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Protein Quality Control and Lipid Droplet Metabolism

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Keywords

lipid droplet, endoplasmic reticulum, triacylglycerol, ubiquitin, proteasome, lipophagy, chaperone-mediated autophagy, metabolism, protein targeting

Abstract

Lipid droplets (LDs) are endoplasmic reticulum–derived organelles that consist of a core of neutral lipids encircled by a phospholipid monolayer decorated with proteins. As hubs of cellular lipid and energy metabolism, LDs are inherently involved in the etiology of prevalent metabolic diseases such as obesity and nonalcoholic fatty liver disease. The functions of LDs are regulated by a unique set of associated proteins, the LD proteome, which includes integral membrane and peripheral proteins. These proteins control key activities of LDs such as triacylglycerol synthesis and breakdown, nutrient sensing and signal integration, and interactions with other organelles. Here we review the mechanisms that regulate the composition of the LD proteome, such as pathways that mediate selective and bulk LD protein degradation and potential connections between LDs and cellular protein quality control.

Contents

1. INTRODUCTION

Lipid droplets (LDs) are neutral lipid storage organelles that function as hubs of cellular lipid and energy metabolism (Olzmann & Carvalho 2019, Renne et al. 2020, Walther et al. 2017). LDs provide a dynamic source of stored lipids that can be rapidly mobilized, releasing fatty acids that can be broken down by β-oxidation for energy, employed as building blocks for membrane biosynthesis, and used as lipid signaling molecules (Olzmann & Carvalho 2019). By sequestering lipids, LDs also play important roles in suppressing lipotoxicity by preventing cell death, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction caused by free fatty acids (Olzmann & Carvalho 2019). Beyond their roles in lipid metabolism, LDs have also been implicated in less conventional roles such as the regulation of gene expression (Gallardo-Montejano et al. 2016, Mejhert et al. 2020), histone sequestration (Cermelli et al. 2006), viral replication (Miyanari et al. 2007), and drug activity (Dubey et al. 2020, Greenwood et al. 2019). The underproduction and overproduction of LDs have been implicated in the etiology of several human diseases (Krahmer et al. 2013a, S. Xu et al. 2018).

LDs have a unique architecture consisting of a core of neutral lipids encircled by a phospholipid (PL) monolayer and a host of resident proteins. LDs are essentially surfactant-stabilized oil droplets suspended in the aqueous cytosol that undergo dynamic cycles of growth and degradation. There are two main steps in LD biogenesis. The first step is the biosynthesis of neutral lipids [e.g., triacylglycerols (TAGs) and sterol esters (SEs)] and their deposition between the leaflets of the ER bilayer. When neutral lipids accumulate to a particular level, a neutral lipid lens forms

in the membrane driven by a liquid–liquid phase separation process (Khandelia et al. 2010, Zoni et al. 2020). This process is modulated by membrane curvature (e.g., ER tubules versus sheets) and the protein and lipid composition at sites of LD biogenesis (Khandelia et al. 2010, Santinho et al. 2020, Zoni et al. 2020). The second step of LD biogenesis involves the budding of the nascent LD toward the cytosol. The directionality of LD budding is driven by asymmetrical surface tension that is mediated by differences in the luminal and cytosolic leaflets of the ER bilayer (Ben M'barek et al. 2017, Chorlay et al. 2019).

Proteins such as seipin and PEX30 localize to sites of LD formation and regulate LD biogenesis (Olzmann & Carvalho 2019, Renne et al. 2020). Seipin-associated factors, such as lipid droplet–associated factor 1 (LDAF1) (also known as promethin and TMEM159) in mammalian cells and lipid droplet organization proteins of 16- and 45-kDa (LDO16 and LDO45) in yeast, also contribute to organized LD biogenesis (Olzmann & Carvalho 2019, Renne et al. 2020). These integral membrane proteins may cooperate with recruited cytosolic factors such as the perilipin (PLIN) family of LD scaffold proteins (Olzmann & Carvalho 2019, Renne et al. 2020). Precisely how the emerging set of LD biogenesis factors influences neutral lipid phase separation and membrane surface tension to promote organized LD formation is incompletely understood. Mature LDs are subject to regulated degradation through two pathways: a lipolytic pathway that employs the consecutive actions of lipases recruited to the LD surface and a selective autophagy pathway termed lipophagy characterized by the autophagosome-mediated delivery of LDs to autolysosomes for breakdown (Schulze et al. 2017, Zechner et al. 2017).

The unique complement of proteins decorating the LD surface (i.e., the LD proteome) regulates the LD life cycle and its functions, including growth, degradation, nutrient signal integration, and the formation of membrane contact sites with other organelles. In this review, we focus on how the LD proteome is established, our emerging understanding of the mechanisms that mediate LD proteome remodeling, and the connections between LDs and cellular protein quality control pathways.

2. LIPID DROPLET STRUCTURE AND PROTEOME

2.1. Physical Properties and Lipid Composition of Lipid Droplets

LDs are an emulsion of neutral lipids stabilized by a shell of surfactants consisting of amphipathic lipids and proteins. The LD core is an amalgam of neutral lipids, predominantly TAGs and SEs, that is devoid of proteins. LDs may also contain retinyl esters, ether lipids, acylceramides, squalene, fat-soluble vitamins, and waxes, with the precise composition differing between cell types and metabolic conditions. The diverse composition of the lipid core suggests that LDs impact many cellular processes beyond their canonical role in energy metabolism. Electron and cryo-electron microscopy studies indicate an additional layer of complexity, providing evidence that the neutral lipid core has an internal structure that depends on the cellular state (Cheng et al. 2009, Czabany et al. 2008, Mahamid et al. 2019, Tauchi-Sato et al. 2002). Although the internal structure of LDs normally consists of an amorphous emulsion of neutral lipids, during mitotic arrest and nutrient deprivation this structure reorganizes into a liquid crystalline shell (possibly composed of SEs) with an amorphous core (Fujimoto & Parton 2011, Mahamid et al. 2019). The impact of the altered internal structure of the LD on lipid access and metabolism, lipid exchange, and protein binding is as yet unknown.

The LD hydrophobic core is bounded by a monolayer of PLs that act as surfactants to maintain the structural integrity of the LD. Lipidomic analyses identify a complex composition of PLs (Bartz et al. 2007a) that is critical for LD biogenesis and the maintenance of LD morphology. The initial PLs surrounding LDs are derived from the outer leaflet of the ER, and continuous PL

synthesis is essential during LD biogenesis both to maintain ER membrane homeostasis and to support directional LD emergence (Chorlay et al. 2019). Similarly, PL synthesis is required for the growth of mature LDs and for the prevention of LD coalescence (Krahmer et al. 2011). How the LD PL composition is maintained and modulated remains for now mostly unknown. As most PLs are synthesized in the ER, ER–LD membrane continuities and lipid-transfer proteins (LTPs) provide mechanisms for PL delivery. The oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) are an important family of LTPs, and ORP2 and ORP5 have been observed at ER–LD contacts (Du et al. 2020, Hynynen et al. 2009). ORP5 mediates the trafficking of both phosphatidylserine from the ER to LDs and phosphatidylinositol 4-phosphate from LDs to the ER (Du et al. 2020). Several members of the chorein domain–containing family of LTPs also localize to ER–LD contacts, including ATG2, VPS13a, and VPS13c (Kumar et al. 2018, Valverde et al. 2019). This unique family of LTPs is distinguished by the ability to bind and transfer tens of glycerophospholipids at once via a large hydrophobic cavity (Kumar et al. 2018, Valverde et al. 2019), providing a mechanism for rapid PL delivery and the equilibration of membrane compositions. Future research is necessary to define the full complement of LD-associated LTPs and their functions at LD–organelle membrane contact sites.

