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REVIEW

Lipid droplets: Open questions and conceptual advances around a unique organelle

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Once viewed as mere lipid inclusions, the past four decades have witnessed an explosion of research into lipid droplet (LD) biogenesis and function. Pioneering cell biology, biochemical, genetics, and lipidomic studies now reveal LDs as active players in lipid metabolism and cellular homeostasis. Here, we discuss some of the major findings that defined LDs as bona fide organelles. However, despite what is known, much needs to be discovered. We highlight five enduring questions that continue to challenge the LD field and discuss a few misconceptions about this remarkable organelle.

More than just fat: Lipid droplets emerge as bona fide organelles

The year 2025 marks the 34th anniversary of the discovery of perilipin-1 (PLIN1) as the first characterized resident protein of lipid droplets (LDs). In the now classic study, the group of Constantine (Dean) Landos and first author Andrew Greenberg used radiolabeling to identify a highly abundant protein in adipocytes that became hyper-phosphorylated during isoproterenol-stimulated lipolysis (Greenberg et al., 1991). Unexpectedly, the phospho-protein was co-purified with the floating "lipid cake" following cellular fractionation. Typically, this lipid fraction was disregarded as the unimportant fat layer, but elegant work by the group revealed that PLIN1 represented a major phosphorylated protein bound tightly to fat droplets. The discovery marked an inflection point in LD biology, as later highlighted by Coleman (2020). Observed for years but generally ignored as mere fat inclusions, the work by Greenberg et al. (1991) indicated that cells encoded specific proteins that coat LDs and that these factors influenced how lipids are stored and utilized by cells. It ushered in a new paradigm that began contemplating LDs as true organelles actively involved in metabolic homeostasis.

In the years following PLIN1 characterization, a flurry of studies revealed other proteins that localize to LDs and influence their function. Pioneering biochemical LD isolations followed by proteomics revealed surprisingly diverse LD proteomes with dozens of abundant proteins (Brasaemle et al., 2004), and isolation protocols were soon standardized, enabling the exploration of LD factors in diverse tissues and organisms (Ding et al., 2013). Genetic screens also began revealing factors that influence LD formation, leading to the first in-depth investigations of LD biogenesis factors like seipin (Szymanski et al., 2007; Fei et al., 2008). More recently, new technologies like mass spectrometry, cryo-electron tomography (cryoET), and advanced live-cell imaging have ushered in a "molecular era" of LD biology, enabling us to understand atomic structures of LD proteins, and how they influence droplet biogenesis. Molecular dynamics simulations and pioneering biophysical work are also peeling away the mysteries surrounding how triglycerides (TG) and steryl esters (SE) are organized into nascent LDs during their creation. As of 2025, our current understanding of LDs as spherical organelles containing hydrophobic cores of TG and SE surrounded by a phospholipid monolayer is well-defined. For more information on LD architecture, we point readers to these recent reviews discussing LD biogenesis, function, lipid and protein contents, and methods for examining them (Olzmann and Carvalho, 2019; Farese and Walther, 2023; Henne, 2023; Wölk and Fedorova, 2024; Dudka et al., 2024; Sapia and Vanni, 2024). The LD field is now a diverse multidisciplinary community with annual international meetings and over >20,000 papers in PubMed discussing this once-orphaned organelle.

With all that is known, what new discoveries await to be uncovered about droplets? Rather than focus on what is known in LD biology, this short review is framed around five open questions about LDs, their contents, and their roles. We also discuss common misconceptions about droplets, which recent findings begin to challenge. These topics highlight an emerging theme of LD research today: that far from being uniform, droplets exhibit remarkable heterogeneity in terms of their lipid and protein compositions, their functions, and how their spatial arrangements within cells influence their functions. Critically, LDs have emerged as an organelle with a unique origin story: despite a number of critical proteins that influence LD formation, their biogenesis appears to be a *lipid-driven* reaction, with proteins providing a mere supportive role as spatial and temporal regulators. This is, of course, a far from comprehensive review of the broad field of droplet biology. We sincerely apologize for any work or references we have omitted due to text limitations. For more in-depth analysis, we point the reader to

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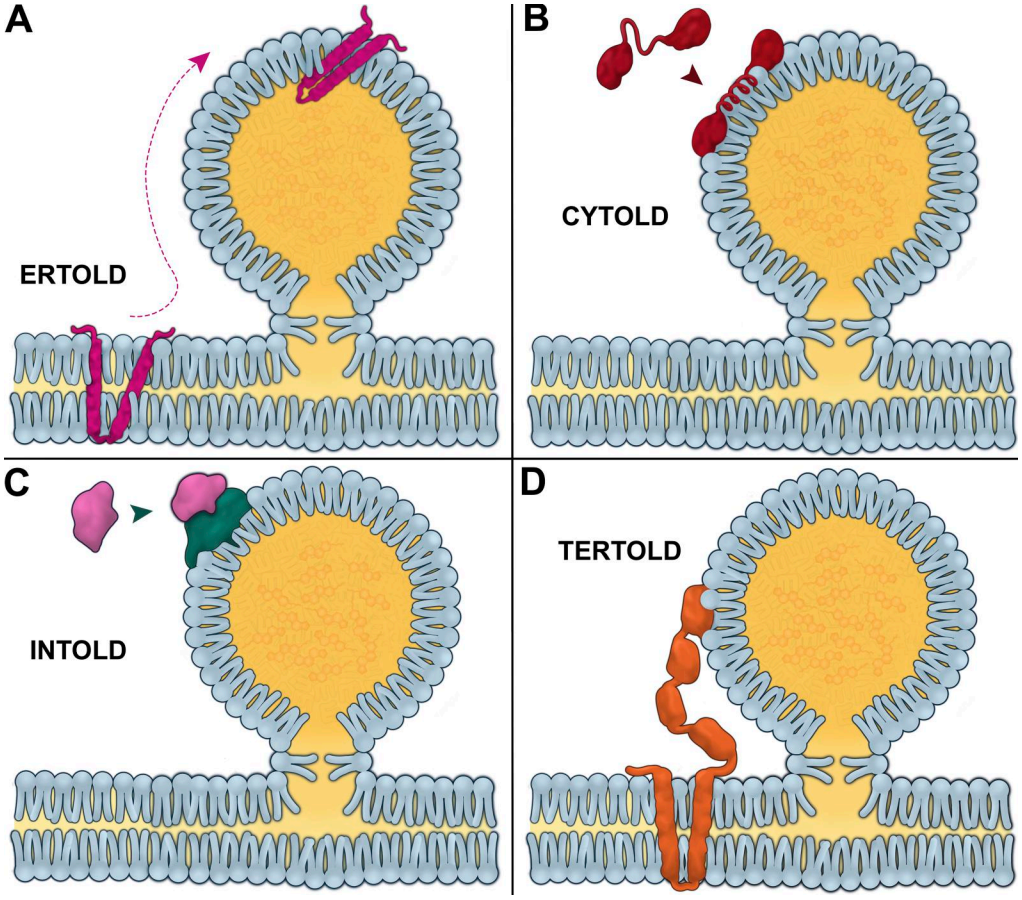

