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Dynamics and functions of lipid droplets

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Abstract

Lipid droplets are storage organelles at the centre of lipid and energy homeostasis. They have a unique architecture consisting of a hydrophobic core of neutral lipids, which is enclosed by a phospholipid monolayer that is decorated by a specific set of proteins. Originating from the endoplasmic reticulum, lipid droplets can associate with most other cellular organelles through membrane contact sites. It is becoming apparent that these contacts between lipid droplets and other organelles are highly dynamic and coupled to the cycles of lipid droplet expansion and shrinkage. Importantly, lipid droplet biogenesis and degradation, as well as their interactions with other organelles, are tightly coupled to cellular metabolism and are critical to buffer the levels of toxic lipid species. Thus, lipid droplets facilitate the coordination and communication between different organelles and act as vital hubs of cellular metabolism.

Lipids, such as fatty acids, are essential building blocks of cellular membranes and mediators of signalling. Fatty acids are also key molecules for energy storage and production in cells. However, if deregulated, lipids can become toxic and ultimately trigger cell death, for example, through prolonged activation of signalling pathways such as the unfolded protein response (UPR)[1]. Perceived as mere cytoplasmic inclusions of fat for a long time[2], lipid droplets have emerged in recent years as fully entitled organelles with key functions in lipid and energy homeostasis[3].

Ubiquitous in cells, lipid droplets have a unique ultra-structure, consisting of a core of neutral lipids encircled by a phospholipid monolayer that is studded with integral and peripheral proteins (Box 1). Lipid droplets are highly dynamic organelles, alternating

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Author contributions

Both authors researched data for the article, contributed to discussion of the content, wrote the article and reviewed and edited the manuscript.

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between periods of growth and consumption (through enzymatic hydrolysis mediated by lipases (lipolysis) or through a selective form of autophagy (lipophagy); see Supplementary Box 1). These processes closely reflect cellular metabolism and cycles of nutrient availability: lipids stored during conditions of nutrient surplus are mobilized for energy production during starvation or for phospholipid synthesis during high demand of membranes. Lipid droplets also buffer cellular amounts of potentially toxic lipids and have prominent roles in preventing lipotoxicity and oxidative stress.

During their life cycle, lipid droplets establish contacts with several other cellular organelles. First and foremost, they make contacts with the endoplasmic reticulum (ER), an organelle that supplies lipid droplets with most of their constituent molecules and has a central role in their biogenesis. Lipid droplets also make contacts with peroxisomes, mitochondria and lysosomes (vacuoles in yeasts). All these interactions are tightly orchestrated and essential for the normal life cycle of lipid droplets and their diverse functions.

In this article, we review recent advances on the mechanisms of lipid droplet biogenesis from the ER as well as their functions. In particular, we focus on how the lipid droplet life cycle is affected and regulated through interactions with other cellular organelles.

Lipid droplet biogenesis

Despite exciting developments in recent years, the mechanisms of lipid droplet biogenesis are still poorly understood. Lipid droplet assembly involves multiple steps and occurs in the ER, an organelle with which lipid droplets maintain a privileged relationship throughout their life cycle (FIG. 1).

Neutral lipid synthesis and lens formation.

The first step in lipid droplet biogenesis is the synthesis of neutral lipids, most commonly triacylglycerols and sterol esters. These result from the esterification of an activated fatty acid to diacylglycerol or a sterol (such as cholesterol), respectively. Different enzymes are involved in each case, all localized primarily to the ER. Sterol esters are made by acyl-CoA:cholesterol *O*-acyltransferases (ACAT1 and ACAT2; Are1p and Are2p in yeast), whereas triacylglycerols are the product of diacylglycerol acyltrans-ferases (DGAT1 and DGAT2; Dga1 and Lro1 in yeast). Yeast cells lacking these four enzymes are devoid of lipid droplets[4,5]. Although under optimal laboratory conditions these cells grow at normal rates, they are extremely sensitive to various stresses, highlighting the roles of lipid droplets in cell physiology (see also below)[6–8]. Expression of any of these enzymes individually is sufficient for lipid droplet formation in yeast, indicating that assembly of these organelles can be triggered by either triacylglycerols or sterol esters (but triacylglycerol-stimulated lipid droplet assembly has been studied in more detail both in yeast and in higher eukaryotes).

At low concentrations, neutral lipids are dispersed between the leaflets of the ER bilayer. As their concentration increases, neutral lipids eventually coalesce, forming an oil lens in a process of demixing. In model membranes, this transition occurs when the triacylglycerol concentration is in the range of 5–10 mol% (REFS[9,10]). These and other studies indicate that simple principles of physical chemistry explain lens formation, with the demixing of neutral

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lipids being energetically favoured by reducing their interaction with other membrane components such as phospholipids or proteins[11]. Consistently, no proteins have been thus far directly implicated in lens formation. Whether neutral lipid lenses form randomly throughout the ER or whether preferential sites for their formation exist is still unclear.

These early lipid droplet intermediates have been difficult to study as they are likely short-lived and very small. However, recent studies in yeast combining a de novo lipid droplet biogenesis assay relying on regulated expression of Lro1 with electron tomography detected, for the first time, lens-like structures embedded in the ER bilayer[12]. These lenses, ranging between 30 and 60 nm in diameter, were detected only soon after Lrol induction, consistent with the lenses being early intermediates. Protein-based fluorescent reporters targeting early lipid droplet intermediates also became available recently[13,14]. These appear to be more sensitive than conventional neutral lipid dyes, but whether they detect oil lenses or later stages of the biogenesis process is not yet clear. However, combined with live-cell imaging, these reporters are powerful tools to dissect multiple aspects of lipid droplet formation, such as their spatial and temporal regulation.

Lipid droplet budding.

Expansion of the neutral lipid lens results in the lipid droplet budding from the ER membrane. The budding of secretory vesicles from the ER requires the involvement of membrane curvature-inducing coat proteins, such as COPII[15]. However, these proteins are not involved in budding of lipid droplets. Instead, in vivo work suggested that ER membrane phospholipid composition is critical for lipid droplet budding[16–18]. This observation was further supported by recent in vitro and modelling studies, which also revealed the importance of membrane surface tension, defined by the energy cost of exposing neutral lipids to the aqueous cellular environment[11,19]. In order to minimize the neutral lipid area potentially in contact with aqueous cytosol, surface tension appears to be particularly important for the acquisition of the rounded shape of lipid droplets, whereas phospholipid composition affects budding efficiency mostly through geometric effects: conically shaped molecules, such as diacylglycerol or phosphatidylethanolamine, disfavour budding, whereas molecules with opposite geometry, such as lysophospholipids, promote budding[19,20]. Membrane tension, in addition to affecting budding efficiency, also appears to impose directionality on the budding process, at least in vitro[21]. Phospholipids and/or proteins mask the oil-water interface, consequently affecting surface tension. Thus, differential protein and/or lipid composition between membrane monolayers is sufficient to induce tension imbalances that ultimately determine budding directionality (FIG. 1, inset). Although in a few cases lipid droplets have been detected in the ER lumen[22], in most cases lipid droplet budding occurs almost exclusively towards the cytosol, suggesting that the tension in lipid monolayers of the ER membrane is tightly controlled.

In cells, lipid droplet budding is facilitated by fat storage-inducing transmembrane (FIT) proteins[12], an evolutionarily conserved family of integral ER membrane proteins[23]. Two highly related FIT proteins, FIT1 and FIT2 (also known as FITM1 and FITM2, respectively), are present in mammals; other metazoans and yeast exclusively express FIT2-related proteins[23]. Depletion of FIT proteins in yeast, worm and mammalian cells inhibits

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lipid droplet budding, resulting in the accumulation of neutral lipid lenses embedded in the ER membrane[12]. However, how FIT proteins promote lipid droplet budding remains unclear. Earlier studies showed that purified FIT2 binds to diacylglycerols and triacylglycerols in vitro, suggesting that FITs could promote lipid droplet budding through direct binding to these neutral lipids[24]. Further support for this hypothesis came from studies in yeast showing that the levels of diacylglycerol in the ER, a lipid that disfavours lipid droplet budding, are increased in FIT-deficient cells[20]. Strikingly, lipid droplet budding in FIT-mutant cells was restored when diacylglycerol levels were reduced by additional mutations[20]. These observations implicate FIT proteins in diacylglycerol metabolism. However, recent studies suggest that FIT proteins may affect lipid droplet budding through a different mechanism. Comparative sequence analyses revealed similarities between FITs and lipid phosphate phosphatases[25]. This activity was confirmed in vitro with purified human FIT2, which was active on phosphatidic and lysophosphatidic acid but inactive on other phospholipids[26]. Consistent with the catalytic requirement in vivo, FIT2 active site mutants did not restore proper lipid droplet budding in FIT-deficient yeast and mammalian cells[25,26]. Whether phosphatidic and lysophosphatidic acid are the relevant FIT2 substrates in vivo remains unresolved. In addition to lipid droplet budding defects, FIT-deficient cells also displayed abnormal ER morphology, with frequent membrane clumps[26]. If challenged with fatty acids, *FIT2*-knockout cells showed reduced triacylglycerol levels and a concomitant increase in phosphatidic acid, suggesting that this phospholipid may be a FIT2 substrate in vivo[23,26]. The rates of fatty acid incorporation into other phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, were also reduced. In agreement with these broader impacts on lipid metabolism, constitutive or postnatal ablation of FITs results in either severe metabolic dysfunctions or death in various animal models[12,23,27,28].

Whether FIT2 activity affects tension imbalance between the leaflets of the ER is not known, but the active site of FIT proteins is predicted to be on the luminal side of the ER membrane[24,26]. In contrast to phospholipids, neutral lipids such as monoacylglycerol and diacylglycerol — which may be generated by FIT2 on the luminal side — are capable of flipping rapidly between membrane leaflets. An appealing possibility is that once on the cytosolic side, these neutral lipids are converted back to phospholipids to replenish the cytosolic leaflet of the ER, which is the major source of monolayer phospholipids during lipid droplet formation[26]. Thus, during periods of intense lipid droplet production, it is possible that phospholipid scramblases[29], which maintain phospholipid balance between ER leaflets, are saturated and this critical FIT2 activity is revealed.

In yeast, ER-lipid droplet budding is facilitated by Pln1 (previously known as Pet10), a protein related to the mammalian perilipins[30]. In mammals, this class of proteins has well-established functions in the regulation of lipid droplet consumption through lipolysis[31] (Supplementary Box 1) and may also facilitate lipid droplet biogenesis[32]. Similar to other perilipins, Pln1 is recruited from the cytosol to the lipid droplet mono-layer (see also next section)[30]. For Pln1, lipid droplet targeting occurs at very early stages during the biogenesis process, suggesting that it facilities budding[30]. This suggestion is also supported by the fact that cells lacking Pln1 show a considerable delay in lipid droplet budding[30]. Interestingly, the binding and stabilizing effect of Pln1 on lipid droplets depends on the presence of triacylglycerols, as it does not recognize droplets containing only sterol esters[30]. How

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exactly Pln1 and perhaps other perilipins promote lipid droplet budding is unclear. However, by accessing only the nascent lipid droplet from the cytosolic side, perilipins may facilitate budding by changing the balance of tension between the two membrane monolayers.