2.2. Lipid Droplet Proteome Composition

A unique complement of integral and peripheral proteins associates with the LD PL monolayer (Bersuker & Olzmann 2017, Zhang & Liu 2019). LD-localized proteins were first observed in the early 1990s, when perilipin-1 (PLIN1) was found in adipocyte LD fractions and localized to the LD surface (Greenberg et al. 1991). More recently, proteomic analyses of LDs isolated by biochemical fractionation on sucrose gradients have enabled the characterization of the LD proteome composition in numerous species, cell types, and tissues (Bersuker & Olzmann 2017, Zhang & Liu 2019). The associated repertoire of LD proteins differs between cell types and reflects the cell's metabolic state, and alterations are observed in cultured adipocytes following induction of lipolysis (Brasaemle et al. 2004) in mice on high-fat diets (Crunk et al. 2013, D'Aquila et al. 2019, Ding et al. 2012, Khan et al. 2015, Krahmer et al. 2018, Liu et al. 2017b) and in human liver and subcutaneous adipose tissue biopsies from patients with nonalcoholic fatty liver disease and insulin resistance, respectively (Su et al. 2014, Xie et al. 2016). These dynamic changes highlight the importance of LD proteome remodeling in modulating LD functions to match cellular energy demands.

LD-associated proteins constitute a variety of functional classes (Bersuker & Olzmann 2017, Zhang & Liu 2019). Many LD proteins are lipid metabolic enzymes that mediate LD growth and degradation. These proteins include enzymes involved in TAG synthesis, such as the acyl-CoA synthetase ACSL3 and the acyltransferases GPAT4 and DGAT2, as well as enzymes involved in TAG breakdown, such as the lipolysis rate–limiting enzyme adipose triglyceride lipase (ATGL) and its associated regulators CGI-58, HILPDA, and G0S2. The PLIN family of proteins (consisting of PLINs 1–5) are invariably found on LDs, with the expression of family members differing by tissue. PLINs are generally thought to function as scaffold proteins that maintain the structural integrity of the LD, but they have also been implicated as integrators of nutrient signaling pathways, regulators of lipolysis, tethering proteins at LD–organelle membrane contact sites, and direct mediators of lipid signaling pathways (Kimmel & Sztalryd 2016). Some cells express members of the cell death–inducing DNA fragmentation factor 45–like effector (CIDE) family of proteins, which mediate the regulated fusion of LDs. Beyond lipid metabolism, other functional classes of proteins are present on LDs, including proteins involved in trafficking (e.g., motor and cytoskeletal proteins) and many Rab guanosine triphosphatases (GTPases) (Bartz et al. 2007b; Liu et al. 2004,

2007). Some of the Rabs affect LD functions, such as Rab18 regulation of ER–LD contacts (D. Li et al. 2019, D. Xu et al. 2018) and Rab10 regulation of the autophagic clearance of LDs (Li et al. 2016, Z. Li et al. 2019), but the effects of most Rab family proteins on LDs is as yet unclear. Several proteins have been implicated in the formation of LD–organelle contact sites that promote fatty acid oxidation. For example, M1 spastin links LDs to peroxisomes through its interactions with peroxisomal ABCD1 and facilitates fatty acid transfer to the peroxisome at these sites by recruiting the membrane-shaping ESCRT-III proteins IST1 and CHMP1B (Chang et al. 2019). Other functional classes include ubiquitination, signaling, transcription, and redox, but their roles in LD biology are not yet well understood.

An ongoing challenge for LD proteomic approaches is distinguishing putative LD proteins from contaminants that copurify in LD-enriched buoyant fractions. Protein correlation profiling (Currie et al. 2014; Krahmer et al. 2013b, 2018) and proximity labeling proteomic analyses of LD proteins using the engineered ascorbate peroxidase APEX2 (Bersuker & Olzmann 2019, Bersuker et al. 2018) provide two methods that are useful for identifying high-confidence LD proteomes and avoid the identification of common, abundant contaminant proteins. These approaches identify approximately 100–150 LD proteins in mammalian cells and 30–40 LD proteins in yeast, some with exclusive LD localization and others found in multiple subcellular compartments. A second challenge is mapping the proteomes of heterogeneous subpopulations of LDs, which can differ in size, localization, function, and organelle contacts. This heterogeneity is lost during bulk isolation of fractionated LDs. Further separation of the isolated LD-enriched buoyant fraction has been used to compare the proteomes of LDs with different sizes (Zhang et al. 2016). Proximity labeling proteomics approaches provide a promising method for profiling separate sets of LD proteomes by tagging differentially localized LD proteins with enzymes that mediate the biotinylation of neighboring proteins.

3. PROTEIN TARGETING TO LIPID DROPLETS

Targeting proteins to LDs presents several unique challenges. In contrast to most other organelles, neither protein insertion machinery (analogous to the ER translocon or peroxisomal importomer) nor specific lipids have been identified on LDs for protein recruitment and insertion. LD proteins also do not contain a signal or targeting sequence that can be predicted by informatic approaches. However, LD proteins exhibit some shared structural features because of the topological constraints imposed by the architecture of the LD monolayer, which requires LD proteins to adopt conformations that avoid exposing hydrophilic domains to the neutral lipid core of the LD. For this reason, integral LD proteins are monotopic proteins that insert into the LD monolayer and orient their soluble domains toward the cytosol. Bitopic and polytopic transmembrane proteins that typically associate with bilayers are excluded from LDs. A general classification system for integral LD proteins has been adopted based on their trafficking pathways: Class I LD proteins insert first into the ER and traffic to LDs, and Class II LD proteins insert directly into LDs from the cytosol (Kory et al. 2016) (**Figure 1**). Both Class I and Class II LD proteins can recruit soluble, peripheral proteins to LDs.

3.1. Class I Lipid Droplet Proteins: Endoplasmic Reticulum to Lipid Droplet Targeting

Class I LD proteins insert into the ER and laterally diffuse from the outer leaflet of the ER to the PL monolayer of nascent LDs (**Figure 1**). In the absence of LDs, Class I LD proteins typically exhibit an ER distribution. Examples of Class I LD proteins include the lipid biosynthesis enzymes
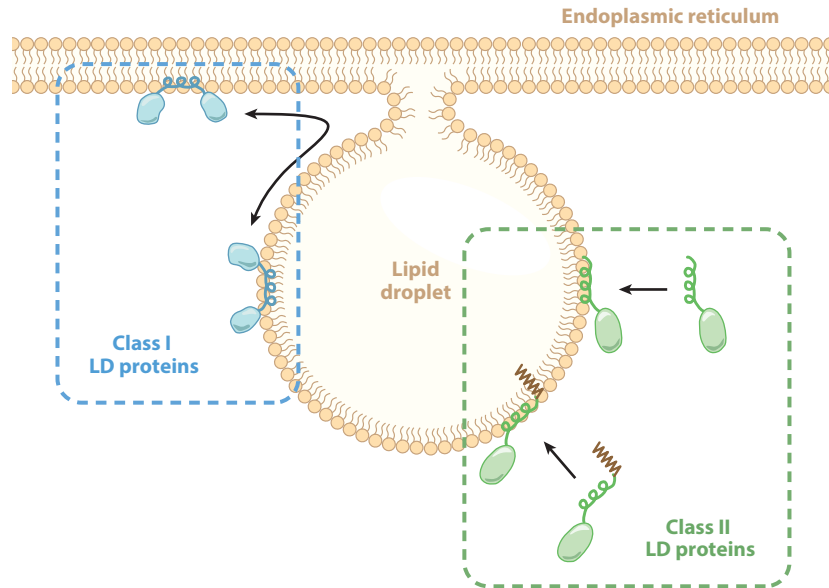
Figure 1

LD protein targeting. Class I LD proteins are inserted into the ER and are trafficked from the ER to nascent LDs. Class II LD proteins are inserted into LDs directly from the cytosol. These proteins include both amphipathic helix-containing proteins that recognize phospholipid packing defects in the LD monolayer and proteins that are modified by conjugated fatty acids. Abbreviations: ER, endoplasmic reticulum; LD, lipid droplet.