Figure 1. **LD protein targeting: Depiction of four different types of LD-targeted proteins.** Canonical class I (ERTOLD) and class II (CYTOLD) proteins are shown, as well as INTOLD and TERTOLD proteins. **(A)** The class I (ERTOLD) targeting. **(B)** Class II (CYTOLD) targeting. **(C)** Indirect LD association (INTOLD) by binding to another LD-resident protein. **(D)** TERTOLD targeting, which involves ER-anchored proteins that interact with LDs at ER–LD interfaces.

excellent recent reviews on LDs (Mathiowetz and Olzmann, 2024; Zadoorian et al., 2023).

What are the molecular determinants of LD protein targeting?

LDs were initially recognized as organelles because through their proteomes it became clear they played roles in metabolism beyond simple lipid storage. However, how proteins are targeted to droplets remains a pervasive question in the field still under active investigation. While some LD protein targeting principles have emerged, the specific molecular determinants dictating this targeting remain incompletely understood. Two major protein-targeting pathways have been identified: one for integral membrane proteins that are not soluble in the cytoplasm, and a second for proteins that are cytoplasm soluble. Proteins that use the former pathway, known as class I or simply as ER-to-LD (ERTOLD)-targeted proteins, must translocate from the ER membrane to the LD monolayer surface (Fig. 1 A and Table 1). While the full mechanism by which ERTOLD proteins associate with the ER and LD is still being studied, an emerging model is that these factors encode membrane-embedded hairpin motifs, allowing them to integrate into both the cytoplasmic leaflet of the ER bilayer and LD surface (Olarte et al., 2020). Protein translocation from ERTOLD is thus achieved through lipidic bridges connecting the ER and LDs, on which ERTOLD proteins flow.

A model ERTOLD peptide is LiveDrop, essentially a minimal LD-targeting peptide derived from the *Drosophila* lipid biosynthetic protein glycerol-3-phosphate acyltransferase 4 (GPAT4) (Wang et al., 2016). The LiveDrop peptide forms a helix-turn-helix hairpin that inserts into the ER membrane but does not fully traverse the membrane bilayer. LiveDrop also decorates the LD surface, indicating it can traverse both compartments. Indeed, recent *in silica* and experimental evidence suggests such hairpins undergo dramatic conformational rearrangements as they move between the ER or LD environments (Dhiman et al., 2024; Olarte et al., 2022). However, these studies diverge somewhat on aspects of the protein conformational dynamics, and it should be noted that recent evidence suggests hairpins deeply integrate into the ER membrane itself (Dhiman et al., 2024), suggesting unexpected complexity in their conformational dynamics. While LiveDrop studies suggest the LD provides a more energetically favorable conformational environment for some hairpins, different proteins may utilize hairpins for ER or other membrane associations. Further work is needed to understand how different hairpin proteins partition between ER, LDs, and other compartments.

Table 1. **Several examples of different classes of LD-resident proteins**

Examples of different types of LD-associated proteins		
Protein	**Notes**	**References**
ERTOLD (ERTOLD targeting, previously class I)		
GPAT4	The LD marker tool LiveDrop is derived from an LD-targeting motif of GPAT4	Wang et al., 2016
ACSL3	Localizes early to nascent LDs	Kassan et al., 2013
PLIN1	Highly abundant LD coat protein in adipocytes, and one of the first discovered LD proteins	Greenberg et al., 1991 Majchrzak et al., 2024
CYTOLD (CYTOLD targeting, previously class II)		
CTP:phosphocholine cytidylyltransferase (CCT)	Relocalizes from cytoplasm to LD surface to support phosphatidylcholine biosynthesis during LD growth	Krahmer et al., 2011
Max-like protein X (MLX)	Transcription factor, activates glycolytic genes	Mejhert et al., 2020
INTOLD		
CGI-58	Recruited to LDs via perilipin binding to regulate ATGL lipolysis	Yamaguchi et al., 2004
VCP/p97	Can be recruited to the LD surface by UBXD8 to regulate lipolysis	Olzmann et al., 2013
TERTOLD		
FATP1	ER-associated protein that can bind to LDs	Xu et al., 2012
Seipin	Promotes the maturation of nascent LDs to mature LDs	Several including Salo et al. (2016) and Wang et al. (2016)
SNX14	Potential lipid transporter that associates with LDs following oleic acid–stimulated LD growth	Datta et al., 2019
Ice2	ER protein that regulates LD growth	Markgraf et al., 2014