Seipin is a widely conserved ER membrane protein required for correct lipid droplet budding[33–35]. In the absence of seipin, lipid droplet formation is delayed[13,36] and there are lower incorporation rates of both proteins and lipids into the lipid droplets that form, resulting in droplets with aberrant morphologies and properties[13,17,34–41]. In addition, in yeast and mammalian cells with seipin mutations, the ER-lipid droplet interface is highly abnormal, suggesting that seipin stabilizes the membrane bridges that normally provide continuity between the two organelles[13,37,38]. Consistent with a function at the ER-lipid droplet interface, seipin is enriched at these regions, as detected by both light and electron microscopy[13,37–39]. Additional clues to seipin function came from recent studies in yeast, which revealed links to the biogenesis of pre-peroxisomal vesicles, structurally distinct organelles that give rise to peroxisomes. Surprisingly, lipid droplet budding in seipin yeast mutants requires Pex30 (REFS[42,43]), an ER membrane protein with well-established roles in peroxisome formation[44]. Normally distributed through the ER in discrete foci, Pex30 concentrates strongly at sites of lipid droplet budding in seipin mutants. Pex30 redistribution is functionally relevant, as simultaneous deletion of seipin and Pex30 inhibits lipid droplet budding and results in neutral lipid accumulation within the ER membrane. This double mutant also shows a strong defect in peroxisome formation. Links between lipid droplet and peroxisome biogenesis are not only seen in seipin mutants but also recently in unperturbed cells[45] (see next section). In addition to defects in lipid droplet and peroxisome budding, cells lacking seipin and Pex30 display abnormal lipid composition, including higher levels of phosphatidylcholine, phosphatidylinositol and diacylglycerol. Interestingly, additional mutations that correct the lipid composition of seipin-Pex30 double mutants restored organelle budding[43]. Consistent with the in vitro data described earlier[19,20], these studies highlight the role of ER lipid composition in lipid droplet budding and suggest that seipin, along with Pex30, may control lipid composition specifically at sites of lipid droplet budding. This control could be achieved through regulation of certain lipid-modifying enzymes, such as glycerol-3-phosphate acyltransferases (GPATs)[46] (FIG. 1). Alternatively, seipin, which forms oligomers[47–49], may bind to and concentrate specific lipids at sites of lipid droplet budding. Consistent with this possibility, cryo-electron microscopy structures of human and fly seipin revealed that the seipin ER luminal domain adopts a β-sandwich fold as observed in other lipid-binding proteins[48,49]. However, the molecular mechanism by which seipin facilitates lipid droplet budding from the ER awaits clarification. The connection between lipid droplet and peroxisome budding should also be further explored.

Lipid droplet growth and maturation.

After budding, lipid droplets expand, which occurs through droplet-droplet fusion (discussed in the section on lipid droplet membrane contact sites below), through transfer of triacylglycerol to lipid droplets via ER membrane bridges or through triacylglycerol synthesis directly on the lipid droplet surface (FIG. 1). Localized synthesis of triacylglycerol is enabled through the relocalization of several enzymes from the ER to the lipid droplet surface[50], with newly made triacylglycerol accumulating in the hydrophobic core. As during

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the budding, the cytoplasmic leaflet of the ER membrane continues to supply the growing droplet with additional phospholipids for the monolayer. Under conditions of very active lipid droplet expansion, new phospholipid synthesis is required to maintain phospholipid homeostasis. For example, stimulation of lipid droplet biogenesis by exogenous fatty acids in fly cells triggers activation of CTP:phosphocholine cytidylyltransferase-α (CCTα), an enzyme producing a rate-limiting intermediate in the synthesis of phosphatidylcholine. Under these conditions, CCTα activation is required to maintain phospholipid homeostasis. Curiously, CCTα is activated directly at the surface of growing lipid droplets, coupling monolayer expansion with phospholipid synthesis[51]. However, in other cell types, CCTα appears to be exclusively nuclear, suggesting that this mechanism is not universal and that coupling between lipid droplet expansion and phospholipid biosynthesis can occur by different means[52,53].

In higher eukaryotes, a population of lipid droplets eventually detaches from the ER. Why, among all the lipids droplets, only a fraction selectively detaches is unclear. Also, the mechanism of membrane fission is not known. It was recently shown that in both insect and mammalian cells, this detachment is reversible, with the re-attachment requiring the COPI coatomer complex[50,54,55]. This complex is recruited to the surface of lipid droplets by the small GTPase ARF1 and its guanine nucleotide exchange factor (GEF), GBF1, and promotes the budding of very small droplets of approximately 60 nm in diameter (‘nano-droplets’) from the existing droplets. The high surface-to-volume ratio of these nano-droplets depletes phospholipids from the monolayer of the ‘mother’ droplet. This depletion results in poor phospholipid coverage of the hydrophobic core and an increase in surface tension, which facilitates lipid droplet fusion with the ER bilayer. How and why COPI facilitates lipid droplets to fuse specifically with the ER is not clear. However, upon fusion, integral membrane proteins such as DGAT2 and GPAT4 are able to diffuse from the ER to lipid droplets, indicating that the membrane bridges between the two organelles have been re-established.

Protein targeting to lipid droplets

Various proteomics-based approaches have determined the complete repertoire of lipid-droplet-associated proteins[56–60]. Although lipid droplet proteomes vary between cell types and depending on the methodology used, a common set ofhigh-confidence lipid-droplet-associated proteins has emerged. This high-confidence lipid droplet proteome consists of 100–150 proteins in a prototypical mammalian cell[60]; in yeast, the numbers drop to 35–40 proteins[61]. Irrespective of the cell types, lipid droplet proteomes are dominated by enzymes involved in lipid metabolism and invariably include members of the peri-lipin family. However, proteins with other functions such as membrane trafficking and protein degradation are also well represented, indicating the diverse set of processes involving lipid droplets. Understanding how these large numbers of proteins influence lipid droplet behaviour and function will be a challenge for many years to come.

Another important challenge is to understand how newly synthesized proteins are targeted specifically to lipid droplets. In contrast to proteins localized to other cellular organelles, lipid droplet proteins lack a specific targeting signal embedded in their sequence. Lipid-

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droplet-associated proteins fall in two main classes. Proteins stably associated with membranes that partition between lipid droplets and the ER belong to class I; proteins recruited directly from the cytosol to the lipid droplet surface define class II (FIG. 2).

Class I proteins.

Proteins of diverse functions can be found among the class I lipid-droplet-associated proteins, from lipid biosynthetic enzymes such as long-chain-fatty-acid-CoA ligase 3 (ACSL3), GPAT4 and DGAT2/Dga1 to proteins involved in ubiquitin-dependent proteolysis such as ancient ubiquitous protein 1 (AUP1) and UBX domain-containing protein 8 (UBXD8; also known as FAF2). Typically, class I proteins associate with membranes through hydrophobic hairpins, which insert midway into lipid bilayers, with both amino and carboxy termini facing the cytosol. These structural elements, by lacking luminal loops or domains typical of polytopic membrane proteins, can be accommodated both in the ER bilayer and lipid droplet monolayer. In some class I proteins, positively charged residues flanking the membrane hairpin are also necessary for efficient lipid droplet localization[62].

Insertion of membrane hairpins typical of class I proteins occurs in the ER, from where the proteins diffuse through membrane bridges to the surface of lipid droplets[50]. Consistent with the ER insertion, class I proteins localize throughout the ER in the absence of lipid droplets[63]. Although the mechanisms of membrane insertion for hairpin-containing proteins have not been extensively investigated, a recent study showed that the ER insertion of UBXD8 requires peroxisomal biogenesis factor 19 (PEX19) and PEX3 (REF[45]) (FIG. 2a), which have well-established roles in the biogenesis of peroxisomal membrane proteins. Upon capture in the cytosol by PEX19, UBXD8 was post-translationally delivered to specific ER domains enriched in PEX3 for membrane insertion[45]. PEX3-enriched ER domains were located close but were distinct from peroxisomes, and it will be interesting to know whether they correspond to sites of lipid droplet and/or peroxisome biogenesis. PEX19 can attach to membranes through farnesylation of a conserved cysteine residue. Interestingly, mutations that prevent PEX19 farnesylation induced UBXD8 mistargeting to peroxisomes[45], suggesting that this post-translational modification confers targeting specificity between lipid droplets and peroxisomes. It remains to be determined whether, in addition to UBXD8, other class I lipid droplet proteins follow the same route to the ER membrane. However, these findings further highlight common steps between the biogenesis of lipid droplets and peroxisomes.

Although the features of class I protein membrane association are well established, they do not explain the enrichment of these proteins at the lipid droplet surface. In fact, many hairpin-containing proteins localize primarily to the ER and do not appear to be particularly enriched in lipid droplets. Thus, how the partitioning of hairpin-containing proteins between ER and lipid droplets is regulated is unclear.

It is possible that some hairpin-containing proteins require active retention in the ER through additional interactions (FIG. 2a). Consistent with this possibility, it has been shown that retention of a pool of UBXD8 at the ER requires its interaction with UBAC2, an ER-resident polytopic membrane protein[64]. Similarly, the GPAT4 membrane hairpin targets lipid droplets much more efficiently than the full-length protein, suggesting that additional domains in the

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protein contribute to its partial retention in the ER[50]. These cases illustrate how ER retention mechanisms offer an important layer of regulation in the targeting of hairpin-containing proteins to the lipid droplet surface. However, for most class I lipid droplet proteins, these regulatory mechanisms are still elusive.

An alternative mechanism to enrich hairpin-containing proteins specifically at the lipid droplet surface is through selective degradation of their ER pool. Studies in both yeast and mammalian cells indicate that such a mechanism operates for at least a subset of class I proteins[60,65]. In both cell types, the ER degradation of class I proteins involves components of Er-associated protein degradation (ERAD)[66,67] (FIG. 2a). Although the best-characterized ERAD substrates are misfolded proteins, several folded proteins are also degraded by ERAD. Once selected, ERAD substrates are ubiquitylated, extracted from the membrane and delivered to cytosolic proteasomes for degradation. Remarkably, ERAD-dependent degradation of class I proteins was triggered by the membrane hairpin in yeast[65] as well as in mammalian cells[60]. It is possible that when in ER bilayers, certain membrane hairpins may present conformation instability, which triggers their recognition by ERAD. By contrast, in the lipid droplet monolayer, they are segregated away from ERAD components and perhaps are in a more favourable environment, avoiding degradation. Although these examples may offer a framework to understand how the partition of hairpin-containing proteins between ER and lipid droplets is regulated, a complete mechanistic understanding of these processes is not yet in reach.

Class II proteins.

Proteins recruited to the lipid droplet surface directly from the cytosol belong to class II (FIG. 2b). For these, various modes of lipid droplet association have been described, such as direct binding to another lipid droplet protein or interaction with the lipid monolayer through a lipid anchor. However, most class II proteins bind to lipid droplets through amphipathic α-helices[68–73]. These motifs are characterized by the segregation of hydrophobic and polar residues to opposite sides of the helix and are thought to bind to membranes displaying phospholipid packing defects, which accommodate the hydrophobic face of the helix in the plane of the membrane[74] (FIG. 2b, inset).

As membrane hairpins are not exclusive to class I lipid droplet proteins, amphipathic helices are also not unique to class II lipid droplet proteins and can be found in a wide range of proteins distributed through various intracellular membranes. Moreover, the amphipathic helices of lipid droplet proteins appear to be highly diverse in various parameters such as amino acid composition or length[71]. Thus, it has been difficult to pinpoint the determinants of their specific targeting to lipid droplets.