GPAT4 and DGAT2, the acyl-CoA synthetase ACSL3, the ER-associated degradation (ERAD) factors AUP1 and UBXD8, the putative methyltransferase AAM-B, caveolin-1 and caveolin-2, HSD17B11, the hepatitis C virus (HCV) core protein, and ALDI.

Class I LD proteins are generally thought to adopt a hairpin conformation in which a hydrophobic domain embedded in the monolayer is flanked by hydrophilic regions that are exposed to the cytosol, forming a V shape extending from the membrane. These proteins lack the luminal loops or domains typically found in transmembrane proteins, allowing them to be accommodated in the outer leaflet of the ER and the monolayer of the LD. Crystal structures of Class I LD proteins are lacking, and the model of hairpin conformation for these proteins is primarily based on the bioinformatic prediction of hydrophobic regions, the biochemical characterization of membrane insertion, and the protease sensitivity of N- and C-terminal tags. The hairpin structure was first noted for plant structural proteins called oleosins, which contain a critical conserved proline knot buried in the hydrophobic sequence (Abell et al. 1997). Although a proline knot is required for LD insertion of oleosins (Abell et al. 1997) and HCV core protein (Hope et al. 2002), mutation of central prolines in the hydrophobic domains of AAM-B, ALDI, and CYB5R3 (Zehmer et al. 2008) does not abolish LD localization, indicating that the proline knot is not a universal requirement for Class I LD protein targeting.

Most secreted and transmembrane proteins are inserted into the ER through well-characterized pathways, with proteins bearing an N-terminal signal sequence inserted cotranslationally via the Sec61 translocon and proteins bearing C-terminal transmembrane domains (i.e., tail-anchored proteins) inserted posttranslationally via the guided entry of tail-anchor (GET)

pathway (Shao & Hegde 2011). The mechanism of ER insertion for Class I LD proteins has not been extensively studied. At least one Class I LD protein, UBXD8, relies on a distinct posttranslational insertion pathway mediated by the peroxisome biogenesis factors PEX19 and PEX3 (Schrul & Kopito 2016). In this pathway, the cytosolic receptor PEX19 binds to the hydrophobic segment of UBXD8 and mediates UBXD8 delivery to specific ER subdomains containing its binding partner PEX3, an ER-resident integral membrane protein (Schrul & Kopito 2016). Whether LD proteins other than UBXD8 use this insertion pathway is unknown.

The mechanisms that control Class I LD protein partitioning between the ER and LDs remain unresolved. The junction between the ER bilayer and the LD monolayer provides a diffusion barrier that is permissive for monotopic protein trafficking but excludes bitopic and polytopic membrane protein movement. However, this diffusion barrier does not explain why some Class I LD proteins concentrate in LDs. The distribution of Class I LD proteins between the ER and LDs can be impacted by selective ubiquitin-dependent degradation of their ER pools (Ruggiano et al. 2016) and by their tethering to protein-binding partners in the ER. ER–LD partitioning of these proteins may be driven by differences in their conformations or mobility in the ER bilayer and the LD monolayer that impact the proteins' stability, degradation, and residence time.

3.2. Class II Lipid Droplet Proteins: Cytosol to Lipid Droplet Targeting

Class II LD proteins are generally thought to be translated on cytosolic ribosomes and post-translationally inserted into the LD directly from the cytosol (**Figure 1**). Examples of Class II proteins include the PLIN family, the rate-limiting enzyme of phosphatidylcholine (PC) synthesis CTP:phosphocholine cytidylyltransferase (CCT), the LD–LD fusogenic protein CIDEA, and the antiviral protein viperin.

How LD proteins are selectively targeted to the LD monolayer as opposed to other membrane-bound compartments has been an enigma. Recent in silico molecular dynamics studies provide an answer, revealing that a PL monolayer encircling a neutral lipid core exhibits persistent and large PL packing defects at the interfacial region in which acyl chains of the bounding PLs and core TAGs are exposed to the cytosol (Bacle et al. 2017, Prévost et al. 2018). This unique feature of LD membranes is exploited by Class II LD proteins, which often contain amphipathic helices with large hydrophobic residues that detect and bind to the hydrophobic PL packing defects (Prévost et al. 2018). Amphipathic helices are not unique to LD proteins, and they are common bilayer-targeting domains found in many proteins that localize to other subcellular compartments. Indeed, promiscuous LD localization of amphipathic helices from a range of proteins is observed when they are ectopically expressed (Prévost et al. 2018). The mechanisms that mediate the selective targeting of LD proteins and exclusion of non-LD proteins that contain amphipathic helices are unclear but may involve differences in their affinities for transiently exposed neutral lipids (Chorlay & Thiam 2020, Prévost et al. 2018), exclusion by macromolecular crowding effects on the LD surface (Kory et al. 2015), and regulation by dominant targeting information present in regions flanking the amphipathic helix (Ingelmo-Torres et al. 2009). The localization of Class II LD proteins may also be favored by a series of amphipathic helices that would increase avidity and binding. The PLIN family of LD proteins localize almost exclusively to LDs and exhibit a series of N-terminal 11-mer repeats that are predicted to form amphipathic helices (Ajjaji et al. 2019, Čopič et al. 2018, Rowe et al. 2016). The PLIN4 11-mer repeat region forms a particularly long amphipathic helix (>60 amino acids), binds directly to TAG, and acts as a potent surfactant to stabilize LDs during periods in which PLs are limiting (Čopič et al. 2018). PLINs 1–3 also contain a C-terminal amphipathic helix consisting of a four-helix bundle domain that can differentially stabilize LD binding and may underlie the observed hierarchal association

of PLINs with the LD (Ajjaji et al. 2019). Not all Class II LD proteins contain amphipathic helices. Some LD proteins contain large hydrophobic residues important for insertion that could still exploit LD PL packing defects as a recruitment mechanism (Boeszoermenyi et al. 2015).

