) has been reported in mammalian cells and yeast to be locally recruited from the ER to the LD surface during LD biogenesis, probably to support local changes in phospholipid composition required for LD budding[67,68].

Whereas PtdCho and PtdEth appear to function as general coat lipids, charged phospholipids such as PtdSer are thought to facilitate the recruitment of specific proteins such as oxysterol-binding protein-related protein 5 (ORP5) to the LD surface, thereby influencing LD growth. In mammals, ORP5 and ORP8 proteins have been proposed to localize to ER–LD contact sites where they regulate the exchange of PtdSer for PtdIns(4)P on the LD surface during LD growth[62]. In budding yeast, PtdIns(4)P has been observed on LDs as well as along the surfaces of micrometre-scale lipid rafts that form on the vacuole surface and serve as platforms where LDs dock during microlipophagy, in which LDs are enveloped into the vacuole[69]. In summary, the phospholipid monolayer provides an outer lipid coat for LDs that promotes their stability and size changes and also acts as a platform for protein recruitment, discussed more in the next section.

LD protein heterogeneity

A key property of membranes is their fluidity, which enables collisions and the mingling of phospholipids over time. These dynamics spontaneously create micro spaces between lipid headgroups that are exposed to the aqueous cytoplasm, resulting in the exposure of hydrophobic regions of the membrane, into which proteins can insert[70]. The LD phospholipid monolayer also exhibits these lipid-packing defects, which heavily influence LD protein targeting and access to the hydrophobic lipid core[54]. LD-associated proteins with amphipathic helices or other

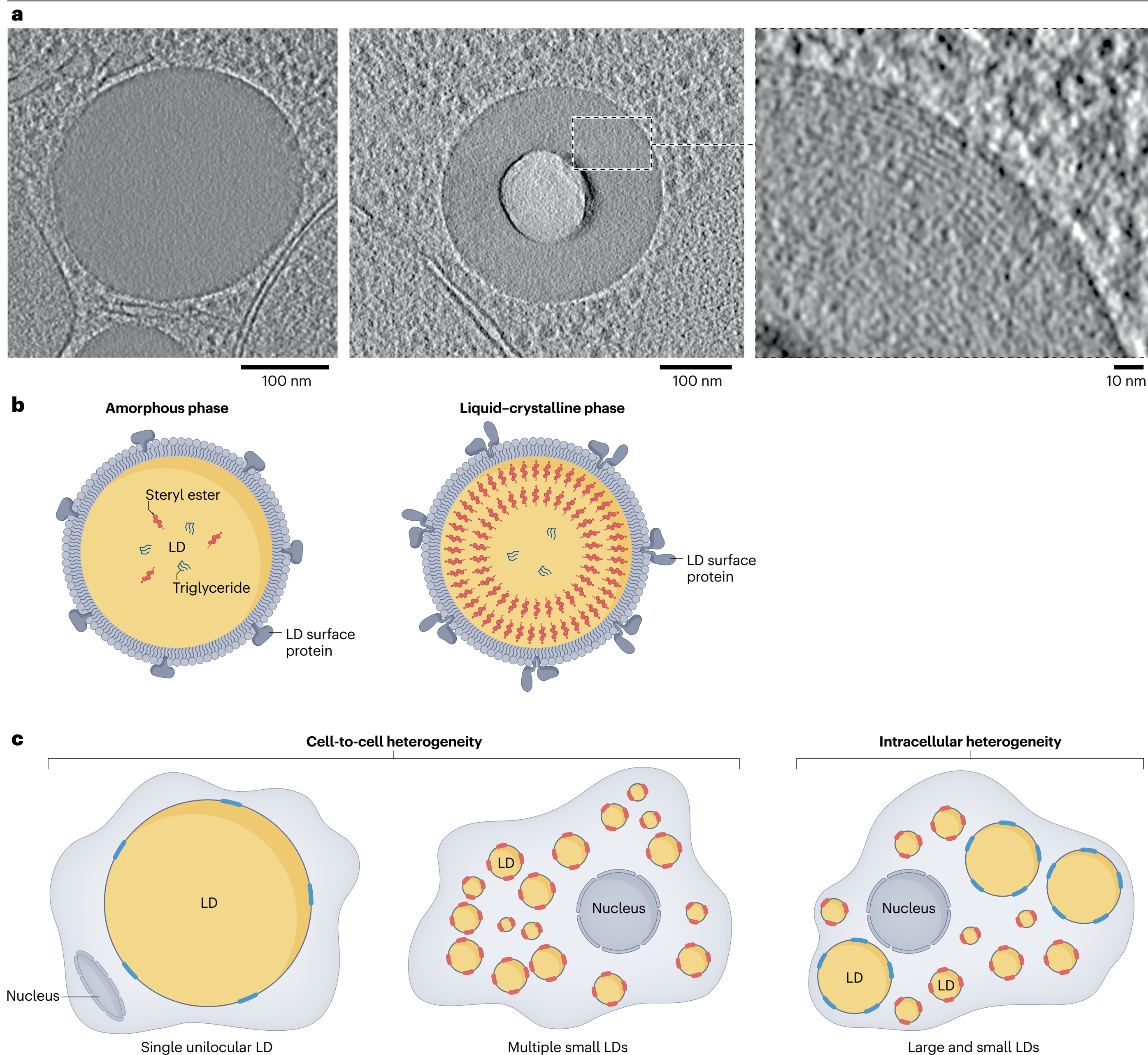


Fig. 2 | LD compositional and spatial heterogeneity. a, Cryo-electron tomograms of lipid droplets (LDs) in budding yeast cells exposed to acute glucose restriction, which triggers metabolic remodelling in yeast favouring triglyceride lipolysis (LD tomograms courtesy of D. Nicastro and W. M. Henne labs, and see published work with additional images in Rogers et al.[56]). The left image shows the amorphous LD hydrophobic core surrounded by the cytoplasm from a yeast provided abundant glucose nutrients. The middle image shows an LD from a yeast grown in low glucose media with layers of smectic liquid–crystalline phase steryl esters in the LD peripheral core, causing the central core region of the LD to appear irregular in the electron microscopy thin section. The right image shows a zoom-in of the smectic liquid–crystalline layers within the LD. **b**, Schematic representations of LDs exhibiting disordered neutral lipid cores (left) or ordered liquid–crystalline lattices of smectic phase steryl esters (right). Triglycerides, steryl esters and the surface phospholipid monolayer are depicted. **c**, A cartoon of cells with different LD spatial organizations. Cells can contain either a single unilocular LD as in a white adipocyte (left), several smaller LDs (middle) or numerous LD subsets decorated by different proteins (denoted as differently coloured surface structures).

hydrophobic residues can attach to the LD surface by inserting into lipid-packing defects, which thus provide anchor points that support protein docking. Critically, lipid-packing defects also expose the amphipathic helix and cytoplasm to the hydrophobic core[71], providing access

to triglycerides for cytoplasmic lipases that drive lipolysis. To limit triglyceride accessibility, the LD surface is densely coated with abundant perilipin (PLIN) proteins, which contain amphipathic helices that fill lipid-packing defects and thus coat the droplet[72–74]. Other LD-targeting mechanisms include the use of hairpin or hydrophobic motifs, protein lipidations such as myristylation groups that may enhance LD anchoring, or docking of proteins to other LD-associated proteins, and have been extensively discussed in other great reviews[14,15].