A second class of LD-targeted protein is the class II or cytoplasm-to-LD (CYTOLD)-targeted protein (Fig. 1 B and Table 1). These proteins equilibrate between the cytoplasm and LD surface. Of note, ERTOLD and CYTOLD nomenclatures initially reflected both where these factors were detected (i.e., either the ER or cytoplasm in addition to LDs) and also implied their LD-targeting mechanisms. Class I LD proteins were proposed to associate with the ER network via hairpin-like motifs, whereas class II proteins were more soluble and only loosely associate with LDs via amphipathic helices (AHs) or lipidation moieties. However, recent studies reveal LD protein classification may be more complicated. For example, some ERTOLD proteins utilize AHs to associate with both ER membranes as well as LDs (Pataki et al., 2018). As discussed further below, as mechanisms of LD targeting are better elucidated, an expansion of the ERTOLD and CYTOLD nomenclatures may be required.

How do CYTOLD proteins insert into the LD surface? Mechanistically, this can occur by the insertion of AHs or other hydrophobic moieties into the LD monolayer surface. LD protein surface binding is thought to be primarily driven by irregularities across the LD monolayer surface that expose lipid-packing defects, regions where the hydrophobic core and its neutral lipids are exposed to the aqueous cytoplasm (Kim and Swanson, 2020). Perilipins were canonically viewed as CYTOLD proteins that are attracted to such packing defects on the LD surface, although whether all perilipins utilize this targeting remains to be determined (Olarte et al., 2022), and recent work even indicates PLIN1 is actually an ERTOLD protein (Majchrzak et al., 2024). This underscores the emerging blurriness regarding whether proteins can be neatly fitted into either ERTOLD or CYTOLD categories based on their mechanism of LD targeting, such as use of AHs. In fact, proteomic analysis suggests amphipathic interfacial alpha-helical regions serve as general membrane and LD anchors, further blurring the models for how amphipathic regions dictate LD enrichment per se (Pataki et al., 2018). More work is warranted to fully understand how specific hairpins and AH regions dictate LD targeting and how the balance of ER and LD localization for a given protein is governed.

It should also be noted that LD-targeting mechanisms beyond hairpin- and AH-based targeting have been observed. For example, LD-associated proteins may indirectly associate with LDs by binding to another resident LD protein, rather than by directly attaching to the LD lipid surface (Fig. 1 C). One example is CGI-58, which is proposed to bind to perilipins on the LD surface to regulate lipolysis, and could thus be denoted as a third class of protein that indirectly docks on LDs (Yamaguchi et al., 2004). Another example is the recruitment of VCP/p97 to the LD surface by UBXD8 (Olzmann et al., 2013). In keeping with the nomenclature, we propose these proteins could be referred to as INdirectly targeted-to-LD, or INTOLD, proteins (Fig. 1 C and Table 1).

Additionally, ER transmembrane proteins can remain anchored in the ER membrane while simultaneously binding the LD surface *in trans* at ER–LD interfaces. Examples include FATP1, seipin when associating with mature LDs, SNX14, and potentially the budding yeast protein Ice2 (Xu et al., 2012; Arlt et al., 2022; Datta et al., 2019; Markgraf et al., 2014; Cao et al., 2019). Topologically, these proteins exhibit transmembrane domains and remain anchored to the ER, but associate with LDs either at lipidic ER–LD bridges or by tethering unattached LDs in close proximity to the ER through distinct LD-binding motifs. Such targeting is distinct from direct ERTOLD protein translocation and could be considered a fourth class of LD targeting. We propose such ER-resident proteins could be called "Tethered-to-ER-and-to-LD" proteins, or TERTOLD proteins (Fig. 1 D and Table 1). Other types of LD targeting can be conceptually envisioned (such as LD-to-LD tethering factors such as Cidec/Fsp27 (Ganeva et al., 2023), and future studies should continue to investigate mechanisms of protein targeting and expand the nomenclature as new principles emerge.

What governs the timing of LD protein targeting? First, it is clear that proteins can be targeted to LDs during their initial

translation or after the protein is fully formed. For example, LD-associated ER membrane proteins can co-translationally associate with LDs via interactions with the EMC complex (Leznicki et al., 2022). Posttranslational ER insertion of LD-associated proteins like UBXD8 via PEX3-dependent machinery also enables LD binding after protein synthesis is complete (Schrul and Kopito, 2016). In terms of targeting sequences, whether LD proteins exhibit specific amino acid signal sequences enabling LD targeting, akin to the K-D-E-L or S-K-L motifs that facilitate ER retention or peroxisome targeting, appears unlikely (Kory et al., 2016). In fact, evidence points to a lack of any specific LD-targeting peptide sequence for proteins. In-depth analysis of the LiveDrop amino acid sequence suggests that while there is not a specific amino acid sequence per se that drives LD association, key hydrophobic amino acids, such as tryptophan, are important to impart LD targeting to the peptide (Olarte et al., 2020). Furthermore, the spatial distribution of tryptophan or other hydrophobic or charged residues within the LiveDrop sequence is important since scrambling the LiveDrop sequence perturbs LD targeting (Olarte et al., 2020). Similarly, exciting work on PLIN4 suggests its polar residues greatly influence its stable association on the LD surface and multimerization status (Giménez-Andrés et al., 2021). Importantly, the LD neutral lipid composition also influences how LD-targeting moieties interact with the droplet surface, as different LD lipid compositions differentially recruit model peptides *in vitro* (Chorlay and Thiam, 2020).