Fatty acid modifications are important for the localization of some Class II LD proteins. The conjugation of fatty acids (e.g., myristate and palmitate) to proteins provides a hydrophobic anchor that facilitates binding to PL membranes. Palmitoylation of the Arf-GTPase activating protein ELMOD2 (Suzuki et al. 2015) and the SNARE protein SNAP-23 (Boström et al. 2007) is required for their LD localization. Palmitoylation is a reversible fatty acid modification that provides a potential switchlike mechanism for regulating the LD association of palmitoylated proteins. Myristoylation is an irreversible fatty acid modification that was recently shown to be required for the LD and plasma membrane localization of FSP1, a Coenzyme Q10 oxidoreductase implicated in preventing oxidative lipid damage and ferroptosis (Bersuker et al. 2019, Doll et al. 2019) and in regulating NADH redox during thermogenesis in brown adipocytes (Nguyen et al. 2020). Additionally, modification by prenylation mediates LD targeting of the aldehyde dehydrogenase ALDH3B2 (Kitamura et al. 2015). It will be important to determine the extent to which other LD proteins are modified by fatty acids and the signals in flanking regions of the protein that contribute to their LD targeting and binding, as several hundred mammalian proteins are modified with fatty acids yet most are not LD localized.

4. UBIQUITIN-DEPENDENT LIPID DROPLET PROTEIN DEGRADATION

Cellular physiology and organelle functions are determined by the composition and integrity of the cellular proteome. Cells have evolved elaborate networks of protein-folding and degradation factors that monitor and maintain the integrity of the cellular proteome by recognizing misfolded proteins and mediating their refolding, sequestration, or degradation (i.e., protein quality control). These same pathways also regulate the levels of folded proteins, modulating the composition of the proteome and enabling cells to adapt to changes in cellular requirements (i.e., protein quantity control). Organelles contain specialized pathways for protein quality and quantity control. For example, proteins in the early secretory pathway can be targeted for clearance either through ERAD (Olzmann et al. 2013a), which mediates the ubiquitin-dependent delivery of substrates to the proteasome, or through a selective ER autophagy pathway known as ER-phagy (Chino & Mizushima 2020), which mediates substrate delivery to the lysosome.

The mechanisms of LD protein quality and quantity control remain poorly understood. LD proteins traffic to and from different subcellular compartments (e.g., the ER and the cytosol), and multiple folding and degradation pathways could be involved in their regulation (**Figure 2**). Several LD proteins are known to be targeted for degradation by the ubiquitin-proteasome system (UPS) under various metabolic conditions, including PLIN1 (Xu et al. 2006), PLIN2 (Eastman et al. 2009, Masuda et al. 2006, Nguyen et al. 2019, Takahashi et al. 2016, Xu et al. 2005), CIDEA (Chan et al. 2007, Qi et al. 2008), CIDEC/FSP27 (Nian et al. 2010, Qian et al. 2017, Zhang et al. 2014), ATGL (Dai et al. 2013, Ghosh et al. 2016, Niyogi et al. 2019, Olzmann et al. 2013b), DGAT2 (Choi et al. 2014, Luo et al. 2018), CGI-58 (Patel et al. 2014), G0S2 (Heckmann et al. 2016, Kamikubo et al. 2019, Yang et al. 2011), PNPLA3 (BasuRay et al. 2017, 2019), and c18orf32 (Bersuker et al. 2018). In the UPS pathway, ubiquitin is conjugated to substrates by the consecutive actions of E1 activating enzymes, E2 conjugating enzymes, and E3 ligases. For most LD substrates, the ubiquitination machinery involved in their degradation is unknown and challenging to characterize, as the cell contains hundreds of E3 ligases and numerous adaptor proteins and accessory factors.
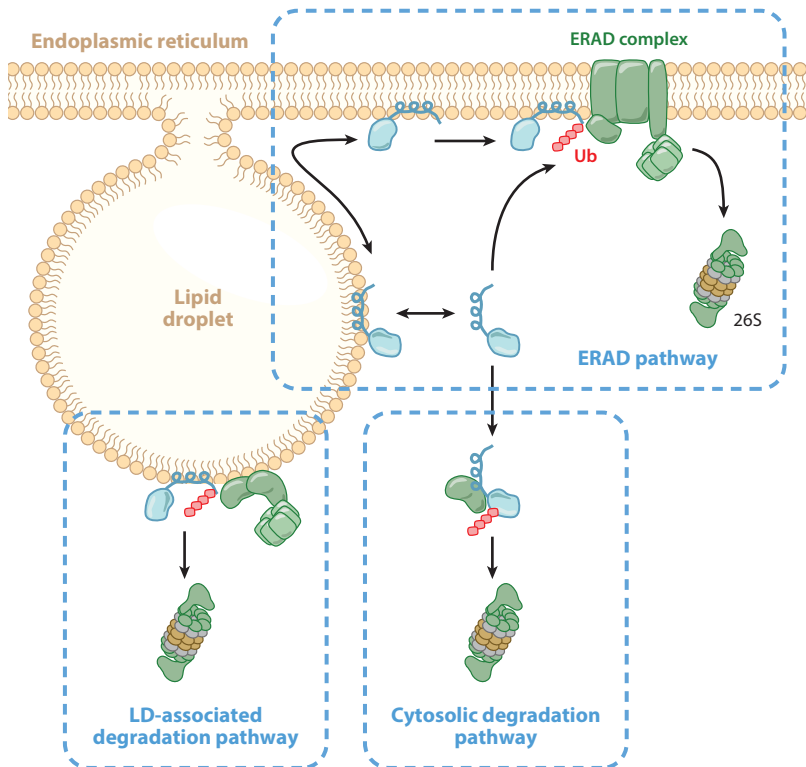
Figure 2

Mechanisms of LD protein degradation. LD proteins are present in multiple cellular compartments and have the potential to be degraded by ubiquitin-dependent pathways in the ER, cytosol, and LDs. Abbreviations: ERAD, endoplasmic reticulum–associated degradation; LD, lipid droplet; Ub, ubiquitin.

4.1. Endoplasmic Reticulum–Associated Degradation

The LD and ER membranes are, at least initially, contiguous, and they continue to be held in close contact by multiple ER–LD tethering proteins (Olzmann & Carvalho 2019). The levels of Class I LD proteins trafficking through the ER en route to LDs may be regulated by ERAD, a process in which a network of factors organized around membrane-embedded E3 ligases mediates substrate recognition, ubiquitination, membrane extraction by the AAA-ATPase p97/VCP (Cdc48 in yeast), and delivery to the proteasome (**Figure 2**) (Olzmann et al. 2013a).

In yeast, the ER-resident E3 ligase Doa10 targets several LD proteins (e.g., Pgc1, Dga1, and Yeh1) for proteasomal degradation (Ruggiano et al. 2016). The hydrophobic hairpins of certain Class I LD proteins act as Doa10 degrons (Ruggiano et al. 2016), possibly because the ER contains machinery capable of recognizing the hairpin as an aberrant structure and/or because the hairpin adopts a distinct, destabilized conformation in the ER bilayer in contrast to a more stable conformation in the LD monolayer. Doa10-mediated degradation of mislocalized LD proteins limits their levels in the ER and leads to their concentration in LDs (Ruggiano et al. 2016), indicating that ERAD is essential for the maintenance of ER and LD proteome compositions. The clearance of mislocalized LD proteins is reminiscent of other forms of spatial quality control such as the degradation of mislocalized ER proteins in the inner nuclear membrane (Foresti et al. 2014,

Khmelinskii et al. 2014) and mislocalized tail-anchored proteins in the mitochondria (Chen et al. 2014, Okreglak & Walter 2014).