Mammals encode five known PLIN proteins that decorate distinct LD populations, making them excellent markers for LD diversity (Fig. 2c). For example, adipose tissues highly express PLIN1, the first characterized LD surface protein, which stably decorates large LDs for long-term fat storage[75]. By contrast, PLIN3 is more ubiquitously expressed and decorates newly formed LDs in various tissue types[76,77]. In adrenal cells, PLIN4 preferentially sequesters to steryl ester-rich LDs, whereas PLIN5 marks LDs enriched in triglycerides[78]. PLIN5 is expressed in brown and beige adipose tissue as well as in heart and skeletal muscle and influences the oxidative turnover of LD lipids during mitochondrial fatty acid oxidation[79]. PLIN1 also influences LD lipolysis through a phosphokinase A-dependent phospho-regulated mechanism that controls adipose triglyceride lipase (ATGL, also known as PNPLA2) lipase activity, highlighting the ancient role for PLIN proteins as LD coats that regulate both lipid storage and mobilization[80]. The non-vertebrate *Drosophila* encodes only two PLIN proteins (called lipid storage droplets surface-binding protein 1 (LSD1) and LSD2) that exhibit binding preferences for large and small LDs, respectively[81], and budding yeast encode just a single perilipin-like protein termed Pln1 (formally denoted as Pet10p) that promotes LD biogenesis and lipid storage[82].

In addition to PLINs, LDs are decorated by dozens of other resident proteins that interact with the LD surface either through the insertion of short hydrophobic motifs such as amphipathic helices or through longer hydrophobic helix-turn-helix folds. At least two mechanisms of LD protein targeting have been identified, which rely on these hydrophobic regions to bind the LD surface. As noted above, the CYTOLD pathway is typically utilized by cytoplasmic proteins that insert an amphipathic helix or hydrophobic wedge into accessible lipid-packing defects on the LD surface. CYTOLD proteins exhibiting covalently attached lipid moieties such as myristyl groups may associate with LDs by inserting these lipid moieties into the LD monolayer[15]. The second targeting system, the ERTOLD pathway, relies on larger helix-turn-helix or hairpin motifs within the protein[83].

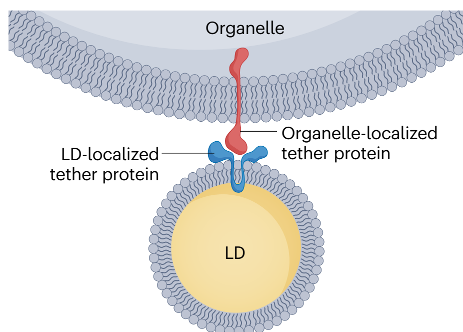
Although the CYTOLD and ERTOLD terms provide the basic framework for discussing LD protein targeting, additional terms have been recently described to further define how different types of proteins interact with the ER and LDs[84]. These newly introduced terms include a category for proteins that do not directly engage the LD lipid surface, but indirectly associate with LDs by attaching to another LD surface protein (denoted indirect-targeted-to-LDs, INTOLD). Another category is introduced for proteins that are permanently anchored to the ER via integral membrane domains, but contain cytoplasmic facing motifs that bind to the LD surface (denoted as tethered-to-ER-and-to-LDs, TERTOLD). Such TERTOLD proteins would include seipin, which does not leave the ER membrane despite tight LD binding. Finally, recent evidence suggests that secreted proteins with an ER signal sequence can target to LDs under some circumstances. Under conditions of lipid synthesis, apolipoprotein E (APOE) avoided translocation into the ER and rerouted to LDs via ER–LD connections, similar to targeting of ERTOLD proteins[85]. The LD proteome contains multiple proteins with putative signal sequences[3], suggesting that targeting of secreted proteins to LDs may be a general phenomenon. These terms represent additional layers of nomenclature as our understanding for how proteins target to LDs becomes more clear.

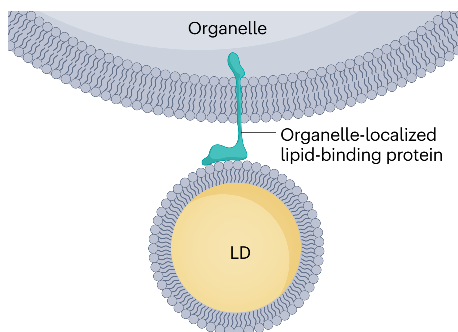
How does conditional targeting of proteins to LDs influence metabolism? Recruiting proteins to LDs enables their participation in various aspects of cellular metabolism, including in lipid synthesis such as by the glycerol-3-phosphate acyltransferase 2 (GPAT2, which catalyses triglyceride synthesis) or CCT (involved in PtdCho synthesis), in lipolysis (for example, ATGL) or even transcriptional regulators such as the transcription factor Mlx that regulates cellular metabolism (see above)[46]. As noted above, proteins enriched at ER–LD interfaces such as seipin have key roles in neutral lipid storage and their loss perturbs LD biogenesis and also drives lipodystrophy and disease[86] (Box 2). Thus, the LD surface in any cell or tissue type can be decorated by dozens of proteins engaged in distinct metabolic or functional roles in cell physiology.

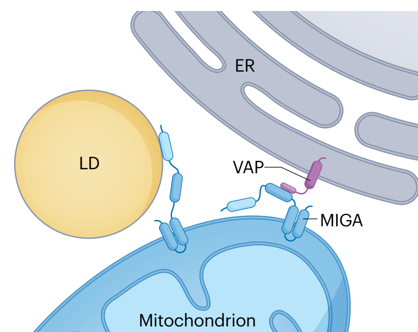
LD intercellular and intracellular heterogeneity

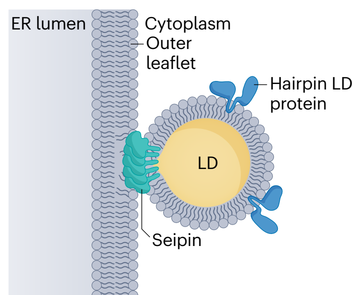
LDs emerge from the ER network and, as such, exhibit an intimate physical connection with their mother organelle. Lipidic bridges connecting LDs and the ER have been observed in electron microscopy and probably serve as conduits for the flow of neutral lipids from the ER into the LD as the droplet expands; this is supported by experiments suggesting triglyceride flow from ER to LDs as they mature[22]. Similarly, LD number, size and spatial arrangement within cells provide functional context for LDs in cell physiology. A classic example is the mature white adipocyte, which contains a single (unilocular) LD that comprises the bulk of the cytoplasm[87] (Fig. 2c). With its smaller surface area-to-volume ratio, it is an ideal long-term storage depot for triglyceride that can be mobilized by ATGL or other lipases during sustained fasting. By contrast, brown adipocytes feature smaller and more numerous LDs that are interspersed in the cytoplasm and often form close functional contacts with mitochondria[88]. In the heart, LDs that form in response to fasting line up in a row between sarcomeres, again interspersed with mitochondria[89]. A similar arrangement of LDs was observed in skeletal muscle, but only early on in postnatal development (stage P1), when LDs were more abundant than later on in development[90]. A heterogeneous LD distribution at the organ level can be observed in the liver. Cells in the nutrient-rich region of a liver lobule near the hepatic artery and portal vein actively oxidize lipids and have few LDs, whereas cells in the nutrient-poor region near the central vein have lower levels of mitochondrial respiration, increased lipid synthesis and increased LD content[91]. Thus, LD association with other organelles can influence cellular metabolism, as discussed below.