Recent molecular dynamics simulations suggest that phospholipid monolayers are particularly prone to display packing defects, specifically under high surface tension[70,75]. In contrast to bilayers, the surface properties of monolayers are easily modified by interdigitating neutral lipids from the lipid droplet core[70,75], leading to the appearance of larger and more persistent packing defects[19,70]. These appear to form the optimal binding site for amphipathic helices containing large hydrophobic amino acids[70], such as the one of the class II protein CCTα. However, studies on the perilipin family member PLIN4 suggest

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that additional mechanisms may also confer specificity of class II protein in lipid droplet binding. Perilipins bind to lipid droplets through a variable number of 11-mer amino acid repeats, which are disordered in the cytosol but fold into an amphipathic helix at the lipid droplet surface[72]. In the case of PLIN4, the amphipathic helix is distinctively long, consisting of approximately 950 amino acids that, once folded, would measure about 140 nm (REF[74]). However, in contrast to CCTa, the PLIN4 amphipathic helix lacks bulky hydrophobic residues. In addition, the polar face of the amphipathic helix has an asymmetrical charge distribution that favours lateral interhelical interactions at the expense of interactions with phospholipids in the monolayer. Together, these features allow PLIN4 to bind directly to neutral lipids and cover portions of lipid droplets by replacing the phospholipid monolayer[71].

Lipid droplet membrane contact sites

In addition to the membrane bridges with the ER, which are established during biogenesis, lipid droplets associate with most cellular organelles (FIG. 3). A recent land-mark study employing multispectral time-lapse imaging of six organelles observed nutrient-regulated (such as starvation and fatty acid treatment) lipid droplet associations with the ER, Golgi, mitochondria, lysosomes and peroxisomes[76]. These interactions likely involve membrane contact sites — regions in which the membranes from different organelles are held in close apposition to facilitate the exchange of lipids, metabolites and ions[77] as well as to regulate organelle division, trafficking and inheritance[76,78]. Protein-based tethering complexes have key roles in establishing and maintaining organelle contact sites[77]. However, for most lipid droplet contact sites, the identity of the tethering complexes, their mechanisms of regulation and their functions remain mostly unknown. In addition, although membrane contacts sites do not typically involve membrane fusion, multiple examples of transient membrane bridges between lipid droplets and other organelles, such as the ER[50,63] and peroxisomes[79], suggest that lipid droplet contact sites might exhibit unique properties. Below, we discuss the current understanding of the molecular basis and function of known lipid droplet membrane contact sites.

Lipid droplet-ER contacts.

In yeast, the lipid monolayer of lipid droplets remains continuous with the ER membrane[63]. In fly and mammalian cells, lipid droplets have often been reported to fully detach from the ER, but an extensive understanding of this process is lacking. Despite the possibility to detach, fluorescence imaging suggests that approximately 85% of lipid droplets remain in contact with the ER in mammalian cells[76]. Electron tomography of human epidermoid carcinoma cells indicated that most lipid droplets exhibit multiple contacts with the ER[37]. However, the membrane profiles at these contacts appear to be thicker and no membrane continuity is observed[37], suggesting that the lipid droplets may completely bud from the ER but remain tethered. Deletion of *BSCL2* (encoding seipin) in human cells disrupted the morphology of ER-lipid droplet contacts and some lipid droplets completely detached from the ER[37]. However, another study found that seipin depletion did not disrupt ER-lipid droplet tethering[13], suggesting that additional proteins contribute to the ER-lipid droplet contacts. In fact, lipid droplet formation was largely normal in cells expressing a seipin

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version lacking all its cyto-plasmic domains, arguing against a putative role of seipin as a tethering factor at the ER-lipid droplet interface[13].

Additional tethering components at the ER-lipid droplet contacts include DGAT2, which pairs with fatty acid transport protein 1 (FATP1)[80], RAB18 together with the NAG-RINT1-ZW10 (NRZ) complex and its associated sNAREs (Syntaxin 18, USE1 and BNIP1)[81] and inheritance of cortical ER protein 2 (Ice2)[82] (FIG. 3). Although deletion of RAB18 affected lipid droplet growth in cultured adipocytes, it had little effect in several other cell lines[81,83], indicating that tethering mechanisms for lipid droplets may be context-specific.

Lipid droplet-nuclear envelope contacts.

Lipid droplets have been observed inside the nucleus in mammalian cells[84] and in yeast[85]. In yeast, it was shown that lipid droplets bud directly from the inner nuclear membrane[85]. The lipid monolayer of the nuclear lipid droplets remains contiguous with the inner nuclear membrane[85]. Furthermore, seipin localizes to the inner nuclear membrane and is necessary for the proper formation of the membrane bridge connecting the inner nuclear membrane and the nuclear lipid droplet[85]. Phosphatidic acid is converted either into diacylglycerol by phosphatidic acid phosphohydrolase 1 (Pah1) for triacylglycerol synthesis or into cytidyldiphosphate diacylglycerol by phosphatidate cytidylyltransferase (Cds1) for phospholipid synthesis. Both Pah1 and Cds1 are present in the inner nuclear membrane, and inhibition of Cds1 promoted nuclear lipid droplet biogenesis[85]. Whether other lipid synthesis genes, such as Dga1 or Lro1, are present in the inner nuclear membrane is unknown.

Nuclear lipid droplets may provide lipids for use in nuclear envelope expansion and may function as a platform for regulating gene expression by scaffolding transcription factors, such as yeast transcriptional repressor of phospholipid biosynthetic genes Opi1, in close proximity to their target genes at the nuclear envelope. Similarly, nuclear lipid droplets in hepatocytes associate with promyelocytic leukaemia nuclear bodies, which function in transcription regulation[84]. Furthermore, mammalian PLIN5 has been observed in the nucleus and functions as part of a transcription regulatory complex that controls mitochondrial gene programmes[86]. However, whether PLIN5 associates with nuclear lipid droplets has not been addressed.

Lipid droplet-lipid droplet contacts.

Lipid droplets in mammalian cells can grow through fusion of two lipid droplets, which is mediated by the cell death-inducing DFFA-like effector (CIDE) family of proteins — CIDEA, CIDEB and CIDEC[87]. Consistent with their role in lipid droplet fusion and growth, depletion of CIDE proteins results in numerous small lipid droplets and their overexpression increases the number of large lipid droplets[88,89]. The CIDE proteins localize to lipid droplets via a carboxy-terminal amphipathic helix and form dimers that diffuse across the lipid droplet surface until encountering another CIDE-positive lipid droplet, which provides an opportunity for the CIDE proteins to form stable trans-organelle oligomers[68,88,89]. Indeed, CIDE proteins are enriched at lipid droplet-lipid droplet contact sites, physically tethering the adjacent organelles[88,89] (FIG. 3). This 'docked' conformation precedes lipid droplet

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fusion. Docking or lipid droplet clustering is also observed in cells expressing low levels of, or even lacking, CIDE proteins, indicating the presence of additional tethering complexes, such as the ubiquitin-dependent AUP1 homodimers[90–92]. These data also raise the possibility that lipid droplet-lipid droplet contacts can serve additional functions beyond fusion. The CIDE oligomers are thought to assemble into a pore or channel that mediates the transfer of triacylglycerol between lipid droplets. Remarkably, triacylglyc-erol transfer invariably occurs from the smaller to the larger lipid droplet, a process proposed to be driven by differences in internal pressure and by surface tension[89].

Detailed analyses of CIDEA indicate that the carboxy-terminal amphipathic helix binds phosphatidic acid[69]. By increasing the local concentration of this cone-shaped lipid, lipid droplet fusion may be promoted[69,93]. Consistent with the importance of phosphatidic acid in lipid droplet fusion, the levels of this lipid directly correlate with fusion efficiency, and a CIDEA mutant that is unable to bind phosphatidic acid fails to induce lipid droplet fusion[17,69]. Although CIDE proteins enable transfer of triacylglycerol between docked lipid droplets, they do not allow for transfer of protein[89]. When lipid droplets shrink, and neutral lipids are transferred to a larger lipid droplet, the fate of the remaining phospholipids and proteins that are not transferred is unknown. In addition, the stoichiometry, size and structure of the putative CIDE pore have not been determined.

CIDE-mediated lipid droplet fusion is regulated by interactions with PLIN1 (REFS[94,95]). In adipose tissue, CIDEC binds PLIN1, a key adipose tissue-specific lipid droplet scaffold protein and signalling integrator that regulates lipid droplet degradation via lipolysis[94,95] (Supplementary Box 1). PLIN1 increases CIDEC-dependent lipid exchange and lipid droplet fusion, possibly by influencing CIDEC oligomerization[94,95]. RAB GTPases are prevalent regulators of membrane dynamics, and many RABs localize to lipid droplets[57,60]. In most cases, the GTP-bound form of an RAB is the active form. Surprisingly, the GDP-bound form of RAB8a appears to be the active form that regulates lipid droplets[96]. GDP-bound RAB8a concentrates at lipid droplet-lipid droplet contacts, directly binds CIDEC and activates CIDEC-mediated lipid droplet fusion in cultured adipocytes[96]. This activity of RAB8a is positively regulated by the GTPase-activating protein (GAP) AS160 (also known as TBC1D4) and negatively regulated by the GEF MSS4 (REF[96]). Finally, CIDEC can be post-translationally regulated by acetylation at Lys56, which is promoted by p300/CREB-binding protein (p300/CBP) and reversed by histone deacetylase 6 in a fatty-acid-sensitive manner[97]. Acetylation increases CIDEC-mediated lipid droplet fusion, possibly by affecting CIDEC stability and/or trafficking[97].

The fusion of lipid droplets facilitates efficient tri-acylglycerol storage, reducing the surface area of the lipid droplet that is exposed to cytosolic lipases for lipolysis (Supplementary Box 1). The physiological importance of CIDE-mediated lipid droplet fusion is underscored by the identification of homozygous *CIDEC* mutations in humans that result in the premature truncation of the protein, causing partial lipodystrophy and insulin-resistant diabetes[98]. Analyses of mice individually lacking CIDEA, CIDEB or CIDEC have also identified extensive metabolic defects associated with the loss of these proteins[87]. The simplest interpretation is that impaired lipid droplet fusion reduces lipid storage and increases

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lipotoxicity (see below). However, the precise connections between CIDE-mediated lipid droplet fusion and disease remain to be deciphered.

Lipid droplet-mitochondrion contacts.

During nutrient deprivation, fatty acids released from lipid droplets through lipolysis or lipophagy (Supplementary Box 1) are used by mitochondria for energy production via β-oxidation and the citric acid cycle (FIG. 4a). The trafficking of these fatty acids likely occurs at contact sites. Consistent with this possibility, the association of lipid droplets and mitochondria increases in response to both nutrient deprivation in cultured cells[76,99–101] and exercise in skeletal muscle[102]. This spatial proximity would reduce the prevalence of cytosolic free fatty acids, preventing lipotoxicity and/or aberrant lipid signalling[99,100] (FIG. 4a). Interestingly, a recent study examining cytoplasmic and lipid-droplet-associated mitochondria in brown adipocytes suggests an opposing model[103], whereby the lipid-droplet-associated mitochondria exhibit reduced β-oxidation and display increased ATP synthesis. Perhaps by supplying ATP for the local fatty acid activation necessary for triacylglycerol synthesis, these mitochondria support the growth and expansion of the associated lipid droplets[103]. In this study, the lipid-droplet-associated mitochondria were elongated and exhibited reduced dynamics[103]. Thus, contacts between lipid droplets and mitochondria could serve as sites for both lipogenesis and lipolysis in different cell types or under different metabolic conditions.