Thus, LD targeting may function conceptually more like the signal peptide sequences that target proteins to the ER or mitochondria. These motifs do not have strict amino acid sequences per se but exhibit physicochemical properties (i.e., bulky hydrophobic resides) that spatially encode targeting information. A clear difference is that, whereas signal peptides are generally at the amino terminus of proteins, LD-targeting motifs can exist throughout a protein's polypeptide sequence (Olarte et al., 2022; Kory et al., 2016; Prévost et al., 2018). Another difference is that while signal sequences that target proteins to particular organelles are recognized by dedicated import machineries (like the ER translocon) (Rapoport et al., 2017), there is limited evidence that proteins targeting to LDs need the assistance of such targeting machineries for LD binding. Instead, targeting is typically driven by the LD protein affinity for the LD lipid surface or by binding other proteins already on LDs. A few exceptions to this make interesting examples, however. One is UBXD8, which localizes to both the ER and LDs and requires a farnesylated PEX19 for proper targeting (Schrul and Kopito, 2016). Indeed, elegant work on UBXD8 indicates it is deeply inserted into the ER bilayer with a V-shaped topology but adopts a shallower conformation on the LD monolayer (Dhiman et al., 2024).

Thus, while much is understood about how LD protein targeting is dictated, many key questions remain. Factors including LD size and lipid composition clearly influence protein targeting, since larger LDs would exhibit less protein crowding and potentially expose more lipid-packing defects by which AH-containing proteins could associate (Fig. 2). Similarly, many proteins exhibit LD targeting preferences based on LD lipid composition, which we are still beginning to understand. Specificity can be dictated by protein affinities for specific lipid substrates like TG, which attract enzymes like ATGL (Londos et al., 2005). Similarly, yeast proteins Pet10/Pln1, Tgl3, and TG-associated LD protein 1 (Tld1) preferentially target to TG-rich LDs, and this is likely due to their direct interactions with TG accessible from the LD surface (Gao et al., 2017; Speer et al., 2024). Additionally, it should be noted that many proteins encode AH motifs that enable binding to organelle membranes and/or LDs. Why some proteins prefer membrane bilayer targeting versus LD association requires additional study, although recent work indicates that LD monolayers exhibit higher degrees of lipid-packing defects than membrane bilayers (Prévost et al., 2018).

Mysterious connections: How do LDs and the ER network physically connect (and do they ever truly separate)?

Numerous studies of LD biogenesis indicate a droplet's life begins in the ER bilayer as TG molecules coalesce and phase separate into a lipid emulsion sandwiched between the ER monolayer leaflets (Choudhary et al., 2015; Wang et al., 2016). We now have a comprehensive working model for the order-of-events for this LD biogenesis. As they are synthesized by DGAT enzymes, TG molecules spontaneously coalesce into a "lens" within the ER bilayer when the local concentration exceeds ∼3 mol% TG (Hamilton et al., 1983; Hamilton and Small, 1981; Choudhary et al., 2015; Zoni et al., 2021; Walther et al., 2023) (Fig. 3). Additional TG molecules are partitioned into this lens as it expands, causing it to bulge into the cytoplasm, distending the cytoplasmic leaflet of the ER bilayer with it. The result is a donation of the cytoplasmic leaflet of the ER onto the LD surface, coating the young LD with a phospholipid monolayer that serves as its surface coat. As maturation continues, the LD remains tethered to the ER surface via a lipidic bridge composed of this phospholipid monolayer, which enables the movement of lipids and proteins between the organelles as the LD grows. During this process, numerous proteins, including PLINs, attach to the LD, regulating its maturation, contents, and expansion (Song et al., 2022). While live-cell imaging and computational simulations generally agree on this step-wise model for LD biogenesis, several questions remain. Among them: how do LDs separate from the "mother membrane," the ER? Do they even need to?

A common misconception is that LDs always separate from the ER during their biogenesis. In fact, imaging and biochemical work particularly in budding yeast suggest LDs remain functionally connected to the ER membrane over long time periods (Jacquier et al., 2011). Topologically, this means the phospholipid monolayer on the LD surface remains continuous with the outer leaflet of the ER bilayer. For example, when fluorescently tagged PLINs were targeted into the ER lumen of yeast or mammalian cells, they robustly labeled a crescent-shaped patch on mature LDs that was exposed to the ER lumen, indicating direct ER–LD contact (Mishra et al., 2016). ER–LD contacts are also observed in electron micrographs in many cell and tissue types, although whether many of these represent bona fide junctions or merely ER encounters with the LD surface is often unclear (Dudka et al., 2024; Mahamid et al., 2019). This supports a model where LDs remain attached to the ER via a lipidic bridge throughout much