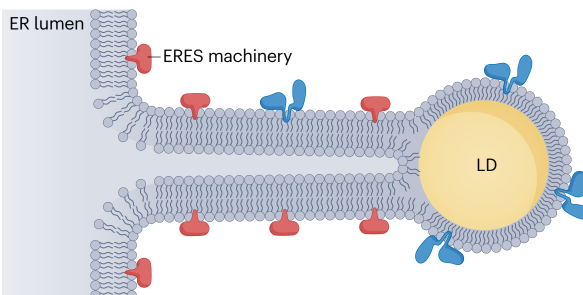
Although LD biogenesis appears to be a lipid-driven reaction, proteins can influence when and where LDs emerge. Biogenesis factors such as seipin closely control the spatial localization of LD biogenesis in the ER network, and thus the spatial arrangement of LDs within cells. For example, the forced relocalization of seipin to the nuclear envelope induced the relocalization of LD biogenesis sites to the nuclear surface, indicating that LD biogenesis is impacted by the presence of seipin[22]. LDs can also cluster or accumulate in specific regions of the cell interior in response to nutrient cues. For example, under glucose exhaustion, yeast cells will exhibit intense clustering of LDs at the interface between their nucleus and the vacuole (the yeast lysosome), a membrane contact site known as the nucleus–vacuole junction[92–94]. In line with this, specific proteins decorate nucleus–vacuole junction-associated LDs including lipid droplet organizing protein 16 (Ldo16), Ldo45,

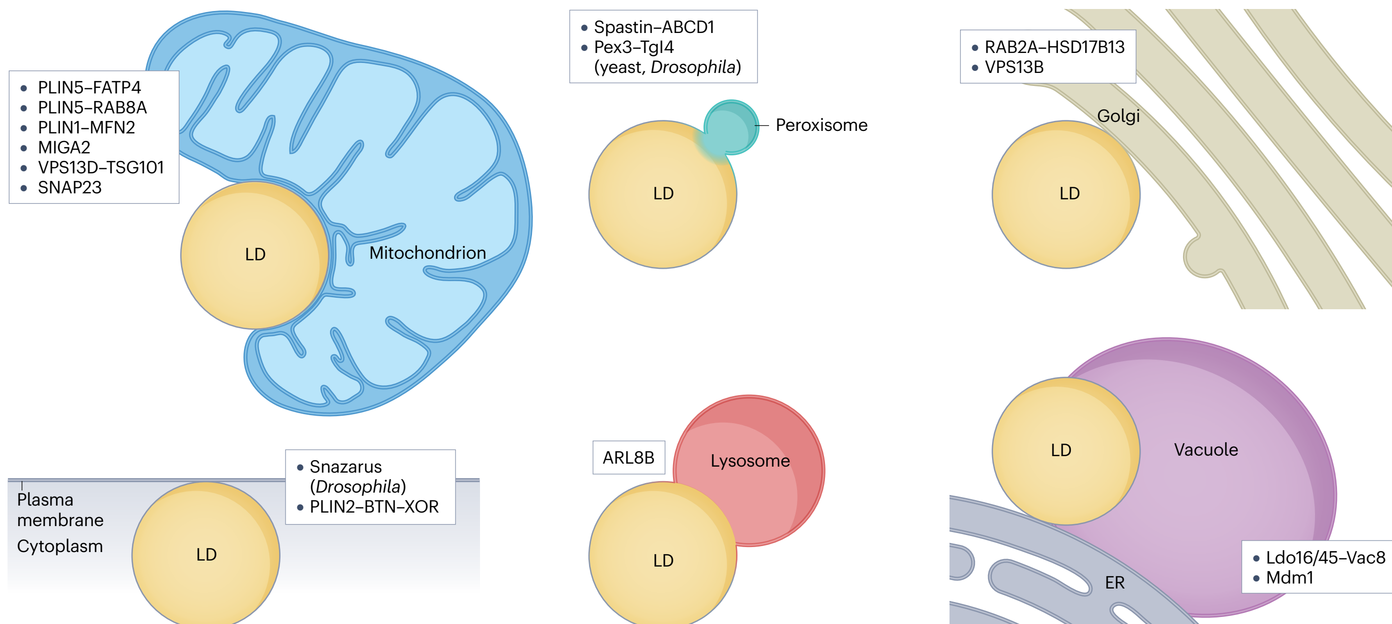












pleiotropic drug resistance protein 16 (Pdr16) and mitochondrial distribution and morphology protein 1 (Mdm1), which influence LD turnover and LD biogenesis, respectively[94–96].

LD spatial organization can be dictated by where along the ER network LDs emerge, but also by motor proteins that influence their movement along the cytoskeleton. LDs move along microtubules with the assistance of kinesin-1, dynein or type V myosins[30]. Microtubule post-translational modifications can regulate LD distribution in response to environmental cues. For example, activation of the energy sensor 5′-AMP-activated protein kinase (AMPK) promotes LD dispersion on detyrosinated microtubules, increasing LD association with mitochondria in the cell periphery and promoting fatty acid oxidation under conditions where ATP is low[97]. Actin also influences the spatial arrangement of LDs, and indeed in sarcoma (U-2 OS) cells, local actin polymerization was proposed to reduce LD clustering by increasing the distance between LDs[98]. Thus, although the ER and its associated

Fig. 3 | Heterogeneity of LD–organelle contacts. a–f, Schematics of protein tethers at lipid droplet (LD)–organelle contact sites. Contacts can be mediated by the interaction of an LD protein with a protein at the partner organelle (for example, fatty acid transport protein 1 (FATP1)–diacylglycerol *O*-acyltransferase 2 (DGAT2) at the endoplasmic reticulum (ER)–LD interface; perilipin 5 (PLIN5)–FATP4 at the LD–mitochondria interface) (**a**). Alternatively, contacts can be mediated by a lipid binding protein, such as the ER protein multiple C2 and transmembrane domain-containing protein 2 (MCTP2), which binds lipids at the LD surface (**b**). Complex interactions of a tether protein with proteins and lipids on multiple organelles can lead to multiway interactions among organelles, shown here for the mitochondrial protein mitoguardin 2 (MIGA2), which has an LD-targeting motif and interacts with VAMP-associated proteins (VAPs) on the ER (**c**). Two types of ER–LD contact sites have been described: LDs contact the ER during LD biogenesis in a process dependent on seipin oligomers (**d**), whereas ER–LD contacts subsequent to LD budding and scission are formed based on membrane bridges that use ER exit site (ERES) protein machinery (**e**). Examples of various LD–organelle contacts and the protein machinery linked to these contacts (**f**). See Table 1 for more detailed discussion of individual tethers. Arf1, ADP-ribosylation factor 1; COPI, coat protein complex I; HSD17B13, 17-β-hydroxysteroid dehydrogenase 13; Ldo16/45, lipid droplet organizing protein 16/45; Mdm1, mitochondrial distribution and morphology protein 1; Pex3, peroxisomal biogenesis factor 3; SNAP23, synaptosomal-associated protein 23; Tgl4, triacylglycerol lipase 4.

proteins may govern sites of LD formation, the cytoskeleton provides conduits for LDs to move within cells, enabling metabolic uses for other organelles. This is also true during organismal development, for example, during *Drosophila* embryogenesis, where LDs are transported from nurse cells into the oocyte by LD–microtubule adaptor proteins that move using microtubule motors proteins[99]. However, many open questions remain regarding the regulation of LD spatial distribution by adaptor proteins and motors in specialized cell types and in response to various physiological conditions.