The perilipin family member PLIN5 is highly expressed in oxidative tissues (liver, skeletal muscle, heart and brown adipose tissue), and overexpression of PLIN5 is sufficient to induce a dramatic recruitment of mitochondria to the periphery of lipid droplets[103,104]. The carboxy-terminal portion of PLIN5 is necessary and sufficient for this mitochondrial recruitment activity[103,104], and PLIN5 is a prime candidate for a tether at lipid droplet-mitochondria contacts (FIG. 3). PLIN5 also regulates lipolysis through its interactions with adipose triglyceride lipase (ATGL) and its activator ABHD5 (1-acylglycerol-3-phosphate *O*-acyltransferase)[105]. Whether PLIN5 mediates tethering of lipid droplets and mitochondria directly through an association with a specific mitochondrial protein or with the mitochondrial membrane remains unknown.

In brown adipocytes, PLIN1 and the outer mitochondrial membrane protein mitofusin 2 (MFN2) have been found to interact, potentially functioning as a tethering complex[106] (FIG. 3). The interaction of PLIN1 and MFN2 is stimulated under lipolytic conditions, and knockout of *MFN2* reduces contacts between lipid droplets and mitochondria[106]. However, because MFN2 is required for mitochondrial fusion, the reduction in lipid droplet-mitochondria contacts in *MFN2*-knockout cells could be secondary to altered mitochondrial fission-fusion dynamics. Given the presence of lipid droplet-mitochondria contacts in a wide variety of cell types, many of which do not express PLIN1 or PLIN5, it is likely that additional complexes exist that tether lipid droplets and mitochondria. Moreover, the mechanisms that regulate lipid droplet-mitochondria tethering and couple their interaction to nutrient abundance remain to be discerned.

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Lipid droplet-peroxisome contacts.

Similar to lipid droplets, peroxisomes have central roles in lipid and energy metabolism. In yeast, they are the sole sites of β-oxidation. In humans, most β-oxidation occurs in mitochondria, but peroxisomes are essential for β-oxidation of very-long-chain fatty acids and branched fatty acids, and mice lacking peroxisomes accumulate enlarged lipid droplets in the liver[107]. In addition to the recent indications of shared steps during biogenesis (see above)[42,43,45], membrane contact sites between lipid droplets and peroxisomes have also been described[42,76,108,109], but the molecular basis and the function of these contact sites are unknown. It is tempting to speculate that these sites facilitate the transfer and breakdown of specific fatty acids. In yeast exposed to fatty acids as their sole carbon source, lipid droplets form prominent and stable associations with peroxisomes[108]. Under these conditions, peroxisomes extend processes (termed pexopodia) into the lipid droplet core[108]. Interestingly, the lipid droplet membrane fuses with the outer phospholipid leaflet of the peroxisome bilayer (FIG. 3), perhaps allowing luminal peroxisome enzymes to directly access lipid-droplet-stored triacylglycerol or facilitating protein transfer[108]. In *Arabidopsis thaliana*, peroxisome extensions mediate the transfer of a triacylglycerol lipase sugar-dependent 1 (SDP1) to lipid droplets (referred to as oil bodies or lipid bodies in plants)[79]. Whether the peroxisome-lipid droplet membrane fusion observed in yeast also occurs in metazoan cells is unknown.

Lipid droplet-lysosome contacts.

Both prolonged and brief kiss-and-run interactions between lipid droplets and lysosomes have been observed in mammalian cells[76,110,111]. Recent data implicate these contacts as sites for the degradation of the perilipin family members PLIN2 and PLIN3 via chaperone-mediated autophagy (CMA)[110]. In CMA, substrates containing a pentapeptide motif are recognized by the heat shock cognate 71 kDa protein (HSC70), delivered to the lysosomal surface and translocated into the lumen of the lysosome via the receptor lysosome-associated membrane protein 2A (LAMP2A)-containing multimeric com-plex[112]. Interestingly, mice lacking LAMP2A exhibit liver steatosis, and fibroblasts lacking LAMP2A display increased lipid droplet and triacylglycerol content as well as reduced fatty acid oxidation, which are all suggestive of decreased neutral lipid turnover[110,113]. PLIN2 and PLIN3 contain CMA consensus motifs, LDRLQ and SLKVQ, respectively[110]. In response to low nutrients, HSC70 associates with PLIN2, promoting its phosphorylation by 5’-AMP-activated protein kinase (AMPK) and its subsequent CMA-dependent clearance[110,114]. Interestingly, the accumulation of PLIN2 levels in response to impaired CMA reduced fatty acid mobilization by both lipolysis and lipophagy[110] (Supplementary Box 1). In addition, mutation of the CMA motif in PLIN2 not only blocked lipid droplet degradation but also resulted in the accumulation of larger, clustered lipid droplets that displayed reduced associations with lysosomes[110]. These results raise the possibility that the direct interaction of CMA machinery with PLIN2 and PLIN3 contributes to the formation of lipid droplet-lysosome contacts (FIG. 3) that in turn allow lipid droplet turnover. Lysosome recruitment to lipid droplets is also regulated by RAB7 (REF[111]), a GTPase implicated in lipophagy. However, whether RAB7 functions in CMA is not known. Furthermore, whether CMA degrades other lipid droplet proteins, in addition to PLIN2 and PLIN3, remains unknown. Previous studies indicate that PLIN2 can also be degraded by the

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proteasome[115–118], in particular in cells with a small number of lipid droplets. The relative contribution of CMA and the proteasome to PLIN2 degradation has not been directly compared, but it may depend on the abundance of lipid droplets.

In yeast, the vacuole functions as the major catabolic organelle, equivalent to the lysosome in metazoans. Under exponential growth conditions, lipid droplets form along the ER throughout yeast cells. However, following starvation, there is a striking 'bloom' of lipid droplet biogenesis around the periphery of the nuclear ER-vacuole junction (NVJ)[119]. The NVJ tethering protein Mdml is an integral ER membrane protein that associates with the vacuole in *trans* through its carboxy-terminal lipid-binding Phox (PX) domain[120]. Mdml has a particularly important role in lipid droplet biogenesis at the NVJ: its overexpression is sufficient to drive lipid droplet biogenesis at the NVJ during exponential growth and in cells lacking other NVJ tethering proteins (nuclear vacuolar junction protein 1 (Nvjl) and vacuolar protein 8 (Vac8))[119]. The mammalian orthologue of Mdm1, sorting nexin 14 (SNX14), is an ER-resident protein that associates with lipid droplets[121]. Thus, Mdm1 may function in tri-organelle contacts between the vacuole, ER and lipid droplets (FIG. 3). In addition, lipid droplet organization protein of 16 kDa (Ldo16) and Ldo45 bind seipin, contribute to the biogenesis of lipid droplets at the NVJ and affect the proteome of these specialized lipid droplets[122,123]. Whether Ldo proteins function as organelle tethers or influence the formation of lipid droplets at the NVJ indirectly is unknown.

During starvation or stationary phase, the vacuole undergoes profound lipid reorganization, with sterols segregating into symmetrical liquid ordered microdomains[124]. Under nutrient-limited conditions, the local biogenesis and tethering of lipid droplets at the NVJ facilitate the clearance of lipid droplets through microlipophagy (Supplementary Box 1), whereby they are directly taken up into the vacuole at the sterol-rich domains[125]. It was also noted that the formation of the sterol-rich domains requires lipid droplets[125], suggesting a feedforward loop in which sterols from lipid droplets contribute to the liquid ordered regions in vacuolar membranes and these regions mediate microlipophagy. In addition, acute glucose deprivation induces AMPK-regulated microlipophagy, which is required for lipid cycling and long-term survival of yeast under starvation[126]. Deletion of the Ldo proteins impairs microlipophagy in starved cells[123], consistent with the importance of NVJ-localized lipid droplets in regulating lipid metabolism under starvation.

Lipid droplet functions

During periods of nutrient deprivation or during cell growth, which requires membrane expansion and high phospholipid biosynthesis, fatty acids sequestered as triacylglycerol in the core of the lipid droplet can be mobilized either by lipolysis or by lipophagy (Supplementary Box 1) to fuel metabolic processes and membrane bio-synthesis. These two lipid droplet catabolic pathways have been recently reviewed[127–129]. Another, and perhaps less appreciated, role of lipid droplets is in the protection against lipotoxicity by sequestering fatty acids (FIG. 4). Free fatty acids can act as detergents that directly disrupt membrane integrity or can be incorporated into lipid species that are cytotoxic at high levels, such as ceramide, acylcarnitine and diacylglycerol. Thus, sequestration of fatty acids as triacylglycerol inside lipid droplets prevents the deleterious activities of fatty acids and their

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derivatives in both a cell autonomous (see below) and non-cell-autonomous manner (see BOX 2). Accordingly, conditions in which fatty acid storage in lipid droplets is impaired or the storage capacity is overwhelmed can result in diseases related to lipotoxicity, such as type 2 diabetes and non-alcoholic fatty liver disease[130,131] (BOX 3).

Lipid droplets protect against ER stress.

ER stress is a general term that refers to imbalances in ER protein folding capacity, calcium uptake and/or lipid composition[132]. The cell has evolved mechanisms to monitor ER stress and to initiate a cellular adaptive response, the UPR, to re-establish ER homeostasis[132]. For example, under conditions of insufficient protein folding capacity, the accumulation of unfolded proteins triggers three independent UPR transducers — inositol-requiring protein 1 (IRE1), protein kinase RNA (PKR)-like ER kinase (PERK; also known as EIF2AK3) and activating transcription factor 6 (ATF6) — which are all ER integral membrane proteins. Their activation triggers signalling cascades that coordinate the slowing of protein translation and upregulation of genes involved in ER protein folding, protein degradation and lipid biosynthesis.

Perturbing lipid droplet biogenesis or simply over-whelming lipid droplet storage capacity often results in the upregulation of the UPR. For example, UPR activation is observed in a yeast strain commonly referred to as LD , which lacks the enzymes required for the biosynthesis of triacylglycerol and sterol esters (dga1, lro1, are1, are2) and is thus devoid of lipid droplets[6,7,133]. In response to a brief starvation, LD cells exhibit altered ER morphology that can be rescued by inhibiting de novo fatty acid synthesis or by inducing ER phospholipid synthesis via deletion of the Opi1 repressor [7], suggesting that phospholipid synthesis is a compensatory response to the inability to form lipid droplets. Interestingly, in the absence of lipid droplets, the ER phospholipid composition is altered and displays an increase in phosphatidylinositol[7,8]. Conditions that impair phosphatidylinositol synthesis (for example, inositol depletion) exacerbate phenotypes, indicating that phosphatidylinositol provides a compensatory buffer for fatty acids in the absence of lipid droplets[7,8]. However, the altered phosphatidylinositol levels are not without consequences and result in impaired autophagosome biogenesis[7]. Thus, lipid droplets in yeast are essential for the maintenance of ER homeostasis by buffering excess fatty acids and preventing UPR activation.

Disruption of triacylglycerol synthesis and lipid droplet biogenesis also results in UPR activation in mammalian cells (FIG. 4a). In adipocytes, the stimulation of lipolysis via a noradrenergic signalling cascade results in the massive release of stored fatty acids and the subsequent generation of numerous small lipid droplets. These small lipid droplets were originally thought to reflect lipid droplet fragmentation or fission[56,134], but more recent studies employing time-lapse microscopy and electron tomography argue against this possibility, and emerging data indicate that the production of these small lipid droplets can be blocked by inhibitors of acyl-CoA synthetases and inhibitors of DGAT1 (REFS[135–138]). Thus, this pool of small lipid droplets likely reflects fatty acid re-esterification and packaging into DGAT1-dependent lipid droplets[135]. DGAT1-dependent fatty acid re-esterification and lipid droplet biogenesis under these lipolytic conditions are essential to prevent ER stress and UPR activation[135]. Disruption of RAB18, a component of an ER-lipid

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droplet tethering complex in preadipocytes, also results in increased UPR activation upon oleate treatment[81].