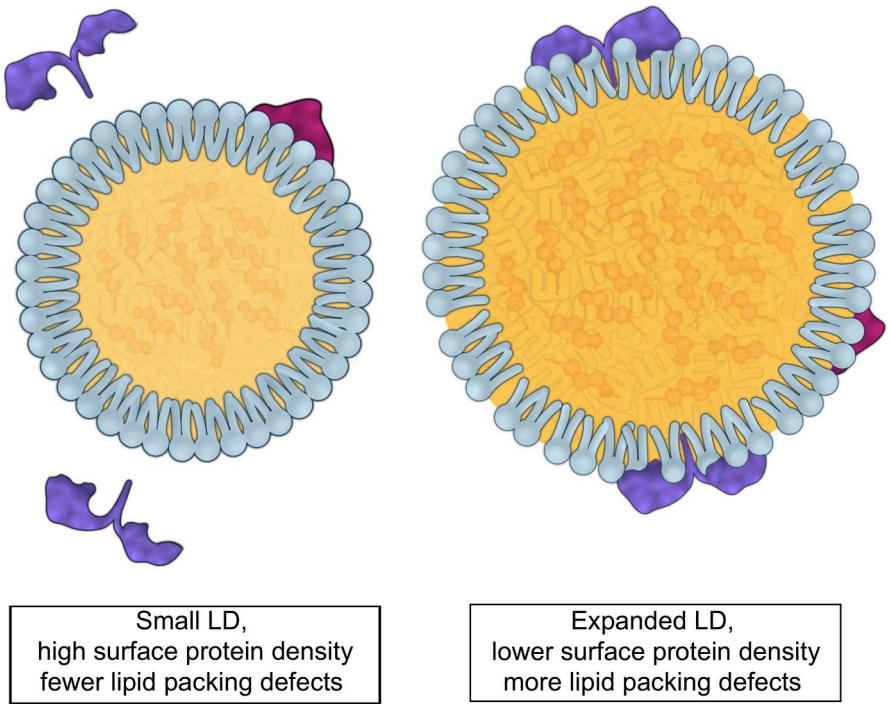

Figure 2. **LD protein targeting due to LD size.** Depiction of LD growth, which increases lipid-packing defects on the LD phospholipid monolayer surface as well as reduces protein crowding of the LD by increasing available surface area on the LD surface.

of their lifetime. Such contacts provide advantages to both organelles and facilitate access of ER proteins to the LD surface, as well as continuous flow of TG from the ER to the LD as it expands (Fig. 3).

If LDs and the ER can remain connected, then how is the timing of protein targeting between the ER and LDs coordinated? To phrase it another way: perhaps the question is not when the ER and LD part ways, but rather how do they regulate traffick between the organelles, or even establish new connections to regulate protein transport between these compartments? Indeed, distinct time-dependent protein targeting to LDs was recently examined by the Farese/Walther group. They stimulated LD biogenesis by treating cells with oleic acid, then monitored whether proteins accumulated on LDs quickly or only after extended time periods. Whereas LiveDrop or PLIN3 target to LDs within minutes of oleic acid stimulation, other LD-resident proteins such as GPAT4 take hours to accumulate on LDs. Surprisingly, genetic screening identified SNARE proteins and related vesicle tethering factors as necessary for these "late arriving" LD proteins. Seipin appears important for this timed protein delivery, suggesting it may selectively regulate LD protein delivery as a "gatekeeper" (Song et al., 2022). As SNAREs are generally transmembrane proteins anchored into membrane bilayers, their recruitment to LD monolayers to mediate such atypical fusion was topologically unexpected. Despite this, they are proposed to act as fusogens, forming new lipidic bridges connecting mature LDs to the ER for late protein delivery (Song et al., 2022). In support of this, previous work from the group

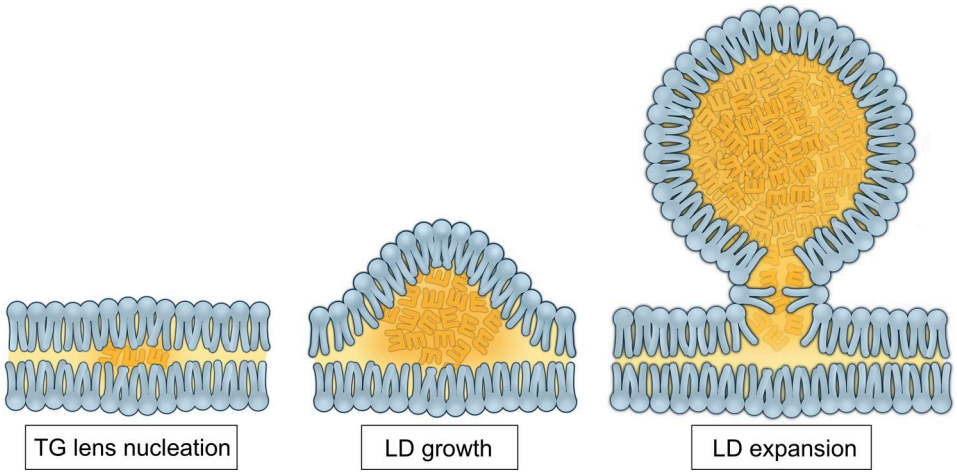

Figure 3. **Stages of LD biogenesis.** Showing nascent LD lens formation (left), LD growth (middle), and LD budding/emergence from the ER bilayer (right).

observed *de novo* ER–LD contacts formed via an Arf1/COPI-dependent process using an elegant cell–cell fusion assay (Wilfling et al., 2014).

Collectively, these findings indicate LDs form stable contacts with the ER, but new ER–LD contacts provide a path to update or alter the LD composition. ER–LD junctions thus provide fine-tuning of LD protein residency on the LD surface, as well as influence lipid flow into LDs. In line with this, lipid flow between LDs can promote LD expansion by Ostwald ripening, which utilizes ER–LD contacts as conduits for TG movement between ER-attached LDs (Salo et al., 2019). Following their maturation, how LDs would sever these contacts with the ER remains unclear. Whether a specific fission factor, analogous to dynamin, would play a role in LD–ER separation is unknown. Further studies are needed to dissect how ER and LD connections are regulated and how this differs in distinct cells and tissues.

What does seipin do exactly, and why does its loss cause multi-organ failure?

A pervasive question remains how LD biogenesis is regulated in different tissues, particularly in tissues that do not manifest large-scale lipid storage in normal physiology. For example, several tissues exhibit LD accumulation only in certain situations, such as during animal fasting (as in the kidney [Scerbo et al., 2017] or in pathologies like neurodegeneration, as Alzheimer noted in 1907 [Alzheimer et al., 1995]). Such cell type–specific LD regulation may be due to the unique assortment of proteins that influence when and how LDs form. One critical LD biogenesis protein is seipin. Genetic screening in yeast identified seipin as important for governing LD size, and it is now appreciated as a critical regulator of LD biogenesis and growth (Szymanski et al., 2007; Fei et al., 2008). Seipin depletion leads to abnormal LD sizes, slower LD formation, and defective LD ripening, and seipin can define sites of nascent LD biogenesis (Wang et al., 2016; Fei et al., 2008; Szymanski et al., 2007; Salo et al., 2016; Salo et al., 2019). Subsequent structural work revealed seipin forms oligomeric cages that enrich at ER–LD junctions that influence LD formation and expansion (Arlt et al., 2022; Klug et al., 2021; Yan et al., 2018). However, why loss-of-function mutations in *BSCL2*, the gene encoding human seipin, causes the general lipodystrophy disease Berardinelli-Seip congenital lipodystrophy type 2 remains poorly understood. This disease impacts multiple organs beyond adipose tissue, and symptoms include muscle and liver failure, hypertrophic cardiomyopathy, and progressive neurological dysfunction (Cartwright and Goodman, 2012). Why would loss of a fat-regulating factor cause such disparate effects?