Heterogeneity in LD–organelle interactions

LDs make physical contacts with nearly every membrane-bound organelle in the cell (Fig. 3). In yeast, split fluorescent reporter systems that fluoresce only when two membranes are in close proximity (<10 nm) have been used to detect contacts among LDs with the ER, mitochondria, peroxisomes, vacuole and plasma membrane[100,101]. In mammalian cells, multispectral imaging of six organelles simultaneously suggested that most LDs remain in close proximity to the ER for sustained periods of time, whereas LD interactions with organelles including mitochondria, peroxisomes, lysosomes and Golgi are far more transient, lasting only seconds[20]. Recent work using dimerization-dependent fluorescent proteins or fluorogen-activated bimolecular complementation has confirmed the presence of bona fide MCSs between LDs with ER, mitochondria, peroxisomes, lysosomes and the plasma membrane in mammalian cells[102,103]. MCSs are typically defined as membrane proximity in the absence of fusion. For LD–ER (LiDER) contact sites, membrane bridges consisting of elongated tubes of ER have been observed; the tip of the ER bridge that is continuous with the LD monolayer represents a hemifused state[21,28,104]. These hemifusion structures are probably unique to LD–organelle contact sites, and are facilitated owing to higher fusogenic properties of the LD phospholipid monolayer membrane compared with a bilayer[31]. Membrane bridges have also been observed at LD–peroxisome contact sites in yeast and plants[105,106], but whether these are a general feature of LD MCSs remains to be determined.

Regardless of their structure, LD–organelle MCSs are mediated or facilitated by one or more tether proteins at the organelle interface (Fig. 3a–c). A tether protein is defined as a protein that localizes to a contact site, binds to proteins or lipids of the opposing organelle at the contact site, and is functionally active at the contact site[107]. MCSs can be regulated rapidly on a timescale of minutes by post-translational modifications (such as phosphorylation) to tether proteins, or on the timescale of hours to days through changes in the expression level of tether proteins[108]. Recently, much progress has been made in identifying tether proteins at LD–organelle MCSs, especially at ER–LD and LD–mitochondria contact sites (Table 1), whereas the machinery is still largely unknown for other LD-based MCSs. A systematic analysis of proteins that localize to and/or affect MCSs in yeast identified 59 LiDER and 37 plasma membrane–LD (pCLIP) proteins; however, it is not yet clear which of these proteins act directly as tethers versus affecting MCSs through alternate mechanisms[109]. LD–organelle MCSs have been implicated in many functions, including LD biogenesis, interorganelle lipid and protein trafficking, lipid signalling and LD turnover. The regulation and function of each LD–organelle MCS is reviewed below.

LD interactions with the ER

LDs form MCSs with organelles of the secretory pathway, including the ER, Golgi and plasma membrane. Of these, ER–LD contacts are by far the best studied. Emerging evidence indicates the presence of two subtypes of ER–LD contacts: membrane continuity that occurs during LD biogenesis, and membrane bridges that form between mature, detached LDs and the ER. Contact sites that form during LD biogenesis are primarily regulated by the multimeric seipin protein complex (Fig. 3d). Current models suggest that ring-shaped seipin multimers of 10–12 subunits initially form a closed cage that promotes triglyceride phase separation, subsequently switching to an open conformation to enable lipid transfer and LD budding[23,110,111]. Seipin has been implicated in the transport of both lipids and proteins into nascent LDs[21,22,112]. However, seipin also seems to prevent premature transfer of certain LD proteins. 'Late' ERTOLD proteins are proteins that traffic from the ER to a subset of LDs at a stage subsequent to LD biogenesis and budding, leading to continued LD expansion and maturation[28]. Knockdown of seipin enhanced LD targeting of the acyltransferase protein GPAT4, which is dependent on 'late' ERTOLD, suggesting that seipin selectively gates the entry of proteins to LDs during biogenesis[112]. Thus, the early and late targeting pathways — hypothesized to occur at distinct types of ER–LD contact sites — are probably an important source of LD heterogeneity. A screen for factors that regulate the late ERTOLD pathway in *Drosophila* cells identified membrane fusion machinery including soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), Ras-related protein RAB1 and multiple ER exit site proteins[112,113] (Fig. 3e). Similarly, previous work in mammalian cells identified the NRZ–SNARE–RAB18 complex as an ER–LD tether[114–116], although this machinery may be cell-type specific[117]. The ADP-ribosylation factor 1 (Arf1)–coat protein complex I (COPI) machinery known for its role in vesicle trafficking has also been implicated in the formation of ER–LD connections and in protein targeting to LDs[118,119]. Protein targeting via the Arf1–COPI machinery is specific, as LD localization of GPAT4 but not lipid storage droplet 1 (Lsd1) was impaired by knockdown of multiple COPI components[119].