The mechanism connecting impaired lipid droplet biogenesis to UPR activation is not clear. One possibility is that aberrant fatty acid storage could activate the UPR by altering ER homeostasis and causing impairments in ER protein folding or ER calcium storage[139,140]. Alternatively, impaired fatty acid sequestration could result in an aberrant ER membrane lipid composition that directly activates the UPR, independent of alterations in protein folding (FIG. 4a). Indeed, recent findings indicate that the UPR can be directly activated by altering the lipid composition of the ER[141,142]. Consistent with the direct sensing of lipid disequilibrium, the luminal sensing domains of yeast Ire1 and mammalian IRE1 and PERK, implicated in sensing unfolded proteins, are dispensable for UPR activation in response to lipid per-turbations[141,142]. Similarly, in reconstituted liposomes, PERK and IRE1 activation is sensitive to fatty acid saturation and requires their transmembrane domains but not their luminal misfolded protein-sensing domains[141]. For IRE1, a luminal, juxta-membrane amphipathic helix has been identified as a sensor of ER membrane physicochemical properties associated with lipid bilayer stress (such as changes in membrane thickness and lipid packing density)[143]. Although UPR activation upon lipid droplet impairments appears to be a consequence of lipid perturbations, we cannot exclude the existence of other mechanisms.

Lipid droplets increase in response to small-molecule UPR inducers (for example, tunicamycin and dithiothreitol) and to genetic perturbations that impair ER protein degradation[144–146], raising the possibility that lipid droplets are induced to alleviate ER stress. Sequestration of excess fatty acids in triacylglycerol or removal of specific phospholipids from the membrane could correct lipid composition and ameliorate ER stress. It has also been proposed that lipid droplets are involved in the clearance of misfolded and aggregated proteins[147,148]. In yeast, lipid droplets form in close proximity to ER aggregates under conditions of impaired phosphatidylcholine synthesis, and these lipid droplets are then degraded via microlipophagy[148]. Polyubiquitylated proteins and some ER chaperones were observed in buoyant, lipid-droplet-enriched biochemical fractions under these conditions[148], suggesting that lipid droplets may sequester some dysfunctional proteins and facilitate their clearance via microlipophagy (FIG. 4b). However, whether these ubiquitylated proteins localize to the lipid droplet surface or to a lipid-droplet-associated ER subdomain is unclear, and specific substrates that employ this putative degradation pathway have not yet been identified. Connections between lipid droplets and select ERAD substrates have been observed in mammalian cells as well. Hydroxymethylglutaryl (HMG)-CoA reductase[149,150] and apolipoprotein B (ApoB)[151,152] undergo ERAD under specific metabolic conditions, and both proteins associate with lipid droplets or a lipid-droplet-associated ER subdomain before their degradation. It may be that these metabolically regulated substrates are degraded from a specialized ER subdomain or associate with cytoplasmic lipid droplets if proteasome function is compromised (or saturated). Lipid droplets were previously proposed to form pores or extract proteins as a route for the retrotranslocation of ERAD substrates[153], but yeast and mammalian cells in which lipid droplet biogenesis is disrupted do not exhibit overall impairments in ERAD[133,154], arguing against this being a general mechanism. Thus,

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a role for lipid droplets in ERAD may be limited to specific metabolically regulated substrates.

Lipid droplets protect against mitochondrial damage during autophagy.

Prolonged nutrient deprivation upregulates autophagy, resulting in the release of amino acids and lipids from the breakdown of proteins and membranous organelles, which is under the control of the master growth regulator, mTOR complex 1 (mTORC1)[155,156]. Interestingly, a portion of autophagy-released lipids is immediately re-esterified to form triacylglycerol, which is then packaged into new pools of lipid droplets[99,100] (FIG. 4a). Consistent with the dependence of this new pool of lipid droplets on autophagy, knockout of ATG5 or treatment with inhibitors of autophagy blocks lipid droplet formation in response to nutrient deprivation or direct mTORC1 inhibition[99,100]. The mechanism by which fatty acids are transported out of autolysosomes and trafficked to the ER for triacylglycerol synthesis is not known. Transport could require an unidentified lysosomal integral membrane fatty acid transporter, or fatty acids protonated in the acidic environment of the lysosomal lumen could exhibit enhanced capacity for flip-flop into the cytoplasm. As mentioned above, in yeast, lipid droplet biogenesis occurs proximal to the NVJ during nutrient stress[119]; analogous ER-autolysosomal contacts may be important for lipid droplet biogenesis during nutrient deprivation in mammalian cells. Although oleate-induced lipid droplets can employ either DGAT1 or DGAT2, autophagy-dependent lipid droplets are exquisitely DGAT1-specific[99]. This specificity for DGAT1 does not appear to be due to altered DGAT1 or DGAT2 expression[99], and the reason why DGAT2 cannot compensate for DGAT1 under these conditions is unclear. It may relate to unique substrate specificities of these enzymes, differential roles in lipid droplet initiation and lipid droplet expansion, differences in localization or post-translational mechanisms of regulation (for example, phosphorylation)[157].

The observation that lipid droplets increase during nutrient deprivation[100] was initially perplexing. Why would cells expend energy to generate triacylglycerol-containing lipid droplets during an energy crisis? Recent findings indicate that lipid droplets function as a buffer to prevent lipotoxic damage to mitochondria[99] (FIG. 4a). Inhibition of lipid droplet biogenesis during starvation-induced autophagy impairs mitochondrial membrane potential, reduces mitochondrial respiration and increases cell death[99]. The failure to sequester fatty acids in lipid droplets results in the aberrant flux of fatty acids into acylcarnitine[99], a fatty acid conjugate that is generated at the mitochondrial outer membrane and is required for fatty acid uptake into the mitochondria for β-oxidation (FIG. 4a). Inhibiting the enzyme responsible for acylcarnitine production rescues mitochondrial membrane potential[99], suggesting that high levels of acylcarnitines may be toxic to the mitochondria. One possibility is that high local levels of acylcarnitine directly permeabilize the mitochondrial membrane. Consistent with this possibility, palmitoylcarnitine reduces the mitochondrial membrane potential of purified mitochondria[99,158] and disrupts the integrity of liposomes[159,160]. This may be a general property of high levels of fatty acid conjugates because *N*-acyl amino acids also disrupt the mito-chondrial membrane potential[161]. Because acylcarnitine is normally consumed in mitochondria, mitochondrial dysfunction could result in a toxic positive feedback loop in which mitochondrial impairment causes acylcarnitine

accumulation, which in turn further disrupts mitochondrial function. It is worth noting that although loss of lipid droplets during starvation results in mitochondrial defects, no ER stress is observed[99]. This finding suggests that although lipid droplet biogenesis may generally prevent lipotoxicity, the precise lipotoxic phenotype depends on a combination of factors that include the cell type, the lipotoxic insult and the type of lipids that accumulate. Although these studies examined the relationship of lipid droplets with starvation-induced autophagy, stimulation of autophagy by chemical inhibition of mTORCl was sufficient to induce lipid droplet biogenesis[99], suggesting that lipid droplets function as a lipid buffer under conditions of high autophagic flux. Thus, lipid droplets are required to prevent lipotoxicity during autophagy, and this may be functionally important, for example, in cancer cells with high autophagic flux[162,163] and during selective organelle autophagy[164].

The relationship of lipid droplets, acylcarnitine and cellular damage may be relevant to human diseases. The heart is particularly susceptible to acylcarnitine-associated damage as cardiac tissue derives a large portion of its energy from fatty acid oxidation. Indeed, fatty acid oxidation diseases and ischaemia are associated with high levels of acylcarnitines[165–167], and improvements in cardiac function in a mouse model of cardiomyopathy were linked to reductions in acylcarnitine, but not diacylglycerol, triacylglycerol or ceramide, suggesting that acylcarnitine can be lipotoxic in vivo[165]. Furthermore, DGAT1 is protective in mouse models of cardiomyopathy[168–170]. Thus, it is advantageous to sequester fatty acids as triacylglycerol in lipid droplets and release these fatty acids gradually through lipolysis rather than risk conversion of fatty acids into toxic lipid species such as acylcarnitine.

Future perspective

Lipid droplets have gained considerable attention in recent years, yielding several important and exciting discoveries on various fronts. Although many of the mechanistic details remain elusive, there has been progress towards understanding lipid droplet biogenesis and many of the proteins involved in the process. The emerging picture suggests a central role for ER phospholipid composition, which appears to be regulated locally by many of the identified biogenesis factors. The description of organelle contact sites involving lipid droplets and their dynamics have also been major steps forward. The challenge here will be the identification of tethering proteins involved at each contact, their functions (such as identification of molecules exchanged) and their regulation by cellular physiology. Detailed understanding of the mechanisms controlling the formation and dynamics of lipid droplets may ultimately help us understand the pathogenesis of metabolic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Lipotoxicity

Deleterious effects of improperly stored lipids on cellular health.

COPII

Complex of proteins that coat vesicles budding from endoplasmic reticulum (ER) exit sites and facilitate anterograde protein transport towards the Golgi

Phospholipid scramblases

Proteins that mediate the bidirectional exchange of phospholipids between two leaflets of a lipid bilayer

Perilipins

Family of proteins that coat lipid droplets and regulate lipid droplet stability and turnover

COPI coatomer complex

Complex of proteins that coat vesicles budding from the Golgi and facilitate retrograde protein transport

Polytopic membrane proteins

Membrane-embedded proteins in which the polypeptide chain crosses the membrane multiple times

Farnesylation

Post-translational modification of a protein by addition of an isoprenoid farnesyl group to a cysteine residue

ER-associated protein degradation (ERAD)

A process that mediates the recognition and delivery of aberrant (for example, misfolded) proteins from the endoplasmic reticulum to the proteasome for degradation

Packing defects

Regions in which the neutral lipid or the hydrocarbon chains of phospholipids are exposed to the aqueous cytoplasm

SNAREs

Family of proteins that mediate membrane fusion

Brown adipocytes

Type of adipocyte that contains large numbers of mitochondria and expresses high amounts of uncoupling protein 1, resulting in the dissipation of the proton motive force and generation of heat

Insulin resistance

Condition in which cells fail to respond appropriately to insulin

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Peroxidative damage

A form of oxidative damage, such as the formation of lipid peroxides

Ferroptosis

Regulated, iron-dependent form of cell death that is characterized by the accumulation of lipid peroxides

Apolipoprotein

Lipid-binding protein that enables lipid transport throughout the body

Retrotranslocation

Movement of endoplasmic reticulum-associated protein degradation substrates across the endoplasmic reticulum membrane back into the cytoplasm for degradation by the proteasome

Fatty acid oxidation diseases

Heterogeneous group of rare, autosomal recessive diseases characterized by defects in fatty acid catabolism