The process of Class I LD protein degradation by ERAD is conserved in more complex eukaryotes, as DGAT2 (Choi et al. 2014, Luo et al. 2018) and c18orf32 (Bersuker et al. 2018) are degraded by ERAD in human cell lines. DGAT2, and the evolutionarily unrelated DGAT1, are enzymes that mediate the conversion of diacylglycerol (DAG) into TAG for storage in LDs. In contrast to DGAT1, which is a polytopic ER-resident enzyme (McFie et al. 2010), DGAT2 consists of a membrane-embedded hairpin with its C-terminal active site oriented toward the cytosol (Stone et al. 2006). DGAT2 is present in the ER and traffics to LDs (Stone et al. 2009, Wilfling et al. 2013) or regions of ER tethered to LDs (McFie et al. 2018), where it mediates local TAG synthesis and LD expansion (Wilfling et al. 2013). Two ERAD pathways using distinct E3 ligases, gp78 (Choi et al. 2014) and Hrd1 (Luo et al. 2018), have been implicated in the ubiquitin-dependent proteasomal degradation of DGAT2. Whether gp78 directly recognizes DGAT2 or uses adaptor proteins to mediate their interaction is unclear. In the case of Hrd1-mediated DGAT2 degradation, Axin interactor dorsalization-associated protein (AIDA) has been implicated as an adaptor that couples Hrd1 and DGAT2 (Luo et al. 2018). AIDA is also required for the degradation of the ER-resident lipid metabolism enzymes MOGAT2 and GPAT3, suggesting a key role for AIDA in regulating the levels of multiple components of a TAG synthesis pathway (Luo et al. 2018).

Another Class I LD protein that is degraded via ERAD is c18orf32 (Bersuker et al. 2018). C18orf32 is a small protein with an N-terminal hydrophobic region that is required for its ER and LD targeting (Bersuker et al. 2018). Cells lacking c18orf32 exhibit changes in the lipid composition of their LDs (Bersuker et al. 2018), but the function of c18orf32 is unknown. The degradation of c18orf32 requires the E3 ligase gp78, the rhomboid pseudoprotease derlin-1, and VCP (Bersuker et al. 2018). Inhibition of gp78 increases the levels of c18orf32 on LDs (Bersuker et al. 2018), consistent with a role for ERAD in regulating the LD proteome composition by controlling the pool of Class I LD proteins available to traffic from the ER to LDs.

The features of DGAT2 and c18orf32 recognized by ERAD machinery are unclear. The data in yeast (Ruggiano et al. 2016) raise the possibility that the hairpin region of the proteins could act as the degron. The first putative transmembrane domain of DGAT2 has been proposed to function as a degron (Choi et al. 2014), but this finding is controversial (Brandt et al. 2016). The presence of a hairpin structure is not always sufficient to induce LD protein degradation in the ER. For example, the Class I LD protein UBXD8 contains a hairpin and is stable both in the ER and in LDs (Olzmann et al. 2013b). This may be due to the association of UBXD8 with ER-binding partners such as UBAC2 (Olzmann et al. 2013b) that could prevent recognition of a degron.

Although examples of ERAD-mediated clearance of Class I LD proteins have been reported, whether ERAD targets Class II LD proteins for degradation is less clear because this would require trafficking of the proteins from LDs to the ER. LDs and the ER form dynamic bridging contacts, fusions of the LD PL monolayer with the outer leaflet of the ER bilayer, which provide a mechanism for protein exchange (Wilfling et al. 2013) and could allow LD proteins to access ERAD machinery. In cells with low basal levels of LDs, uninserted PLIN2 is degraded through a UPS pathway that involves the ERAD E3 ligase MARCH6 (Nguyen et al. 2019). MARCH6 and its yeast ortholog Doa10 have been implicated in the N-degron pathway (also referred to as the N-end rule pathway), which recognizes proteins with destabilizing N-terminal amino acids that act as degrons (Varshavsky 2019). Indeed, the initiator methionine of PLIN2 is cleaved, yielding an alanine that is acetylated (Nguyen et al. 2019, Takahashi et al. 2016). This N-terminal acetylated alanine facilitates the binding of MARCH6 and the ubiquitin-dependent degradation of PLIN2 (Nguyen et al. 2019). MARCH6 and Doa10 have also been reported to degrade cytosolic proteins, and, in

some cases, the protein first associates with the outer leaflet of the ER through an amphipathic helix (Stefanovic-Barrett et al. 2018). Whether, in the absence of LDs, PLIN2 inserts into the ER to associate with MARCH6 or cytosolic PLIN2 associates with MARCH6 for clearance is unknown. Oleate-induced LD biogenesis prevents PLIN2 degradation (Masuda et al. 2006, Xu et al. 2005), indicating that PLIN2 LD insertion can impair recognition by the MARCH6-mediated N-degron pathway, despite the presence of the N-terminal destabilizing residue. Whether PLIN2 that has been inserted into LDs can be degraded by MARCH6 or another ERAD pathway during lipolytic degradation of LDs is not yet known.

4.2. Lipid Droplet–Associated Degradation

The association of ubiquitination machinery with LDs raises the possibility of an LD-associated degradation pathway (**Figure 2**). Several proteins implicated in ERAD traffic from the ER to LDs, including UBXD8 and UBXD2, which recruit p97/VCP to LDs through their UBX domains (Olzmann et al. 2013b, Suzuki et al. 2012), and AUP1, which recruits the E2 enzyme UBE2G2 to LDs through its C-terminal UBE2G2-binding motif (Klemm et al. 2011, Spandl et al. 2011). This set of ubiquitination and VCP-recruitment factors is suggestive of a complex that could extract ubiquitinated proteins for proteasomal degradation. However, an LD-associated E3 ligase that cooperates with these factors and substrates for this putative degradation pathway has yet to be identified. AIP4/5, which are recruited to LDs by spartin (Eastman et al. 2009, Edwards et al. 2009, Hooper et al. 2010, Tokunaga et al. 2013), and SMURF1 (Petrasek et al. 2019) have been proposed to function as LD-associated E3 ligases. These proteins have not been observed in most proteomic analyses of LD fractions, although their association may be lost during the biochemical isolation of LDs or be limited to specific cell types. RNF213 (also known as mysterin), which is mutated in the rare cardiovascular disorder moyamoya disease, also localizes to a subset of LDs (Sugihara et al. 2019). RNF213 is a large protein that contains two AAA-ATPase domains and a RING finger domain that confers E3 ligase activity (Liu et al. 2011, Morito et al. 2014). RNF213 competes for LD localization with ATGL, the rate-limiting enzyme in lipolysis, and thereby impacts LD abundance and turnover (Sugihara et al. 2019). The localization of RNF213, and hence its ability to displace ATGL, requires its RING finger and AAA-ATPase domains (Sugihara et al. 2019), but ATGL does not appear to be an RNF213 substrate. Whether RNF213 ubiquitinates any LD proteins is unknown at this time.

An LD-associated protein degradation pathway has also been proposed in plants (Deruyffelaere et al. 2018, Kretzschmar et al. 2018). Oleosins are plant LD scaffold proteins that stabilize LDs and are degraded by the UPS following seed germination. Studies in *Arabidopsis thaliana* indicate that the removal of ubiquitinated oleosins from LDs and their subsequent proteasomal degradation are dependent on PUX10, a UBXD8 ortholog that recruits the VCP ortholog Cdc48 to LDs via its C-terminal UBX domain (Deruyffelaere et al. 2018, Kretzschmar et al. 2018). The signals that recruit PUX10 to the LD, as well as the machinery required for oleosin ubiquitination, remain to be determined.