Table 1 | LD–organelle tethers

Protein names[a]	Description
ER–LD contacts	
Seipin (Sei1 (also known as Fld1) in yeast)	Key protein in LD biogenesis; facilitates ER-to-LD lipid and protein transfer[21,22,193,194]
Ldb16 (yeast)	Seipin interaction partner at ER–LD contact sites[193,194]
LDAF1 (also known as promethin) (Ldo16/Ldo45 in yeast)	Seipin interaction partner at ER–LD contact sites[93,195,196]
RAB18	LD-localized small GTPase that induces ER–LD contacts in adipocytes[115]
NAG–RINT1–ZW10 (NRZ) complex	The ER-localized NRZ tethering complex interacts with SNARE proteins (syntaxin 18, USE1, BNIP1) and RAB18 to promote LD growth[114]
DFCP1	DFCP1 interacts with the RAB18–ZW10 complex to mediate ER–LD contacts[116]
FATP1–DGAT2	The ER-localized acyl-CoA synthetase FATP1 interacts with diacylglycerol acyltransferase DGAT2 on LDs to promote triglyceride synthesis and LD expansion[122]
HILPDA–DGAT2	The ER-localized hypoxia-inducible lipid droplet-associated protein HILPDA interacts with diacylglycerol acyltransferase DGAT2 on LDs to promote lipid storage in hepatocytes[123]
SNX14 (Mdm1 in yeast)	The ER protein SNX14 localizes to ER–LD contact sites and promotes LD biogenesis and growth[124,197]
MCTP2 (Pex30 in yeast)	The ER-shaping protein MCTP2 binds LD phospholipids to promote ER–LD tethering and growth[126,198]
MOSPD2	An ER-localized VAP family protein that mediates LD–ER contacts[125]
ORP2, ORP5, ORP8	Lipid transfer proteins at the ER–LD interface that regulate phosphoinositide levels[62,199]
VPS13A, VPS13C	Bridge-like lipid transfer proteins that localize to ER–LD contact sites and influence LD abundance[120,121]
Lec1 (yeast)	Putative lipid transfer protein that localizes to and increases the number of ER–LD contact sites, modulating sterol distribution[109]
LD–Golgi contacts	
RAB2A–HSD17B13	Golgi-localized RAB2A interacts with HSD17B13 on LDs to promote membrane contacts required for VLDL lipidation and secretion in hepatocytes[132]
VPS13B	Bridge-like lipid transfer protein that localizes to and promotes LD–Golgi contacts[134]
LD–plasma membrane contacts	
Snazarus (*Drosophila*)	ER-associated protein that localizes to ER–plasma membrane contact sites and interacts with LDs to modulate a peripheral pool of LDs[137]
PLIN2–BTN–XOR	PLIN2 on LDs may interact with BTN and XOR at the apical plasma membrane to promote LD secretion in mammary glands[200]
LD–mitochondria contacts	
PLIN5	LD protein that recruits mitochondria via its C-terminal region[89]
FATP4	Mitochondrial acyl CoA synthetase that interacts with PLIN5 to mediate LD-to-mitochondria fatty acid transport in myoblasts[148]
RAB8A	Small GTPase that interacts with PLIN5 to promote LD–mitochondria contacts in myoblasts[149]
PLIN1–MFN2	PLIN1 on LDs interacts with the mitochondrial fusion protein MFN2 to promote membrane contacts and fatty acid oxidation in brown adipocytes[201]
MIGA2	Mitochondrial protein with an LD targeting domain and a domain that binds VAP family proteins in the ER; MIGA2 promotes de novo triglyceride synthesis in white adipocytes[155]
VPS13D–TSG101	The bridge-like lipid transfer protein VPS13D localizes to LD–mitochondria contacts and interacts with TSG101 to mediate ESCRT-dependent LD remodelling and fatty acid transfer[153]
SNAP23	SNAP23 promotes LD–mitochondria contacts in fibroblasts[151]
LD–peroxisome contacts	
Spastin–ABCD1	M1 spastin on LDs interacts with the ABCD1 transporter on peroxisomes to promote LD-to-peroxisome fatty acid trafficking (ESCRT dependent)[161]
Pex3–Tgl4 (yeast, *Drosophila*)	The peroxisome protein PEX3 interacts with the LD-localized lipase Tgl4 to promote LD–peroxisome contacts[159]
LD–lysosome contacts	
ARL8B	LD-localized ARL8B forms a heterotypic complex with lysosome-localized ARL8B to promote contact site formation and lipolysis in macrophages[189]

DGAT2, diacylglycerol O-acyltransferase 2; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; FATP1/4, fatty acid transport protein 1/4; HSD17B13, 17-β-hydroxysteroid dehydrogenase 13; LD, lipid droplet; Ldo16/45, lipid droplet organizing protein 16/45; MCTP2, multiple C2 and transmembrane domain-containing protein 2; Mdm1, mitochondrial distribution and morphology protein 1; MIGA2, mitoguardin 2; MOSPD2, motile sperm domain-containing protein 2; ORP2/5/8, oxysterol-binding protein-related protein 2/5/8; Pex3, peroxisomal biogenesis factor 3; PLIN1/2/5, perilipin 1/2/5; SNAP23, synaptosomal-associated protein 23; Tgl4, triacylglycerol lipase 4; VAP, VAMP-associated protein; VLDL, very-low-density lipoprotein. [a]Mammalian homologue specified unless otherwise indicated.

Other proteins implicated in LD biogenesis or growth at the ER–LD interface include intermembrane lipid transfer proteins VPS13A and VPS13C (refs. 120,121), the fatty acid transport protein 1 (FATP1)–DGAT2 complex[122], hypoxia-inducible lipid droplet protein (HILPDA)–DGAT2 in hepatocytes[123], SNX14 (ref. 124) and motile sperm domain-containing protein 2 (MOSPD2)[125]. Interestingly, some of these MCS proteins may have functions at multiple LD life stages. Multiple C2 and transmembrane domain-containing protein 2 (MCTP2) is an ER-shaping protein with a reticulon homology domain and multiple C2 domains. The reticulon homology domain induces membrane curvature required for LD biogenesis, whereas the C2 domains tether mature LDs to the ER, facilitating continued growth[126]. Most studies of protein and lipid trafficking at the ER–LD interface have documented roles in ER-to-LD transfer. However, a recently published, non-peer-reviewed study used single-molecule tracking of a model LD-targeting protein, LiveDrop, to show bidirectional trafficking of LiveDrop across seipin-mediated ER–LD contact sites[127]. LD-to-ER trafficking is also possible for lipids: Ice2 is an ER protein that facilitates transfer of triglyceride from LDs to the ER for membrane synthesis during exponential growth phases in yeast[128]. Thus, ER–LD MCSs function in bidirectional trafficking of proteins and lipids between the ER and LDs. The importance of ER–LD contact sites to human health is illustrated by Warburg micro syndrome, a neurological disease linked to altered lipid metabolism that results from mutations in several genes including *RAB18* (refs. 129,130). Patients manifest microcephaly, brain and vision abnormalities, and developmental delays. RAB18 is associated with the ER network and growing LDs, and RAB18-depleted cells exhibit LD accumulation, although the precise role of RAB18 in LD biology remains unclear[114,117,131].

LD interactions with the Golgi

In addition to extensive interactions with the ER, LDs form MCSs with the Golgi. Recently, a novel function for LD–Golgi contacts was identified in the lipidation of very-low-density lipoprotein (VLDL) in the liver. Golgi-localized RAB2A interacts with 17-β-hydroxysteroid dehydrogenase 13 (HSD17B13) on LDs; RAB2A depletion or genetic inhibition reduced LD-to-Golgi lipid transfer and impaired VLDL secretion, resulting in reduced serum triglyceride and cholesterol levels[132]. HSD17B13 loss-of-function mutations are associated with a decreased risk of metabolic dysfunction-associated steatotic liver disease[133], indicating the clinical relevance of the LD–Golgi MCS. The lipid transfer protein VPS13B has also been identified as a putative LD–Golgi MCS protein, although the functional relevance of VPS13B-mediated contacts remains to be determined[134].

LD interactions with the plasma membrane

LD–plasma membrane MCSs are among the least well-characterized LD–organelle contacts, and their function is currently unknown. Systematic analysis in yeast revealed 37 proteins that localized to or affected this contact site, including Mdm1 (ref. 109), a contact site protein known to localize to a three-way LiDER–vacuole contact[96]. This study also identified a new family of VPS13-related proteins at the LD–plasma membrane contact site: Hobbit homologues 1 (Hob1) and 2 (Hob2), and cold sensitive fermentation protein 1 (Csf1)[109]. Like VPS13, these proteins may localize to multiple MCSs[135,136], and could have similar roles in lipid transport between membranes. The *Drosophila* homologue of Mdm1, Snazarus, is also part of a triple contact that involves LDs and the plasma membrane: Snazarus localizes to the cell periphery at ER–plasma membrane contact sites and binds to LDs[137]. Interestingly, these three-way contact sites contributed to the biogenesis of a distinct class of LDs at the cell periphery. These peripheral LDs were composed of dietary lipids taken up by the adipose tissue, in contrast to larger cytoplasmic LDs composed of lipids synthesized by de novo lipogenesis[137]. LD contacts with the plasma membrane or caveolae have been observed in mammalian cells using dimerization-dependent fluorescent proteins[102] and electron microscopy[138], but the machinery that mediates these contacts is unknown. Hob1, Hob2 and Csf1 have mammalian homologues (bridge-like lipid transfer protein family members BLTP2 and BLTP1, respectively) but whether they localize to LD–plasma membrane contact sites remains to be determined.