Ichthyosis

Condition characterized by skin that is thickened, dry and scaly

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Box 1 |

Lipid droplet structure

Lipid droplets are universal storage organelles for neutral lipids that can be found in most cells, from yeast to man. Despite their ubiquitous presence, the number, size and composition of lipid droplets varies widely between cells or even within the same cell. The differences often reflect metabolic states within those cells. Although morphologically diverse, all lipid droplets have a similar structural organization that sets them apart from all other organelles. Instead of the typical phospholipid bilayer, lipid droplets are surrounded by a phospholipid monolayer enclosing a core filled by neutral lipids, most commonly triacylglycerol and sterol esters[171] (see figure part **a**). Lipid droplets emerge from the endoplasmic reticulum (ER) (see figure part **b**, showing a 2D tomogram of lipid droplets in yeast, where the lipid droplet phospholipid monolayer and the close proximity to the nuclear ER (arrows) are evident). in the monolayer, which has a composition reminiscent of the ER bilayer, phospholipids have their polar head groups oriented towards the cytosol whereas their acyl chains contact the hydrophobic neutral lipid core. Associated with the monolayer are a variety of proteins, which decorate the surface of the lipid droplet but are absent from the hydrophobic core (see figure part **c**, depicting a fluorescence microscopy image of cultured liver cancer cells indicating the presence of the neutral lipid core labelled with a fluorescent fatty acid (BODiPY 558/568 C12 dye) and of a perilipin family member (PLiN2-GFP) around the periphery of the lipid droplet; image courtesy of M. Roberts). These proteins associate with the membrane through hydrophobic hairpins, amphipathic helices and fatty acid modifications (see figure part **a**) and have many different functions that control diverse aspects of lipid droplet dynamics. For example, lipid droplet growth and degradation are controlled by enzymes that, at the monolayer surface, promote triacylglycerol synthesis and hydrolysis, respectively. Proteins associated with the monolayer are also thought to control lipid droplet positioning inside the cell and association with other organelles. importantly, the rapid and dramatic changes in lipid droplet size, number and distribution in response to cellular metabolism and nutrient availability likely reflect alterations in the lipid droplet proteome. Moreover, it is also clear that within a cell, distinct lipid droplet populations can be identified on the basis of the proteins present on their surfaces in yeast[122,123] and humans[172,173]. these observations indicate that, as in the case of other organelles, the targeting of proteins to lipid droplets is tightly regulated.

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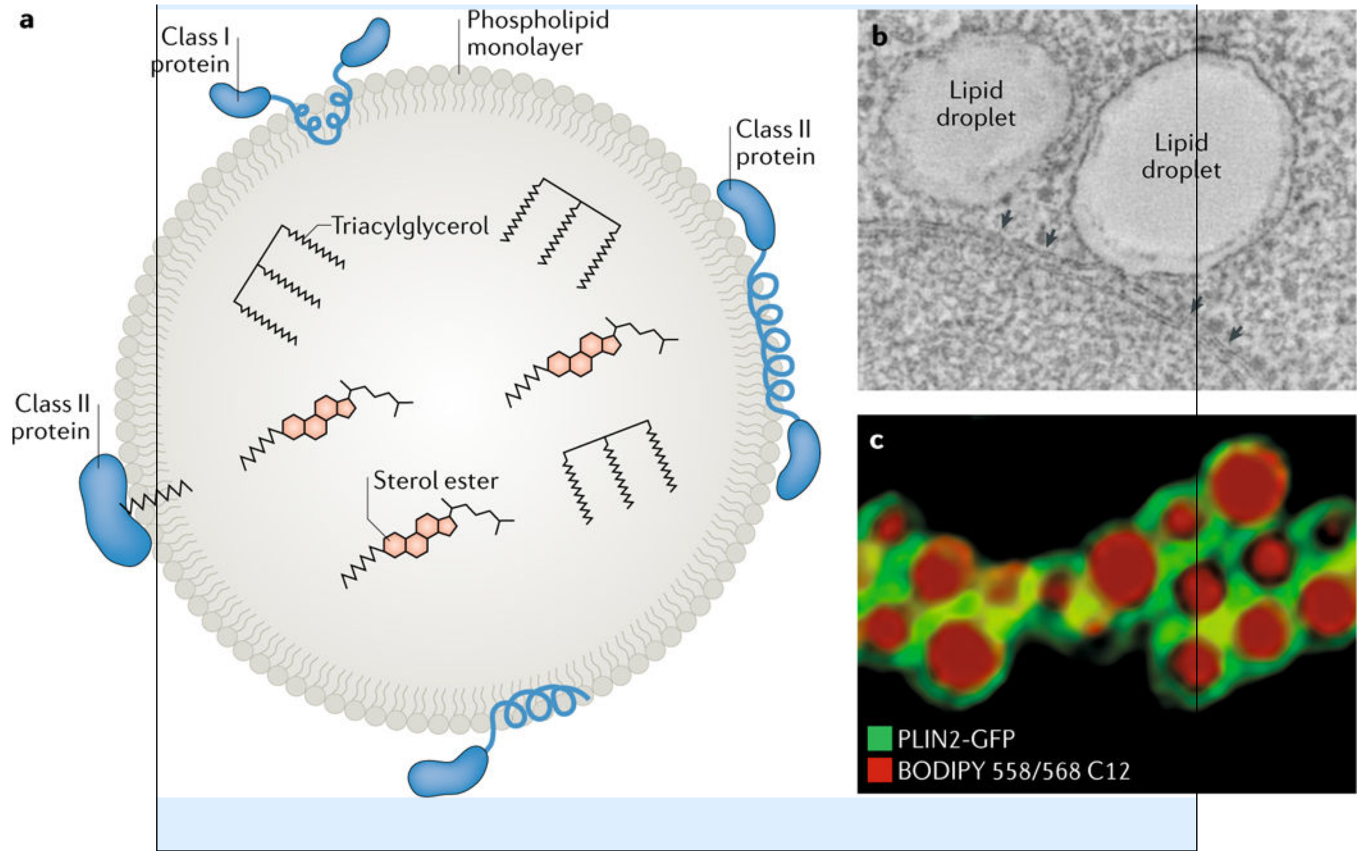

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Box 2 |

Cell non-autonomous prevention of lipotoxicity by lipid droplets

Lipid droplet biogenesis functions as a cell autonomous response to prevent lipotoxicity. In addition, lipotoxicity can also be averted in a non-cell autonomous manner. The best-known example is adipose tissue, which is highly specialized for the storage of excess fat in lipid droplets. impairments in fat storage in adipose tissue result in ectopic fat deposition (for example, fatty liver) and lead to high levels of circulating fatty acids in plasma, which is closely linked to the development of insulin resistance. More recently, cell-to-cell heterogeneity in lipid droplet accumulation has been found to prevent damage in cell populations and tissues. in liver, hepatocytes exhibit a variable amount of lipid droplets. this distribution can also be observed in cultured hepatocytes, and this heterogeneity appears to form spontaneously within the culture[174]. intriguingly, cells that contain higher levels of lipid droplets exhibit higher levels of reactive oxygen species (ROs), suggesting that they protect their neighbouring cells from lipotoxicity[174]. the formation of lipid droplets by glia also protects neighbouring cells from lipotoxicity, such as neural stem cells (neuroblasts) during fly development[175]. During development, glia facilitate the health and growth of neuroblasts by sequestering polyunsaturated fatty acids (PuFAs) in lipid droplets[175]. PuFAs are particularly susceptible to rOs-induced peroxidative damage, and storage of PUFAS in lipid droplets prevents their incorporation into glial and neuroblast membrane phospholipids that can be damaged during oxidative stress[175]. A similar relationship between neurons and glia has also been observed in other fly and murine co-culture models of disease. During periods of increased ROS production, fatty acids are transferred from neurons to glia for storage in lipid droplets through a mechanism involving fatty acid transporters and apolipoprotein E[176,177]. the glial lipid droplets have been proposed to protect neurons by sequestering the damaged, peroxidated lipids[176,177]. interestingly, peroxidation of PuFA-containing phospholipids is integral to a newly described non-apoptotic, regulated cell death pathway termed ferroptosis [178,179]. the emerging data identify an intriguing relationship between lipid droplets and lipid peroxidation, but whether ferroptosis resulting from insufficient sequestration of PuFAs and/or peroxidated lipids in droplets drives cell elimination during fly development or neurodegeneration remains to be determined. understanding of the mechanisms of cell-to-cell communication that modulate lipid transfer and lipid storage is still in its infancy, but these mechanisms are likely to be broadly important for tissue and organismal homeostasis.

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Box 3 |

Lipid droplet dysregulation and disease

Several human pathologies have been associated with the dysregulation of the lipid droplet life cycle and physiological functions.

Obesity

Obesity is characterized by the excessive accumulation of fat in lipid droplets. Obesity is also associated with resistance to insulin and is a major risk factor for type 2 diabetes, steatohepatitis and cardiovascular disease. Adipose tissue is specialized to buffer excess lipids by safely sequestering large amounts of fatty acids as triacylglycerol in lipid droplets. Conditions in which the lipid droplet storage capacity is exceeded result in an increase in circulating fatty acids and ectopic deposition of lipids in lipid droplets in the liver, skeletal muscle and heart. The amount of fat that can be appropriately stored in lipid droplets is affected by lipid-droplet-associated proteins. For example, patients with mutations in cell death-inducing DFFA-like effector C (CIDEC) have impairments in the formation of the characteristic large, unilocular lipid droplets in adipocytes, and the reduced ability to store lipids results in partial lipodystrophy and insulin-resistant diabetes.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is characterized by the aberrant accumulation of lipid droplets in hepatocytes and is the most prevalent form of chronic liver disease in the world. NAFLD can result from a wide variety of conditions, such as the failure to sequester lipids in obesity, the stabilization of lipid droplets during hepatitis C viral infection and impaired secretion of very-low-density lipoproteins. NAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH) and potentially to cirrhosis, which is a risk factor for liver cancer. The mechanisms that influence NAFLD progression are a current area of active investigation, and the precise role of lipid droplets in this process is an open question. Mutations in the lipid droplet lipase PNPLA3 (REF[180]) and TM6SF2 (REFS[181,182]), which is involved in very-low-density lipoprotein lipidation, have been implicated in NAFLD.

Cardiovascular disease

Atherosclerosis is linked to accumulation of cholesterol and cholesterol esters in arteries. Cholesterol esters can be stored in the lipid droplets of foam cells, which are macrophages that have internalized and degraded large amounts of high cholesterol-containing lipoproteins. The cholesterol esters stored in macrophage lipid droplets can be hydrolysed and released for efflux to circulating high-density lipoproteins, which then transport cholesterol from peripheral tissues to the liver. This process of reverse cholesterol transport is thought to be protective and is inversely correlated with atherosclerosis. If the capacity of the macrophages is overwhelmed, free cholesterol can accumulate and can lead to atherosclerotic plaque formation, thrombosis and infarction.

Neutral lipid storage disease

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Neutral lipid storage disease (NLSD) is characterized by the deposition of triacylglycerol in ectopic lipid droplets in multiple tissues, including liver, muscle, skin, central nervous system and leukocytes. NLSD is caused by autosomal recessive mutations in adipose triglyceride lipase (*ATGL*), which encodes a rate-limiting enzyme in lipolysis, and 1-acylglycerol-3-phosphate *O*-acyltransferase (*ABHD5*), which encodes ATGL activator[183]. *ATGL* mutations cause NLSD associated with myopathy, whereas mutations in *ABHD5* cause NLSD associated with ichthyosis, suggesting that they have additional functions. Consistently, mice lacking ATGL or ABHD5 exhibit different phenotypes, and ABHD5 is able to regulate triacylglycerol metabolism in the absence of ATGL[184].