4.3. Cytosolic Degradation Pathways

Class II LD proteins synthesized in the cytosol may be degraded via cytosolic degradation pathways prior to insertion (**Figure 2**). In addition, as LDs are degraded during lipolysis, they shrink, and protein crowding forces weakly bound proteins off of the LD into the cytosol (Kory et al. 2015). Whether these LD proteins employ cytosolic UPS pathways is mostly unexplored. Nonetheless, ATGL is reported to be targeted for degradation by the E3 ligase COP1 (Ghosh et al.

2016, Niyogi et al. 2019), and G0S2 is targeted for degradation by the E3 ligase RNF126 along with the chaperone Bag6 (Kamikubo et al. 2019). Bag6 and RNF126 play a well-characterized role as a cytosolic complex that recognizes hydrophobic regions of mislocalized secretory proteins that have failed to insert into the ER or have been retrotranslocated in certain ERAD pathways. Whether LD proteins that fail to insert into LDs and mislocalize to the cytosol engage this pathway remains an open question.

5. AUTOPHAGIC DEGRADATION OF LIPID DROPLET PROTEINS

5.1. Bulk Degradation by Lipophagy

Orchestrated by a host of autophagy-related (ATG) proteins, autophagy is an evolutionarily conserved process that mediates the delivery of cytoplasmic components to the lysosome for the catabolism and recycling of macromolecules (Mizushima 2018). Macroautophagy (hereafter referred to as autophagy) involves the sequestration of portions of cytoplasm within double-membrane-bounded vesicles (i.e., autophagosomes) that then fuse with lysosomes to enable the degradation of cargo by lysosomal enzymes. Autophagy can nonselectively degrade portions of cytoplasm, or it can be harnessed for the selective clearance of specific cargo such as protein aggregates and damaged organelles. A seminal study revealed that LDs in hepatocytes are broken down by a selective form of autophagy now known as lipophagy, resulting in the nonselective, bulk degradation of LD proteins and lipids (Schulze et al. 2017, Singh et al. 2009, Zechner et al. 2017).

Lipophagy is regulated by two small Rab GTPases, Rab10 and Rab7, that localize to LDs and are activated under autophagy-stimulating conditions (Li et al. 2016, Schroeder et al. 2015). Rab10 is required for the recruitment of LC3-positive autophagic membranes to LDs. It also forms a complex with the adaptor protein EH domain–binding protein 1 and the membrane-deforming ATP EH domain–containing 2, which together promote phagophore expansion and the engulfment of LDs within the autophagosome (Li et al. 2016). Rab7 regulates the trafficking of multivesicular bodies and lysosomes to LDs (Schroeder et al. 2015). The selective autophagy of many organelles involves organelle-specific receptors that bind to the autophagosome protein LC3 and initiate the assembly of the phagophore around the target organelle. The recruitment of autophagosome membranes can be mediated by organelle-resident receptors that contain Atg8 family–interacting motifs or LC3-interacting regions, such as Atg32, NIX, and BNIP, or by LIR-containing adapter proteins, such as OPTN, NBR1, TAX1B1, p62, and NDP52, that bind to ubiquitinated cargo (Pickles et al. 2018). Whether lipophagy employs a specific receptor or an alternative mechanism for the recruitment of autophagic membranes remains unclear. P62 is one possible candidate for a lipophagy receptor, as it associates with LDs and is required for lipophagy (Lam et al. 2016, Tapia et al. 2019, Wang et al. 2017, Yan et al. 2019). To date no ubiquitination machinery has been implicated in either p62 recruitment to LDs or lipophagy. Deciphering what the mechanisms are that mediate the recruitment of autophagic machinery to LDs and if it is ubiquitin-dependent remain important questions.

Lipolysis and lipophagy can act synergistically in LD catabolism. The removal of PLIN2 by chaperone-mediated autophagy (CMA) (discussed in detail in the next section) is an early step in LD catabolism that is important for the recruitment of ATGL and autophagic membranes to LDs (Kaushik & Cuervo 2015). In hepatocytes, ATGL-dependent lipolysis functions upstream of lipophagy, reducing the size of existing LDs while liberating fatty acids that are reesterified and packaged into small LDs (Schott et al. 2019). LDs in hepatocytes can reach sizes >5 µm in diameter. Lipolysis-mediated reductions in LD volume may be necessary to meet a size threshold compatible with autophagosome engulfment and fusion with the lysosome during lipophagy. The fatty acids released during lipolysis also function as signaling molecules to upregulate lipophagy

gene programs through SIRT1 (Sathyanarayan et al. 2017). These studies indicate that LD size and fatty acid–regulated gene programs are important regulators of lipophagy, although their overall contribution to LD catabolism likely differs according to cell type and metabolic conditions.

In yeast, LDs are degraded by microlipophagy in response to nutrient-depleted conditions, including nitrogen starvation, stationary phase growth, and acute glucose restriction (Graef 2018). During microlipophagy, LDs are not enveloped within an autophagosome as in lipophagy. Instead, LDs are delivered directly to the vacuole (the yeast equivalent of the lysosome) for internalization and breakdown. LD transport to the vacuole may be facilitated by the biogenesis of LDs proximal to the vacuole under nutrient stress conditions and the physical tethering of the ER, vacuole, and LDs at the nuclear ER–vacuole junction by Mdm1p (Hariri et al. 2018, 2019). An essential event in microlipophagy is the striking partitioning of vacuolar lipids and proteins into microdomains, yielding large sterol- and sphingolipid-rich, raft-like liquid-ordered (Lo) domains that mediate LD docking and internalization (Toulmay & Prinz 2013, van Zutphen et al. 2014, Wang et al. 2014). LDs bound at these Lo domains are internalized by ESCRT-mediated inward budding of the vacuolar membrane, and subsequent membrane scission liberates LD-containing luminal vesicles for degradation by vacuolar hydrolases (Vevea et al. 2015). The catabolism of internalized LDs releases sterols that are required for the formation of the Lo domains, indicating a feedforward loop and a reciprocal relationship between LDs and microlipophagy (Seo et al. 2017, Wang et al. 2014). The NPC orthologs Ncr1p and Npc2p also play important roles in facilitating the formation and expansion of the Lo domains by transferring sterols from intraluminal vesicles formed by the multivesicular body pathway (Tsuji et al. 2017). Although LDs are not encapsulated within autophagosomes during microlipophagy, this process still requires core autophagy machinery and a subset of ATG proteins. Autophagy may impact microlipophagy indirectly due to its roles in the trafficking of Ncr1p, Npc2p, and sphingolipids to the vacuole for the formation of vacuolar Lo domains (Tsuji et al. 2017). However, some ATG proteins play direct roles in microlipophagy. For example, during acute glucose starvation, Atg14p transits from ER exit sites to Lo domains in an AMPK/Snf1p-dependent manner (Seo et al. 2017). Atg14p localizes to the edges of the Lo domains and, together with Atg6p, drives the enlargement of Lo domains (Seo et al. 2017). Microlipophagy has also been observed to occur independently of core ATG machinery in response to defects in PC synthesis (Vevea et al. 2015) and the transition through diauxic growth (Oku et al. 2017). Whether microlipophagy occurs independently of Lo domains under these conditions or if Lo domains form through an alternate mechanism are not known. Furthermore, there is currently a lack of evidence demonstrating microlipophagy in mammalian cells.