LD interactions with mitochondria

In addition to making contacts with secretory pathway organelles, LDs also interact with mitochondria and peroxisomes. Mitochondria and peroxisomes have multiple roles in lipid metabolism, including in β-oxidation of fatty acids. In yeast, peroxisomes are the major site of β-oxidation. In mammals only β-oxidation of very long-chain fatty acids occurs in peroxisomes, whereas oxidation of medium-chain and long-chain fatty acids occurs in mitochondria. LD–mitochondria contacts have been implicated in both anabolic and catabolic processes. PLIN5 was the first LD–mitochondria contact site protein to be identified[89]. PLIN5 is expressed in oxidative tissues including heart, skeletal muscle, brown adipose and liver[139]. It was initially shown that PLIN5 expression increases in the heart in response to fasting, resulting in the recruitment of mitochondria to the LD surface via the PLIN5 C-terminal domain[89]. Whether PLIN5-mediated LD–mitochondria contacts promote lipid storage or oxidation has been controversial. Studies overexpressing or knocking out PLIN5 have led to contradictory results, with some studies suggesting a role for PLIN5 in promoting neutral lipid synthesis[140–142], whereas others implicate PLIN5 in fatty acid oxidation[143–145]. Whether PLIN5 channels metabolites from mitochondria to LDs for lipid synthesis or channels fatty acids from LDs to mitochondria for oxidation depends on its phosphorylation status and mitochondrial binding partner, which are probably cell-type specific[146]. PLIN5 can be phosphorylated at serine 155 by phosphokinase A, which alters the ability of PLIN5 to interact with the lipase ATGL and its co-activator CGI-58 on LDs[147]. In myoblasts, LD-localized PLIN5 interacts with the mitochondrial acyl-CoA synthetase long-chain fatty acid transport protein 4 (FATP4) to promote LD-to-mitochondria fatty acid transport in response to starvation[148]. This transfer depends on phosphorylation of PLIN5 and on the PLIN5 C-terminal mitochondrial tethering domain. PLIN5 binds fatty acids, but whether it acts as a lipid transfer protein at LD–mitochondria contact sites remains to be determined. Interestingly, knockdown of FATP4 completely prevented the ability of PLIN5 overexpression to stimulate fatty acid transfer but did not abrogate PLIN5-induced LD–mitochondria contact sites, indicating redundancy in tether function[148]. PLIN5 was also shown to interact with RAB8a in myoblasts[149]; it is currently unclear whether RAB8a and FATP4 act at the same contact sites or at distinct populations of MCSs. In contrast to muscle cells, a recent preprint found that expression of phospho-null PLIN5 in the liver promoted LD–mitochondria interactions and triglyceride synthesis, and expression of phospho-mimetic PLIN5 reduced LD–mitochondria contacts and promoted fatty acid oxidation[150]. FATP4 is not highly expressed in liver, suggesting that interaction with an alternate mitochondrial binding partner may reverse the function of phosphorylated PLIN5. Like FATP4, the acyl-CoA synthetase ACSL1 on mitochondria is also implicated in LD–mitochondria contacts, interacting with synaptosomal-associated protein 23 (SNAP23) on LDs in hepatocytes[151,152]. This indicates that ACSLs may have a general role in metabolic channelling at membrane contact sites.

The large lipid transfer protein VPS13D also promotes LD-to-mitochondrial fatty acid trafficking in response to starvation in embryonic kidney (HEK293) cells[153]. Localization of endogenous VPS13D revealed that this protein localizes to the LD–mitochondria interface; targeting to the LD occurs via two amphipathic helices in the VPS13_C domain, whereas the N-terminal region targets mitochondria[153]. Intriguingly, the VPS13D VAMP-associated protein (VAP) domain was found to interact with TSG101, a component of the endosomal sorting complex required for transport (ESCRT) machinery[153]. The ESCRT complex remodels membranes during cytokinesis, endosome maturation, autophagy, membrane repair and viral budding[154]. The canonical role of ESCRTs is to facilitate inverse budding reactions on negatively curved membranes, but the ESCRT proteins charged multivesicular body protein 6 (CHMP6), CHMP4B and CHMP1B, and vacuolar protein sorting-associated protein IST1 also mediate membrane-shaping reactions on positively curved membranes[154]. Knockdown of any of these ESCRT components, or of ALIX or TSG101, reduced LD-to-mitochondria fatty acid trafficking during starvation[153]. These data, together with electron microscopy imaging showing budding or tubulation of LDs decorated with TSG101, led to a model in which VPS13D recruits ESCRT machinery to LD–mitochondria contact sites to mediate membrane remodelling, facilitating fatty acid transfer by VPS13D. As this mechanism was discovered in cells that do not express PLIN5, it is likely that PLIN5–FATP4 or PLIN5–RAB8a and VPS13D–TSG101 act at distinct LD–mitochondria MCSs; whether both types can be present in the same cell type remains to be determined. Finally, in white adipocytes, mitoguardin 2 (MIGA2) is the main LD–mitochondria contact protein. MIGA2 is a mitochondrial outer membrane protein that contains an LD-targeting amphipathic helix and also bridges to the ER through interaction with the ER tether proteins VAP-A and VAP-B[155]. MIGA2 has a hydrophobic cavity that can bind phospholipids and fatty acids and transfers phospholipids between membranes in vitro[156,157]. In white adipocytes, MIGA2 links de novo lipogenesis in mitochondria with triglyceride synthesis in the ER and storage within LDs[155]. Interestingly, knocking out MIGA2 in preadipocytes prevented LD expansion and reduced PLIN1 expression, suggesting an important role for MIGA2 in adipocyte differentiation[155].

LD interactions with peroxisomes

LD interactions with peroxisomes have been observed in yeast, plants and mammals. Peroxisomes have diverse roles in lipid biosynthesis and catabolism, as well as in the metabolism of reactive oxygen species[158]. Extensive LD–peroxisome contacts were observed in *Saccharomyces cerevisiae* cultured in oleic acid-rich medium, and biochemical fractionation of LDs resulted in co-purification of multiple peroxisomal β-oxidation enzymes, suggesting that these contacts function in LD-to-peroxisome fatty acid transfer[105]. A recent study identified yeast peroxisomal biogenesis factor 3 (Pex3) as a peroxisomal transmembrane protein that mediates homotypic peroxisome–peroxisome contacts, as well as peroxisome–LD contacts, through an interaction with the LD-localized triacylglycerol lipase 4 (Tgl4)[159]. In plants, LD–peroxisome contacts have been implicated in lipid mobilization in seedlings. Here, the peroxisome-localized E3 ubiquitin ligase MIEL1 ubiquitinates the LD coat protein oleosin-1 (OLE1), targeting it for degradation and promoting lipid mobilization[160]. The LD-localized triacylglycerol lipase SDP1 and peroxisomal long-chain fatty acid import protein 2 (PXA1) were proposed to function at this contact site[160], but whether any of these proteins act as true tethers remains to be determined. In mammalian cells, the AAA ATPase M1 Spastin localizes to LDs and interacts with the fatty acid transport protein ABCD1 on peroxisomes to form MCSs[161]. In addition, M1 Spastin recruits the ESCRT proteins IST1 and CHMP1B to LDs through its MIT domain, and knockdown of either M1 Spastin or CHMP1B reduced LD-to-peroxisome fatty acid trafficking, suggesting that ESCRTs function in LD remodelling and fatty acid trafficking at multiple LD–organelle contact sites. Spastin is implicated in hereditary spastic paraplegia and expression of M1 Spastin carrying a Lys388Arg mutation commonly found in hereditary spastic paraplegia caused reduced LD–peroxisome contacts, reduced LD-to-peroxisome fatty acid trafficking and increased peroxidated lipids within LDs[161]. This highlights the relevance of LD–peroxisome contacts in preventing accumulation of lipid peroxides, which neurons may be especially sensitive to.