At the molecular level, several models for seipin function have emerged, which may provide insights into disease pathology in different tissues. Fundamentally, seipin appears to promote the local phase separation of TG into a lipid lens that is probably the first step of LD biogenesis. How precisely this occurs is still being explored and can be viewed from several perspectives related to the proteins and lipids seipin interacts with. From the lipid perspective, seipin appears to act as a "corral" or molecular fence by forming an oligomeric scaffold that promotes TG accumulation in the ER bilayer to nucleate a nascent LD lens (Fig. 4 A). Later, this seipin cage stabilizes the growing LD and the ER–LD lipidic bridge so additional TG can flow into the LD (Kim et al., 2022a; Salo et al., 2016; Wang et al., 2016) (Fig. 3). As stated above, from a protein perspective, seipin may also act as a "gatekeeper" at the ER–LD interface, regulating which proteins pass between these compartments, thus serving as a key regulator of LD protein composition (Song et al., 2022) (Fig. 4 B). This gatekeeper role may also regulate phospholipid movement from the ER to the LD surface, but this needs further investigation. From a protein–protein interaction perspective, a third model for seipin posits that it stimulates LD biogenesis by acting together with specific adaptor proteins that modulate its ability to corral TG (Fig. 4 C). A few of these seipin cofactors have been described, including LDAF1. In this model, seipin is an active spatial driver of where LDs form within the ER network, with the seipin:LDAF1 oligomeric complex lowering the energetic barrier for TG phase separation (Chung et al., 2019; Klug et al., 2021). In support of this, seipin exhibits polar residues (i.e., Ser76 in humans) that directly bind TG in simulations and promote TG clustering (Renne et al., 2022; Prasanna et al., 2021). Mutation of these residues attenuates seipin function, supporting the role of seipin in binding and corralling TG during LD formation. No doubt new seipin-binding proteins will continue to be identified, which will further reveal how the seipin oligomer may be modulated to interact with lipids.

A fourth model views seipin as a regulator of phospholipid biosynthesis that indirectly influences LD biogenesis. This focuses on the observation that seipin loss correlates with elevated phosphatidic acid (PA) levels in the ER network (Fig. 4 D). PA is a central phospholipid and precursor of TG and phospholipids, and increased PA abundance can drive defects in ER homeostasis, promote ER membrane expansion, and alter LD morphology. Indeed, several studies suggest seipin depletion correlates with elevated PA levels, suggesting seipin negatively regulates PA biosynthesis (Fei et al., 2008; Pagac et al., 2016; Yan et al., 2018). A potential mechanism is through influencing glycerol-3-phosphate acyltransferases (GPATs), which display increased activity in seipin-depleted cells (Pagac et al., 2016). In support of this model, recent work suggests that LDs formed in the nucleoplasm (so called nuclear LDs, nLDs) are elevated when seipin is deleted. Seipin appears unable to move onto the inner nuclear envelope, indicating that seipin may influence these nLDs from afar, potentially through PA regulation across the ER network (Sołtysik et al., 2021). As an additional note, purified human seipin was reported to directly bind anionic phospholipids including PA *in vitro*, although whether seipin interacts with PA directly *in vivo* remains unknown (Yan et al., 2018).

It should be noted that these models for seipin function are not mutually exclusive. Seipin may simultaneously corral TG and regulate PA metabolism through GPAT interactions in different cell types. Despite this, how seipin loss impacts different tissues and leads to disease remains unclear. It is important to note that, on the length scale of single cells, seipin depletion in cultured cells can often result in no significant change in total TG levels (Wang et al., 2016). Therefore, seipin does not dictate TG biosynthesis nor abundance per se, but rather spatially organizes TG pools and promote TG storage into LDs. A tempting