Lipodystrophy

Lipodystrophies are a heterogeneous group of disorders characterized by the variable loss of fat tissue, including nearly the whole body (generalized) or just certain regions of the body (partial). Depending on the extent of fat loss, the disease may also be accompanied by metabolic abnormalities, such as insulin resistance and associated metabolic phenotypes (for example, diabetes and hypertriglyceridaemia). Some forms of lipodystrophy result from mutations in genes involved in lipid metabolism and lipid droplet function[185]. Congenital generalized lipodystrophy can be caused by autosomal recessive mutations in *AGPAT2* (acyl-glycerol-3-phosphate acyltransferase 2), *BSCL2* (seipin), *CAV1* (caveolin 1) and *PTRF* (encodes cavin 1). Familial partial lipodystrophy can be caused by heterozygous mutations in *PLIN1*, *PPARG* and *AKT2* as well as by homozygous autosomal recessive mutations in *CIDEC* and *LIPE* (encoding hormone-sensitive lipase).

Hereditary spastic paraplegia

Hereditary spastic paraplegia (HSP) refers to a group of inherited neurological disorders characterized by the degeneration of upper motor neurons, eventually resulting in progressive weakness and spasticity in the legs. The disease may be accompanied by neurological symptoms such as ataxia, cognitive impairments, peripheral neuropathy and epilepsy. Mutations in over 60 genes have been implicated in HSP, some of which affect lipid droplet biogenesis and dynamics[186]. For example, mutations in seipin, atlastin, spartin, spastin and REEPl cause HSP[186]. Atlastin, spastin and REEPl likely affect lipid droplet biogenesis indirectly through their roles in the shaping of the endoplasmic reticulum network[186–188]. Spartin has been implicated in the ubiquitylation of lipid droplet proteins through the recruitment of the E3 ligase AIP4 (REFS[118,189,190]). Whether dysregulation in lipid droplet functions contributes to the aetiology of the disease is unclear.

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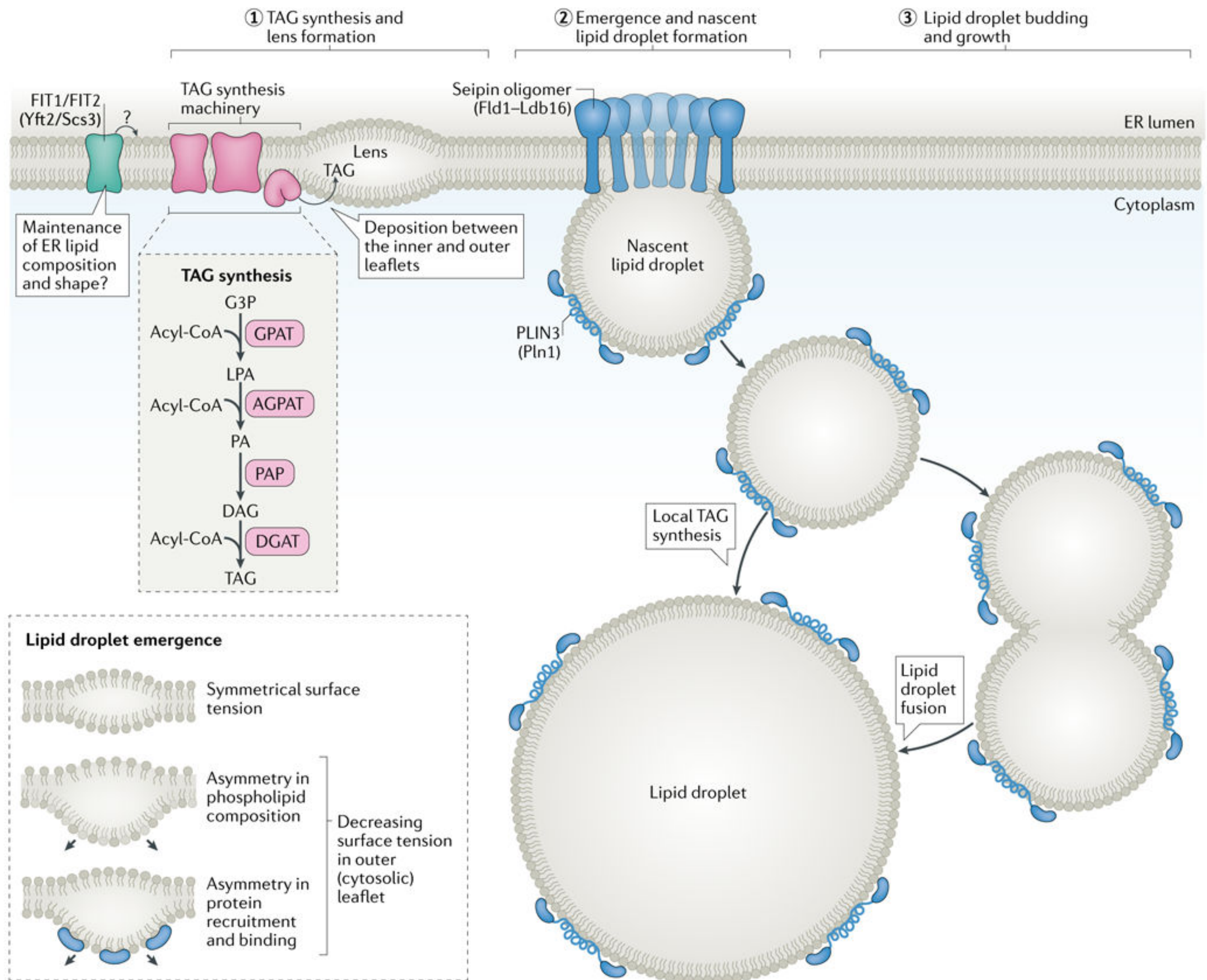

Fig. 1 |. Steps in lipid droplet biogenesis.

Lipid droplets emerge from the endoplasmic reticulum (ER). The correct shape and composition of the ER membranes, which are likely affected by the fat storage-inducing transmembrane 2 (FIT2) protein and other ER-resident proteins, are important determinants of organized lipid droplet biogenesis. Step 1: triacylglycerol (TAG) synthesis (see inset) and cholesterol ester synthesis enzymes deposit neutral lipids in between the leaflets of the ER bilayer. Beyond a certain concentration, the neutral lipids demix and coalesce into a lens. Step 2: seipin and other lipid droplet biogenesis factors are recruited to the lens structure and facilitate the growth of the nascent lipid droplet. The emergence of the lipid droplet into the cytosol is affected by differences in surface tension of the luminal and cytosolic leaflets of the ER bilayer, likely determined by asymmetrical protein binding and phospholipid composition (shown in inset). Step 3: in some mammalian cells, lipid droplets bud from the ER and grow through fusion or local lipid synthesis. AGPAT, acylglycerolphosphate acyltransferase; DAG, diacylglycerol; DGAT, acyl-CoA:diacylglycerol acyltransferase; G3P,

glycerol-3-phosphate; GPAT, glycerol-3 phosphate acyltransferase; LPA, lysophosphatidic acid; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PLIN, perilipin. Yeast orthologues of mammalian proteins are given in parentheses.

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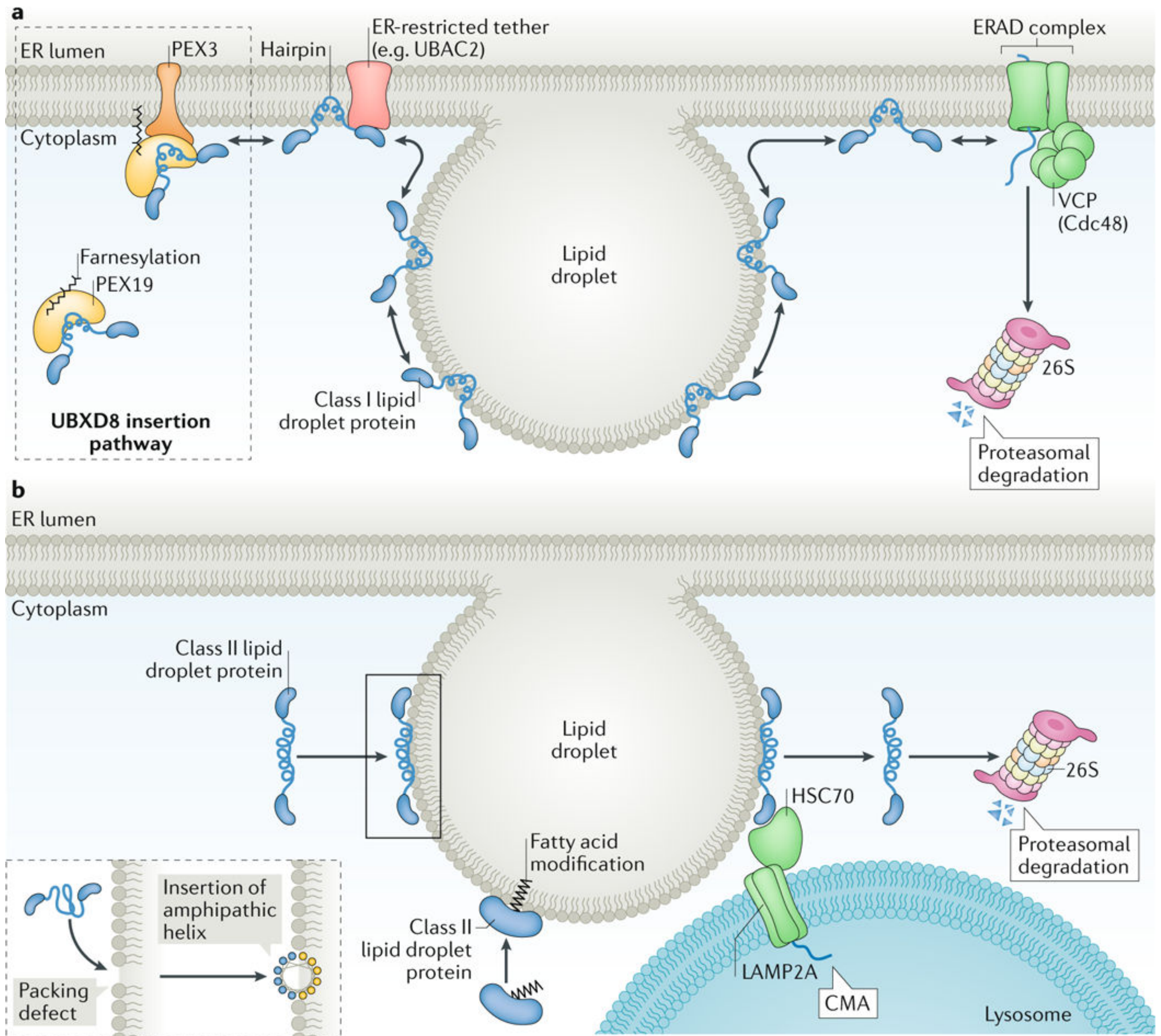

Fig. 2 |. Mechanisms of lipid droplet protein targeting and degradation.

a | Class I lipid droplet proteins insert into the endoplasmic reticulum (ER) and laterally diffuse within the membrane entering the forming lipid droplets. In at least one case, the insertion of a class I protein is mediated through the association with farnesylated peroxisomal biogenesis factor 19 (PEX19) and its ER receptor PEX3. Most class I lipid droplet proteins contain a hydrophobic hairpin, through which they associate with the lipid monolayer. The asymmetrical distribution of class I proteins between the ER and the lipid droplets may be facilitated by targeting some proteins to ER-associated protein degradation (ERAD). **b** | Class II lipid droplet proteins insert directly from the cytosol into the lipid droplet via amphipathic helices that recognize packing defects in the membrane (inset) or through fatty acid modifications. In the absence of lipid droplets, these proteins may be degraded by the ubiquitin–proteasome system. These proteins may also be susceptible to

degradation by the proteasome if they are forced off the surface of the lipid droplet, for example, by molecular crowding during lipid droplet lipolysis. The heat shock cognate 71 kDa protein (HSC70) can extract select proteins bearing a characteristic pentapeptide consensus sequence directing proteins for chaperone-mediated autophagy (CMA) from the lipid droplet for degradation in the lysosome. CMA of lipid-droplet-associated proteins may potentially occur at lipid droplet–lysosome contacts. L AMP2A, lysosome-associated membrane protein 2A; UBXD8, UBX domain-containing protein 8; VCP, transitional endoplasmic reticulum ATPase.