Intriguingly, ER–LD, LD–mitochondria and LD–peroxisome contacts can form higher-order structures that have been implicated in inflammatory lipid signalling[162]. A study in bone marrow-derived mouse macrophages found that LDs responded more strongly to activation with lipopolysaccharide and interferon-γ than other organelles[162]. Mitochondria–ER–LD clusters formed first, followed by peroxisome–ER–LD clusters, and finally mitochondria–ER–peroxisome–LD clusters[162]. MIGA2-dependent mitochondria–ER–LD clusters supported the trafficking of fatty acids from the ER and mitochondria to LDs in activated macrophages, whereas LD–peroxisome interactions were required for LD lipolysis and prostaglandin synthesis[162]. LDs are stores of arachidonic acid, the precursor for the key lipid mediator prostaglandin E2 (PGE2)[50]. Depletion of the LD–peroxisome tether ABCD1 reduced both arachidonic acid release from triglyceride stores and PGE2 synthesis[162]. Together, these results indicate that prostaglandin synthesis in activated macrophages requires coordinated lipid trafficking among at least four organelles: initial funneling of fatty acids from ER and mitochondria into triglycerides within LDs, followed by lipolysis at the LD–peroxisome interface to liberate arachidonic acid required for PGE2 synthesis.

Mechanisms of LD turnover

LDs interact with the endolysosomal system via multiple mechanisms, including MCSs, in the context of LD catabolism. The turnover of LDs occurs by two general mechanisms: cytoplasmic lipases and autophagy[34,35] (Fig. 1). Autophagy mechanisms include CMA, macroautophagy (also called macrolipophagy when LDs are the substrate) and microautophagy (also known as microlipophagy)[163]. CMA facilitates the turnover of LD surface proteins, whereas cytoplasmic lipases hydrolyse triglyceride and phospholipids, and macrolipophagy and microlipophagy mediate the degradation of both proteins and lipids through lysosomal proteases and lipases[35]. CMA, macrolipophagy and microlipophagy all involve physical interactions of LDs with the endolysosomal system that includes endosomes, autophagosomes and lysosomes. Lipolysis typically involves interactions of LDs with mitochondria and/or peroxisomes as described above, but owing to the interplay between lipolysis and autophagy, it is useful to discuss these mechanisms together. Each turnover pathway can contribute to LD heterogeneity by removing specific proteins or lipids from a subset of LDs, which results in an LD subpopulation of distinct composition and potentially function. Of note, lipolysis and autophagy can also fine-tune LD size, which is highly heterogeneous in different cell types (see above). Indeed, these pathways are proposed to act sequentially in hepatocytes to first shrink LDs through lipolysis before lipophagic engulfment, suggesting LD size control by lipid mobilization is highly regulated[164].

LD turnover by CMA and lipolysis

CMA is a proteolytic mechanism that selectively targets proteins to the lysosome for degradation. LD proteins can also be turned over by other pathways that utilize the proteasome, which has been reviewed elsewhere[33]. In CMA, proteins with a KFERQ motif are bound by the chaperone heat shock cognate 71 kDa protein (HSC70) and threaded into the lysosome for degradation by a multimeric translocation complex that includes the CMA receptor lysosome-associated membrane protein type 2A (LAMP2A)[163]. For example, in response to nutrient deprivation, the LD proteins PLIN2 and PLIN3 were degraded by CMA in fibroblasts in vitro and in the liver in vivo[165]. Blocking CMA-dependent proteolysis of PLIN2 and PLIN3 reduced triglyceride lipolysis by preventing the recruitment of the lipase ATGL to LDs, and also reduced LD turnover by macrolipophagy[165]. This degradation by CMA was dependent on phosphorylation of PLIN2 by AMPK[166]. CMA has roles in both development and ageing. For example, CMA activity increases during adipocyte differentiation, and blocking CMA or expressing a CMA-resistant mutant of PLIN2 altered transcriptional programmes and prevented adipocyte differentiation[167]. This indicates that CMA-mediated degradation of PLIN2 is required for the maturation process that results in unilocular, PLIN1-coated LDs in white adipocytes. Activity levels of CMA were found to change during ageing in a sex-specific and tissue-specific manner: adipose tissues exhibited the most notable sexual dimorphism in CMA, with CMA increasing with age in females, and remaining unchanged or decreasing in males[168]. How these changes impact LD function and lipid metabolism in adipose and other tissues remains to be explored.

Following CMA-mediated remodelling of the LD proteome, cytosolic lipolysis is initiated. ATGL catalyses hydrolysis of triacylglycerol to diacylglycerol and is the rate-limiting enzyme in the lipolysis cascade[34,169]. Diacylglycerol is then hydrolysed to monoacylglycerol by hormone-sensitive lipase, followed by the action of monoacylglycerol lipase, resulting in glycerol and free fatty acids[34]. Intriguingly, ATGL acts preferentially on large LDs, releasing fatty acids that can then be re-esterified into small, nascent LDs, thereby contributing to LD heterogeneity[170,171]. In hepatocytes, lipolysis and macrolipophagy and microlipophagy (see below) occur sequentially, with cytoplasmic lipases acting on large LDs and autophagy breaking down small LDs that remain after lipolysis[164]. Biochemical fractionation to separate large and small LDs revealed their unique protein signatures: large LDs were enriched for ATGL, whereas small LDs were enriched for autophagic and lysosomal markers such as LC3 and LAMP2A[164]. These two populations also had distinct lipid compositions. Although they contained near-equivalent levels of triglyceride and steryl ester relative to total lipid abundance, diacylglycerol was enriched in the smaller LDs, as expected given that these LDs had previously undergone lipolysis by ATGL, and the small LDs were also enriched for PtdCho, PtdEth and PtdIns, consistent with having a higher surface-to-volume ratio than large LDs[164]. Thus, in addition to heterogeneity in protein and lipid composition that arises during LD biogenesis, heterogeneity can also be introduced during LD turnover.