5.2. Chaperone-Mediated Autophagy

CMA is a protein-degradation pathway that mediates the delivery of substrates to the lysosome for proteolysis (Kaushik & Cuervo 2018). In CMA, substrate proteins containing specific pentapeptide (KFERQ-like) motifs are recognized by the cytosolic chaperone heat shock–cognate 70-kDa protein (Hsc70) and shuttled to the lysosomal surface, where the Hsc70-substrate complex binds to lysosome-associated membrane protein type 2A (LAMP2A). Hsc70 facilitates substrate unfolding and translocation into the lysosomal lumen for proteolytic breakdown by lysosomal proteases. KFERQ-like CMA motifs were identified in PLIN2 (LDRLQ) and PLIN3 (SLKVQ), which target them for CMA (Kaushik & Cuervo 2015). Brief kiss-and-run as well as prolonged interactions between LDs and lysosomes have been observed; these organelle contact sites allow PLIN2 and PLIN3 to be transferred from LDs to lysosomes (Kaushik & Cuervo 2015, Schroeder et al. 2015, Valm et al. 2017). Mutation of the PLIN2 CMA-targeting motif reduces LD–lysosome contacts

(Kaushik & Cuervo 2015), suggesting that a portion of these contacts may be mediated by interactions between PLINs and the CMA machinery. The small GTPase Rab7 has also been implicated in the formation of LD–lysosome contacts, the transfer of PLIN2 from LDs to lysosomes, and lipophagy (Schroeder et al. 2015), raising the possibility that Rab7 may regulate the CMA of LD proteins or a piecemeal autophagic event. The CMA-mediated degradation of PLIN2 is coupled to cellular energetics by 5′-AMP-activated protein kinase (AMPK), which phosphorylates PLIN2 and promotes its CMA during starvation (Kaushik & Cuervo 2016). Interestingly, the CMA-mediated removal of PLIN2 and PLIN3 facilitates the recruitment of lipolytic and lipophagic machinery (Kaushik & Cuervo 2015), suggesting that CMA degradation of PLINs is an upstream, initiating event for LD degradation by lipolysis and lipophagy. The disruption of CMA in cultured cells and in mouse models results in TAG and LD accumulation (Kaushik & Cuervo 2015), likely due to a combination of altered LD breakdown and reduced degradation of CMA substrates associated with lipid metabolism. Whether LD proteins other than PLIN2 and PLIN3 are targeted by CMA remains to be determined.

6. LIPID DROPLETS AND CELLULAR PROTEOSTASIS

While the canonical function of LDs is to store energy as TAG and suppress lipotoxicity, LDs have also been linked to less conventional roles in regulating cellular proteostasis and stress responses. The role of LDs in these processes is unclear but many possibilities have been suggested, including LDs sequestering aggregation-prone proteins prior to their degradation and regulating the ER lipid landscape to promote homeostasis.

6.1. Lipid Droplets and Endoplasmic Reticulum Stress

An imbalance between the ER protein-folding load and the capacity of quality control machinery can lead to the buildup of misfolded proteins in the ER lumen and membrane, a condition generally referred to as ER stress (Walter & Ron 2011). The cell is equipped with an adaptive homeostatic feedback mechanism, collectively referred to as the unfolded protein response (UPR), whose function is to alleviate ER stress (Walter & Ron 2011). The UPR encompasses three pathways initiated by ER transmembrane proteins (Ire1p in yeast; ATF6, PERK, and IRE1 in mammals) that sense the aberrant accumulation of unfolded ER proteins and activate downstream signaling cascades that lead to an expansion in the ER protein-folding capacity by coordinately inhibiting protein translation and upregulating the expression of ER folding machinery, ERAD factors, and lipid biosynthesis enzymes. The UPR can also be activated directly by alterations in the ER lipid composition that cause membrane bilayer stress independent of protein misfolding (Promlek et al. 2011, Volmer et al. 2013). Increased UPR signaling has been observed in yeast treated with saturated fatty acids or lacking key lipid metabolic enzymes (Jonikas et al. 2009, Pineau et al. 2009, Thibault et al. 2012), cultured mammalian cells treated with saturated fatty acids or following inhibition of steroyl-CoA desaturase (SCD1) (Ariyama et al. 2010, Cunha et al. 2008, Minville-Walz et al. 2010), animal models of metabolic diseases (Fu et al. 2011), and humans with obesity (Gregor et al. 2009).

The upregulation of LD biogenesis is a conserved response to ER stress. Yeast upregulate LD biogenesis under genetic or pharmacologically induced ER stress, such as treatment with the N-glycosylation inhibitor tunicamycin or the protein transport inhibitor Brefeldin A (Fei et al. 2009). Mammalian cells also increase LD production under pharmacologically induced ER stress (e.g., tunicamycin, thapsigargin, or bortezomib) (Hartman et al. 2010, Lee et al. 2012), as do the livers of transgenic mice that lack either Ire1 or ATF6 (Yamamoto et al. 2010, Zhang et al. 2011).

Although LD biogenesis appears to be a conserved response to ER stress, it is not yet known why cells increase lipid storage under these conditions.

LDs may serve a protective role that suppresses ER stress. Consistent with this possibility, impairments in LD biogenesis are associated with an upregulation of UPR signaling. LD-deficient yeast strains (LDΔs) lacking the enzymes required for neutral lipid biosynthesis (Δare1, Δare2, Δdga1, and Δlro1) exhibit upregulated UPR signaling (Olzmann & Kopito 2011, Petschnigg et al. 2009) and sensitivity to oleic and palmitoleic acid but not palmitate (Garbarino et al. 2009, Petschnigg et al. 2009). In the absence of neutral lipid synthesis, oleate largely accumulates as a free fatty acid, whereas palmitate is shunted into PLs (Garbarino et al. 2009). Thus, the ability to buffer free fatty acids as neutral lipids or PLs is important for yeast to avoid fatty acid–mediated toxicity. Similarly, LDs are required to prevent ER stress and to provide a buffer for fatty acids to suppress lipotoxicity in mammalian cells. In adipocytes, protein kinase A–induced lipolysis releases fatty acids that are rapidly reesterified and sequestered as TAG in numerous small LDs (Chitraju et al. 2017). Inhibition of DGAT1 (but not DGAT2) during periods of lipolysis impairs LD biogenesis and is associated with increases in UPR markers (Chitraju et al. 2017). The role of DGAT1 in preventing ER stress is not limited to adipocytes, as inhibition of DGAT1 during oleate treatment in preadipocytes is accompanied by ER stress (D. Xu et al. 2018). Disruptions in LD regulation that do not directly impact neutral lipid synthesis can also cause ER stress. For example, the reduction of ER–LD contacts by the depletion of Rab18 or NAG, a component of an ER–LD tethering complex, induces the upregulation of ER stress markers in preadipocytes treated with oleate (D. Xu et al. 2018). In addition, the loss of FIT2, an ER-resident protein that supports LD biogenesis, is associated with ER stress (Becuwe et al. 2018). However, it is unclear if the effect of FIT2 on ER stress is due to the regulation of LD biogenesis or ER morphology and membrane lipid composition through its putative activity as a lipid phosphatase (Becuwe et al. 2018, Hayes et al. 2017). Disruptions in LD biogenesis do not always lead to ER stress, and the importance of LDs in preventing ER stress is likely to be condition specific. For instance, the loss of DGAT1-dependent LD biogenesis during nutrient stress–induced autophagy has no effect on ER stress and instead causes mitochondrial dysfunction (Nguyen et al. 2017). It remains to be determined if the loss of LD biogenesis leads to UPR activation due to alterations in ER protein-folding capacity or in ER membrane lipid composition. The simplest explanation is that the inability to form LDs results in the accumulation of free fatty acids, which can then act as membrane detergents or be channeled into toxic lipid species in the ER. LD biogenesis may also be important to remove specific lipids from the ER to reestablish membrane homeostasis during periods of membrane stress.