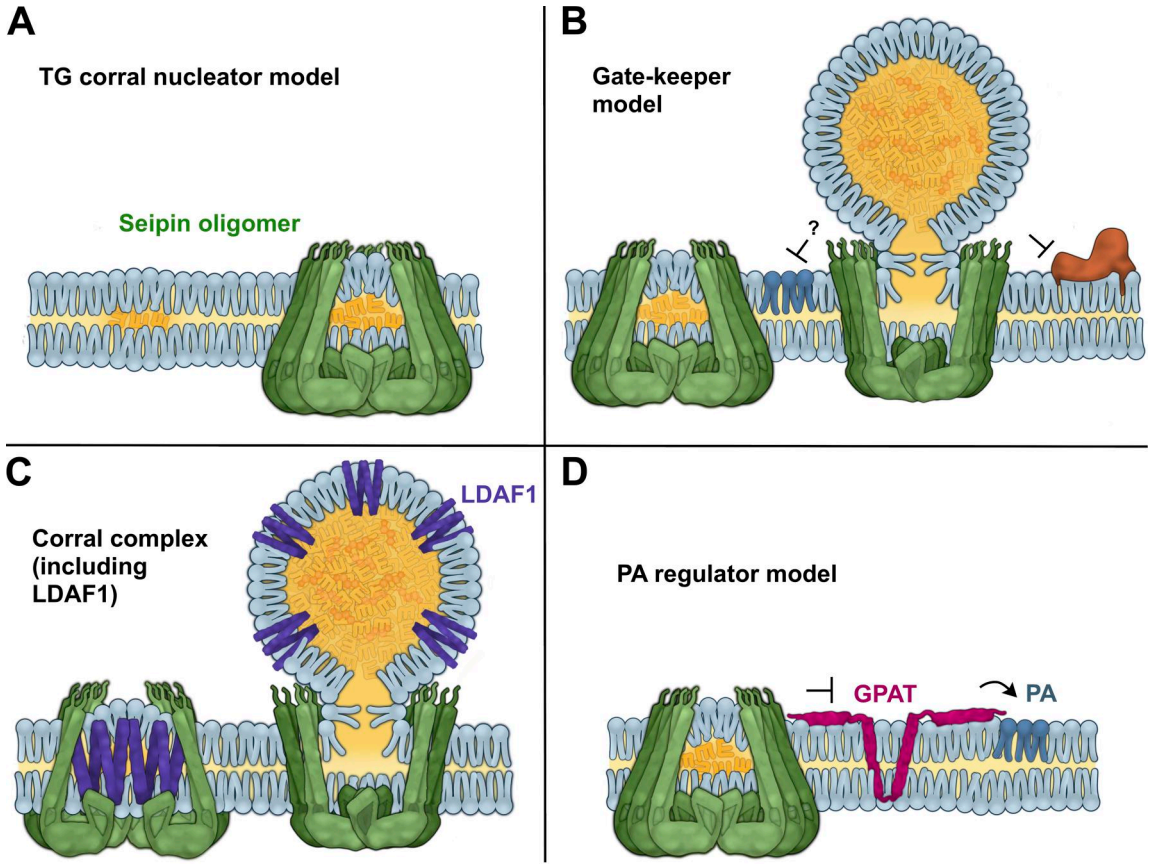

Figure 4. **Models of seipin function from lipid and protein perspectives. (A)** Model of seipin as a LD-nucleating agent that promotes nascent TG lens to form in the ER membrane. **(B)** Model of seipin as a protein gatekeeper that regulates the movement of proteins and potentially some lipids between the ER and LDs. **(C)** Corral complex model of seipin as an oligomer that complexes with various adaptor proteins like LDAF1 to modulate its structure and interactions with lipids. **(D)** Model of seipin as a regulator of PA biosynthesis. Seipin loss is associated with PA accumulation in the ER network.

possibility is that loss of seipin perturbs not just TG storage in LDs but also indirectly perturbs TG loading into ApoB-lipoproteins that are secreted into the blood, which would compound pathologies in tissues like liver or intestine, where ApoB-containing lipoproteins are produced.

It should also be noted that seipin mutations drive a broad variety of clinical manifestations (Cartwright and Goodman, 2012). For example, seipin loss can lead to general lipodystrophy, but the gain-of-function mutations N88S and S90L, which perturb protein glycosylation and protein aggregation, promote the autosomal dominant motor neuron disease Silver syndrome/SPG17 (Ito and Suzuki, 2009). Recent commentary suggests fat loss caused by loss of seipin may actually promote tissue pathology by generally elevating cellular lipotoxicity, rather than disrupting TG stores, since defects in storing lipids would lead to elevated fatty acids and membrane stress (Rao and Goodman, 2021). Further work is needed to unravel how our understanding of seipin at the cellular level influences organism-wide physiology and pathology.

How are LD size, abundance, and spatial position determined?

Different cell and tissue types display dramatic differences in LD size and abundance. Several models have been proposed explaining how LD size and number are influenced, but much remains to be determined regarding how these properties are ultimately regulated. In one model, LD size is governed by the flow of newly synthesized TG from the ER into growing LDs. Computational work indicates LDs form stochastically in local regions of the ER network where the TG concentration reaches a threshold of ~3 mol% (Hamilton et al., 1983; Hamilton and Small, 1981; Kim et al., 2022b). If local TG production at the ER remains high, LDs expand in size. Thus, LD size and number are tightly coupled to rates of TG synthesis. Low rates of TG synthesis would lead to TG incorporation into already formed LDs, whereas elevated TG synthesis would favor nascent LD biogenesis events and an increase in total LD number (Fig. 5, A and B). This is supported by experiments where LD number and size both increase if cells are treated with oleic acid, a stimulant of TG biogenesis (Wang et al., 2016; Datta et al., 2019).

LDs can also grow through LD–LD homotypic fusion or direct lipid exchange between droplets (Fig. 5 C). Both mechanisms provide means for LDs to exchange lipids, the first (fusion) through combining two or more separate LDs into a single LD, whereas the second mechanism provides a means to retain separate droplet compartments but have them exchange lipids through lipid flow. Both mechanisms have been proposed to act in cell physiology and are currently undergoing active research. LD–LD homotypic fusion is almost certainly utilized in