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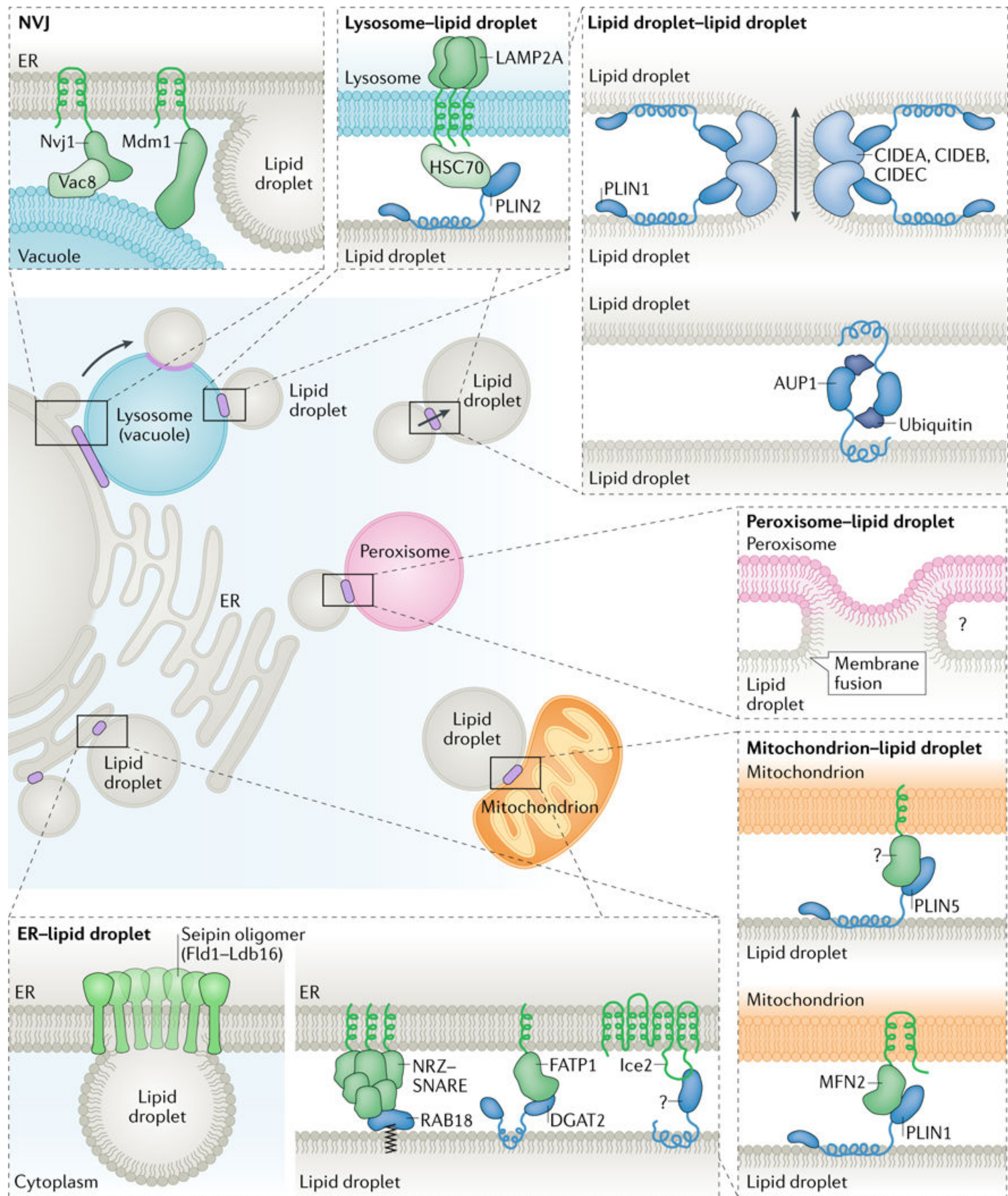

Fig. 3 |. Lipid droplet–organelle contacts.

Lipid droplets interact with nearly all organelles in the cell. The molecular basis for many of these contacts remains poorly understood. Select proteins implicated in tethering or organization of lipid droplet contact sites are shown, with lipid droplet proteins in blue and all other proteins in green. Although the organelle contacts are depicted as distinct and spatially separate, it is likely that individual lipid droplets participate in contacts with multiple organelles simultaneously. Tethering mechanisms that are thus far undefined are indicated by a question mark. AUP1, ancient ubiquitous protein 1; CIDEA, cell death-

inducing DFFA-like effector A; DGAT2, diacylglycerol acyltransferase 2 protein; ER, endoplasmic reticulum; FATP1, fatty acid transport protein 1; HSC70, heat shock cognate 71 kDa protein; Ice2, inheritance of cortical ER protein 2; L AMP2A, lysosome-associated membrane protein 2A; Mdm1, structural protein MDM1; MFN2, mitofusin 2; NVJ, nuclear ER–vacuole junction; NRZ, NAG–RINT1–ZW10 complex; Nvj1, nuclear vacuolar junction protein 1; PLIN, perilipin; Vac8, vacuolar protein 8.

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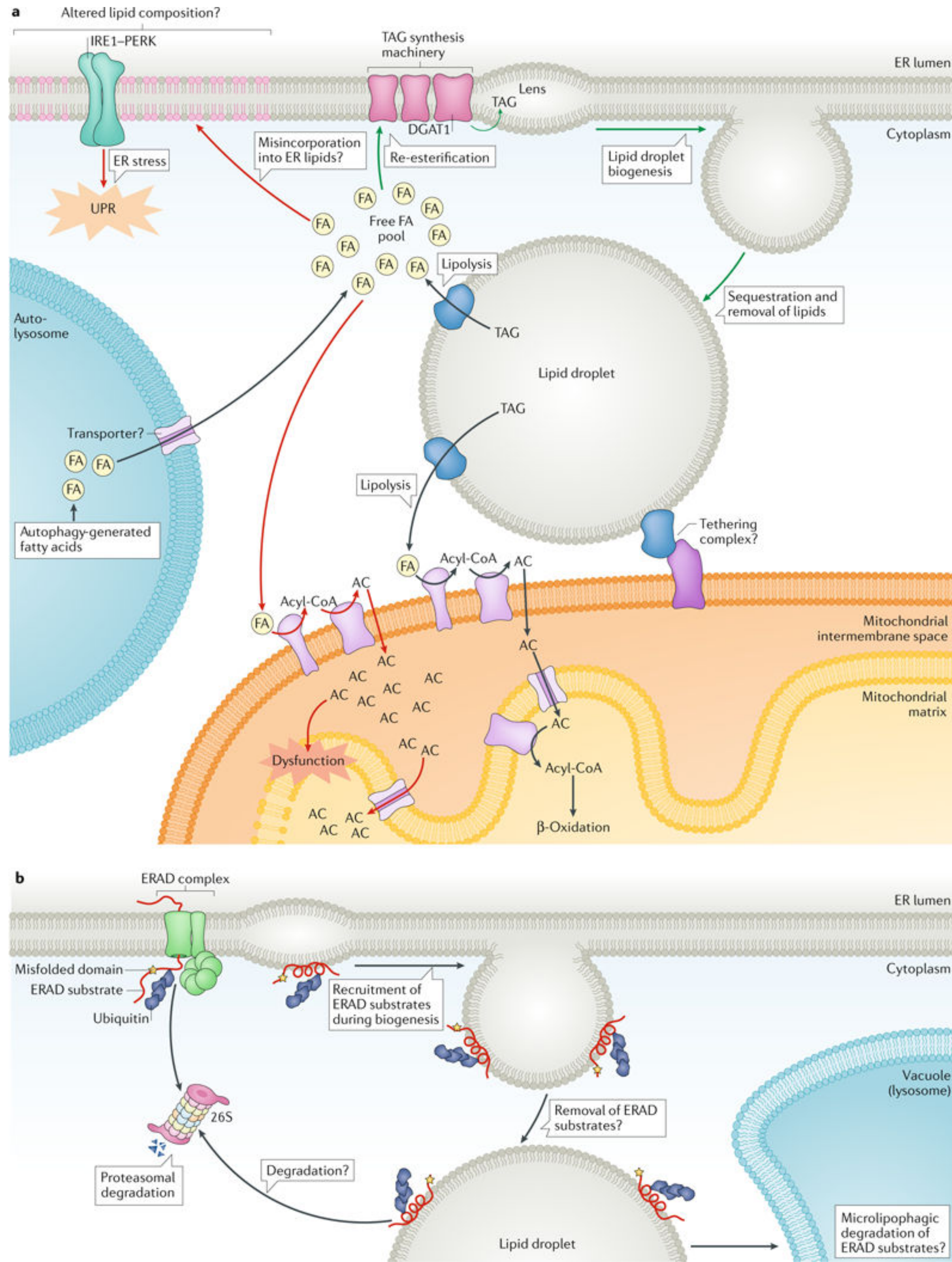

Fig. 4 |. Lipid droplets as buffers against stress.

a | Fatty acids (FAs) are important sources of cellular energy. FAs derived from the LipoLysis of lipid droplets are converted at the mitochondrial membrane into acylcarnitine (AC), which is then taken up by mitochondria and broken down via β-oxidation. However, increased FA levels can be toxic to cells, and sequestration of FAs by lipid droplets provides a buffering capacity that prevents lipotoxicity. In adipocytes, induction of lipolysis by external stimuli (such as noradrenergic signalling) releases an enormous amount of FAs, many of which are immediately re-esterified and packaged as triacylglycerol (TAG) into new

diacylglycerol acyltransferase 1 (DGAT1)-dependent lipid droplets. When lipid droplet biogenesis is impaired, lipolytic release of FAs from the existing lipid droplets results in endoplasmic reticulum (ER) stress, leading to the activation of the unfolded protein response (UPR) through inositol-requiring protein 1 (IRE1) and protein kinase RNA (PKR)-like ER kinase (PERK). UPR activation may involve alterations in the ER lipid composition, which could directly activate IRE1 and PERK. Large amounts of FAs are also released during starvation owing to the breakdown of membranous organelles via starvation-induced autophagy. Loss of DGAT1-mediated lipid droplet formation under these conditions results in the flux of FAs into AC, which accumulates and disrupts mitochondrial integrity and function, likely by inducing mitochondrial membrane permeabilization. **b** | Lipid droplets have also been connected with ER protein homeostasis. In certain cases, lipid droplets may facilitate removal of ER-associated protein degradation (ERAD) substrates. ERAD substrates may be targeted to the proteasome from lipid droplets or lipid-droplet-associated ER subdomains. It has also been proposed that ERAD substrates associated with the lipid droplet may be degraded through microlipophagy.

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