6.2. Lipid Droplets as Mediators of Endoplasmic Reticulum Protein Degradation

LDs may mediate ER protein degradation through both proteasomal and autophagic pathways. One model suggests that LDs may temporarily sequester retrotranslocated ERAD substrates en route to the proteasome. Studies in yeast and mammalian cells have demonstrated that LD biogenesis is not generally required for ERAD (Nakatsukasa & Kamura 2016, Olzmann & Kopito 2011, To et al. 2017). Nonetheless, LDs have been reported to transiently associate with two different ERAD substrate proteins prior to their proteasomal degradation: apolipoprotein B-100 (Apo B-100) and HMG–CoA reductase. Polyubiquitinated Apo B-100 accumulates on the surface of LDs or in close proximity to LDs upon proteasome inhibition (Ohsaki et al. 2006, 2008; Suzuki et al. 2012). The close association of ubiquitinated Apo B-100 with LDs suggests that degradation-bound proteins may interact with LDs en route to the proteasome, potentially as a strategy to

prevent their aggregation. However, it remains unclear whether LDs represent an on-pathway intermediate in Apo B-100 degradation or an off-pathway detour that is only evident under conditions of impaired proteasomal clearance. HMG–CoA reductase, the rate-limiting enzyme in cholesterol synthesis, localizes to LD-associated ER subdomains prior to its retrotranslocation to the cytosol (Hartman et al. 2010, Jo et al. 2013). Given that LDs are not generally required for the degradation of many ERAD substrates (Nakatsukasa & Kamura 2016, Olzmann & Kopito 2011, To et al. 2017), only a subset of ERAD substrates may associate with LDs or be degraded from LD-associated ER subdomains. Apo B-100 and HMG–CoA reductase are important regulators of lipid metabolism, and their association with LDs and/or degradation from LD-associated ER subdomains could be related to their cellular functions.

In yeast, LDs may facilitate ER protein degradation by mediating their delivery to the vacuole via microlipophagy (Vevea et al. 2015). In microlipophagy, LDs are delivered directly to the vacuole at sterol-rich subdomains in an autophagosome-independent manner (Seo et al. 2017, van Zutphen et al. 2014, Wang et al. 2014). Yeast strains deficient in the synthesis of PC, the predominant membrane PL, exhibit alterations in their lipid profiles and upregulate LD biogenesis (Vevea et al. 2015). This increase in LDs likely reflects the channeling of excess lipids into TAG due to the inability to convert DAG into PC. The LDs formed under these conditions are degraded through microlipophagy (Vevea et al. 2015). Interestingly, LD fractions were enriched in both polyubiquitinated proteins and the ER chaperone Kar2p, leading Vevea and colleagues (2015) to propose a role for LDs in physically removing unfolded proteins from the ER and facilitating their subsequent degradation by microlipophagy. However, the enrichment in polyubiquitinated proteins detected in the LD fraction may represent proteins found in regions of extensive LD–ER contacts, similar to the structures that harbor proteasome-bound HMG–CoA reductase (Hartman et al. 2010, Jo et al. 2013). Furthermore, the identities of substrate proteins that use this putative pathway for their degradation have yet to be identified.

6.3. Lipid Droplets and Toxic Protein Aggregates

Emerging findings implicate dysregulated lipid metabolism as a key factor in the etiology of Parkinson's disease (PD) and suggest a possible role for LDs in suppressing PD-associated lipotoxicity (Fanning et al. 2019, 2020; Imberdis et al. 2019; Soste et al. 2019; Vincent et al. 2018). α-Synuclein is a small, brain-enriched protein that is implicated in sporadic and familial forms of PD and is the primary aggregated protein within the PD-associated inclusion bodies known as Lewy bodies (Shahmoradian et al. 2019). α-Synuclein binds PLs and oleate (Sharon et al. 2001, Stöckl et al. 2008), and unsaturated fatty acids promote α-synuclein membrane binding, aggregation, and cytotoxicity (Fanning et al. 2019; Jo et al. 2002; Sharon et al. 2001, 2003). The expression of α-synuclein leads to alterations in the cellular lipid landscape, including increases in oleate, DAG, and TAG, as well as an increase in LD biogenesis (Fanning et al. 2019, Outeiro & Lindquist 2003, Vincent et al. 2018). Yeast strains deficient in LD biogenesis are more sensitive to α-synuclein-induced toxicity (Fanning et al. 2019), indicating a protective role for LDs under these conditions. LDs may act to sequester oleate and DAG as TAG, thereby mitigating a portion of α-synuclein toxicity and inhibiting its effects on membrane trafficking. SCD1 (Ole1 in yeast) inhibitors, which reduce the production of monounsaturated fatty acids such as oleate, strongly suppress α-synuclein toxicity in both yeast and cultured human induced pluripotent stem cell–derived neurons (Fanning et al. 2019, Imberdis et al. 2019, Vincent et al. 2018). The mechanism by which α-synuclein expression causes changes in lipid abundance is unknown, as is the mechanism by which oleate promotes α-synuclein toxicity. Neurons have a limited capacity to generate LDs, and recent findings demonstrate that these cells actively traffic damaged lipids via

apolipoprotein E particles to astrocytes, which sequester these lipids in LDs prior to degradation (Ioannou et al. 2019, Liu et al. 2017a). Determining whether this neuronal lipid clearance pathway impacts α-synuclein toxicity and aggregation by reducing neuronal oleate concentrations will be important for future therapeutic research. LDs may also directly promote the clearance of inclusion bodies containing aggregated proteins in yeast, where LDs associate with inclusion bodies and increase their turnover via the release of an SE-derived detergent-like metabolite (Moldavski et al. 2015). Whether this is a general role for LDs remains unexplored.

7. PERSPECTIVES AND OPEN QUESTIONS

Numerous proteomic analyses of LDs have established a catalog of LD proteins in a variety of species, cell and tissue types, and metabolic conditions. The LD proteome composition reflects its role as a central player in cellular lipid and energy metabolism. Determining the composition and functions of heterogeneous LD subpopulations, possibly by leveraging proximity labeling methods, will be an important next step. While defining the LD proteome is crucial, we are still at the beginning of the process of understanding the mechanisms that regulate the LD proteome composition. Recent findings have established two classes of LD proteins defined by their initial insertion into the ER or LDs. The mechanisms that mediate their selective insertion into the ER or LDs and control their partitioning between the ER and LDs remain important areas of investigation. Similarly, while emerging data indicate that LD proteins can be degraded by CMA and the UPS, little is known about the degradation signals presented by substrates, the ubiquitination factors that recognize substrates and target them for degradation, and the mechanisms that regulate degradation under different metabolic states. Furthermore, while LDs clearly function in regulating cellular lipid homeostasis and responses to lipotoxicity, whether they play direct roles as mediators of protein quality control remains unresolved. Given the integral role of LD proteins in regulating all aspects of LD biology, defining the mechanisms that establish and remodel LD proteome composition is essential to understanding the cellular functions of LDs and their connections to human health and disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

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