LD turnover by macroautophagy or microautophagy

LD proteins and lipids can also be turned over by macroautophagy or microautophagy. Macroautophagy, also referred to simply as autophagy, is a process in which a double-membraned autophagosome forms in response to a range of cues, including nutrient starvation. The autophagosome engulfs cytoplasmic cargo in a selective or non-selective manner, and then fuses with the lysosome, delivering the cargo and inner autophagosome membrane for degradation by lysosomal proteases and lipases[172]. Autophagy was first shown to selectively degrade LDs via lipophagy in hepatocytes cultured in methionine-deficient and choline-deficient medium, and in vivo in the livers of starved mice[173]. Electron microscopy revealed double-membraned autophagosomes engulfing parts of LDs or even entire small LDs, and live-cell imaging revealed dynamic associations of LDs with lysosomes[173]. In selective autophagy, adaptor proteins bind to ubiquitylated proteins on organelle-specific cargo or to protein aggregates, and to LC3 on the autophagosome surface, targeting substrates for degradation by autophagy[172]. The ubiquitin-binding protein spartin was recently identified as a selective autophagy receptor for LDs[174]. Intriguingly, spartin also has glycerophospholipid transfer activity that contributes to LD turnover[175]. It is not clear in which direction spartin transfers phospholipids during LD breakdown: it could transfer phospholipids from the ER or autophagosome to the LD monolayer to increase surface area and facilitate extrusion of LD fragments, or transfer phospholipids from the LD to the autophagosome membrane to promote autophagosome growth during LD engulfment. Several members of the RAB family of small GTPases mediate targeting of LDs to autophagosomes. RAB7 promotes autophagy of LDs under starvation or alcohol exposure in hepatocytes, and during β-adrenergic stimulation in adipocytes[176–178]. RAB10 interacts with the endocytic adaptor EH domain-binding protein 1 (EHBP1) and the membrane-deforming EH domain-containing protein 2 (EHD2) protein,

Glossary

Birefringence
An optical feature of some materials that allows polarized light to have different refractive indices when it passes through the material, revealing a sometimes rainbow-like diffraction pattern to the polarized light. When used in optical imaging of lipid droplets, smectic liquid–crystalline lipid droplets display a distinctive Maltese cross pattern.

BODIPY
Refers to several fluorescent dyes 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene that partition inside lipid droplets and are used for lipid droplet labelling.

Fluorogen-activated bimolecular complementation
A microscopy technique used in studying organelle contacts where non-fluorescent protein segments are fused to proteins of interest localized on different organelles. When these proteins come together the non-fluorescent segments dimerize, forming a docking site for a fluorescent organic dye molecule that then labels the interaction site.

Glucose exhaustion
A metabolic condition characterized by the depletion of available glucose for cells to utilize in metabolism.

Macrophage foam cells
A pathogenic cell type that accumulates in cardiovascular disease when macrophage cells absorb excess LDL particles and accumulate intracellular lipid droplets rich in cholesteryl esters.

NRZ–SNARE–RAB18
A tethering complex mediated by RAB18 at the lipid droplet interacting with the endoplasmic reticulum-localized NAG–RINT1–ZW10 (NRZ) complex and their associated SNAREs.

Phase separation
The process where a mixture demixes into two distinct liquid phases, driven by interactions between molecules.

Smectic
A phase of a liquid crystal where molecules are arranged in layers.

recruiting autophagosomes and mediating extension of the autophagic membrane around the LD[179].

Highlighting the importance of lipophagy in disease, spartin is mutated in Troyer syndrome, a complex form of spastic hereditary paraplegia[180]. It has long been thought that neurons do not readily form LDs[181], but expression of a dominant-negative spartin construct in neurons in vivo caused accumulation of LDs without affecting triglyceride synthesis[174]. This suggests that LDs constitutively form in neurons but have a higher autophagic turnover rate than in other cell types, making neurons uniquely vulnerable to defects in lipophagy. Lipophagy is also crucial in macrophage foam cells. LDs in macrophages are enriched in steryl esters, and lysosomal acid lipase was found to mediate a large fraction of total steryl ester hydrolysis following autophagy of LDs[182].

Finally, microlipophagy is another type of autophagy that can break down LD proteins and lipids. In microautophagy, organelles are targeted directly to the lysosome without an autophagosome intermediate. Microautophagy of LDs was first observed in yeast where it requires the core autophagy machinery and ESCRT components[183–185]. Glucose restriction was shown to cause a striking phase separation between liquid-ordered microdomains enriched in sterols and liquid-disordered microdomains in the yeast vacuole membrane[186]. In response to glucose restriction, AMPK interacts with the class III PI3K complex regulator Atg14p, causing its redistribution to liquid-ordered microdomains on the vacuole membrane, where LDs are then internalized by microlipophagy[184,187]. Mammalian microlipophagy was recently discovered in hepatocytes[188]. Live-cell imaging revealed transient, ‘kiss-and-run’ encounters between LDs and lysosomes that transferred entire LDs or portions thereof into the lysosome, and electron microscopy identified injection of lipids from LDs directly into the lysosome at sites of contacts between the organelles[188]. The small GTPase ARL8 was identified as an LD–lysosome contact protein required for microlipophagy in macrophages[189]. The GDP-bound state of ARL8 associates with LDs, whereas the GTP-bound state associates with lysosomes; the two states form a complex at the organelle interface that is required for lysosomal lipolysis of LDs and may be the predominant lipolytic pathway in macrophages[189].

Conclusions and future perspectives

LDs are remarkable organelles with functions in energy storage, metabolic channelling, lipid signalling and protection against lipotoxicity. An emerging theme is that structural and functional heterogeneity underlies the ability of LDs to contribute to these various aspects of cell physiology. This heterogeneity occurs in both space and time, at the level of cells, tissues and organisms. Structurally, LDs are heterogeneous in lipid and protein composition[2–4]. The LD core typically contains an amorphous mixture of triglyceride and steryl esters, but conditions that alter the triglyceride:steryl ester ratio lead to the phase transition of the steryl esters into a smectic liquid–crystalline phase[55–57]. This phase transition in turn affects the recruitment of proteins to the LD surface[56], with potential implications for lipid channelling and interactions with other organelles[57,190]. LD lipid and protein composition is a two-way street, with LD surface proteins affecting the composition of the LD core and phospholipids. Spatially, heterogeneity can occur at the cellular or organ level. LDs within the same cell can have distinct lipid and protein compositions and can exist as subpopulations that interact with other organelles. Whether these represent stable LD subpopulations or temporal intermediates remains an open question in many circumstances. It is likely that LD subpopulations can convert over time, as part of the normal maturation of an LD during its life cycle (Fig. 1), during development (for example, during adipocyte differentiation), or in response to conditions such as changes in nutrient availability or inflammation. Excitingly, recent studies have identified pools of nuclear LDs distinct from cytoplasmic LDs, indicating a previously unappreciated level of intracellular LD spatial compartmentalization that requires additional study[191,192]. At the tissue level, cells may be heterogeneous in their LD composition and function; for example, in liver lobules, cells near the hepatic artery versus cells near the central vein contain LDs with strikingly distinct compositions and functions[91]. How LD heterogeneity arises during LD biogenesis, is maintained and changes throughout LD maturation in different cell types, during development and in disease contexts, are exciting open questions for the field. Historically, many methods for studying LDs have relied on techniques such as biochemical fractionation, which often averages measurements across all LDs. As new methods emerge for studying LD heterogeneity in situ (Box 1), we are optimistic that it will become possible to answer these questions and to understand how LD heterogeneity facilitates the many functions of LDs, in health and disease.

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Competing interests

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