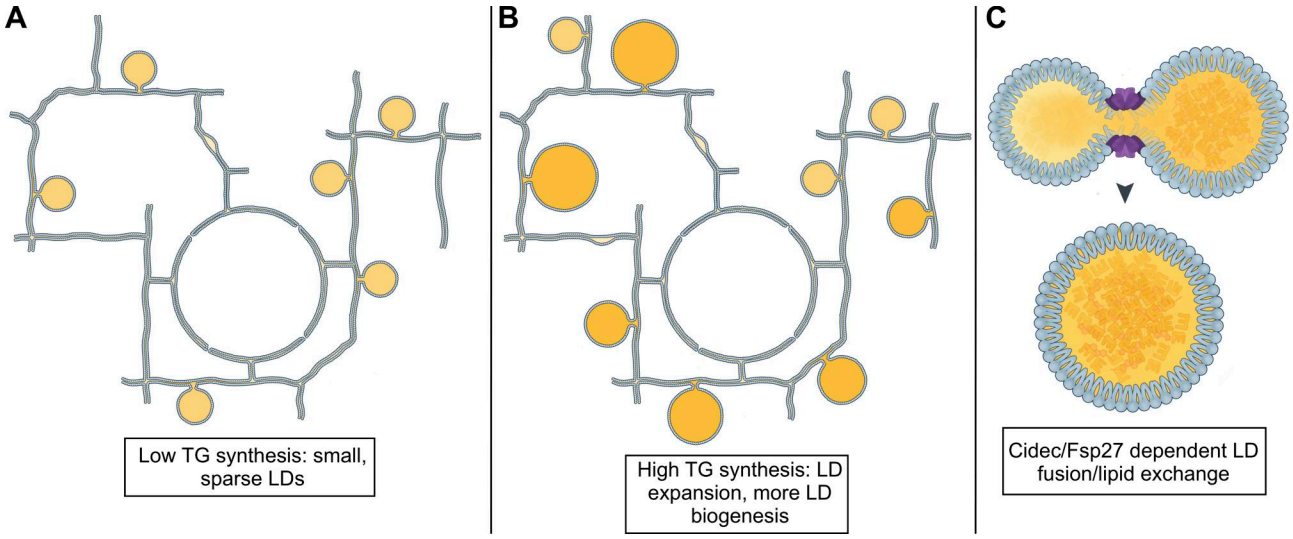

Figure 5. **Mechanisms of LD size control and spatial organization. (A)** Model of LD biogenesis as it is affected by TG biosynthesis. Low rates of TG synthesis in the ER results in small, sparse LDs. **(B)** Elevated TG biosynthesis in the ER drives both more LD formation as well as LD growth as TG is deposited into LDs for storage. **(C)** Depiction of Cidec/Fsp27-mediated LD–LD fusion.

mammalian white adipocytes to form the singular unilocular LD (Sun et al., 2013), although further work is needed to investigate this process. While LD fusion and lipid exchange is not well understood, some molecular machinery has been identified. Cidec has been implicated in mediating the docking and fusion and/or exchange of lipids between LDs (Wu et al., 2014; Gong et al., 2011). Cidec detectably enriches at LD–LD contacts (Ganeva et al., 2023). Of note, pressure differences between small and large LDs can naturally drive LDs to fuse. Since the pressure within a smaller LD is higher than a larger LD, such pressure differences can promote LD–LD fusion (Jüngst et al., 2013). How proteins like Cidec or other factors regulate such spontaneous fusion or lipid exchange remains to be explored. Relatedly, how LDs are able to reorganize their densely crowded protein coat, which could inhibit LD–LD fusion by acting as a shell and sterically hindering direct lipid flow, remains unknown.

A third mechanism for determining LD size posits that distinct LD pools are maintained by organizing them into spatially distinct subsets. This LD spatial positioning and organization appears to be largely regulated by LD associations with actin or microtubule networks (as nicely reviewed by Kilwein and Welte [2019]). *Drosophila* embryos contain LDs that traffic on microtubules in a process requiring *Drosophila* perilipin LSD2 (Welte et al., 2005). Some *Drosophila* tissues also exhibit distinct LD subpopulations arranged in spatial patterns. The *Drosophila* larval fat body (FB) contains large and small LDs that are polarized to different regions of the FB cell interior. Small peripheral LDs (pLDs) are polarized to the cell surface, whereas larger LDs reside in the perinuclear and central cytoplasmic space (Ugrankar et al., 2019). Remarkably, pLDs are encircled by a dense network of cortical actin cytoskeleton, which appears to anchor pLDs near the cell surface and limits their growth. This actin meshwork is primarily composed of actin subunit Act5C, and its tissue-specific depletion leads to defects in LD morphology, including larger LDs, suggesting the actin network regulates LD size and position (Fig. 6 A) (Diaconeasa et al., 2013; Ugrankar et al., 2022).

LD–cytoskeleton interactions also clearly govern how LDs move within the cell. In mammalian cells, LDs move along microtubule tracks with motor proteins (Targett-Adams et al., 2003) (Fig. 6 B). LDs can also "hitchhike" by attaching to early endosomes that actively move along microtubules through dynein-dependent transport (Guimaraes et al., 2015). Interesting work in *Drosophila* shows that during embryo development, LDs migrate via cytoskeletal interactions from nurse cells into developing oocytes to serve as platforms for histone storage prior to embryo cellularization (Giedt et al., 2023; Yu et al., 2011; Li et al., 2012). In human cells, such as U2OS, the actin cytoskeleton, non-muscle myosin II, and formin-like 1 are required to spatially separate LDs, indicating the actin network provides LDs with organization and spatial positioning (Pfisterer et al., 2017).

LD sizes can also be actively tuned by lipid mobilization, whether by enzymes on the surface of LDs like ATGL or by hydrolases in lysosomes after LD autophagy (i.e., lipophagy), and indeed, ATGL activity can promote both lipolysis and lipophagy in hepatic tissues (Sathyanarayan et al., 2017). Human hepatocytes also display decreased size in response to nutrient depletion, and it has been proposed that TG lipolysis may shrink large LDs so they can then be subsequently digested via macroautophagic turnover when they reach a smaller diameter (Schott et al., 2019). Thus, LD size can be tuned by lipolysis to influence their function in response to metabolic cues (Fig. 6 C).

What functions do LDs of different sizes provide for cells? Since small LDs have a higher surface area-to-volume ratio compared with larger LDs, it is advantageous to store TG in many small LDs if a cell needs to quickly mobilize lipids during acute bioenergetic demand. This is consistent with LD morphologies observed in mammalian brown adipocytes, where LDs provide fatty acids to mitochondria for fatty acid oxidation (Benador

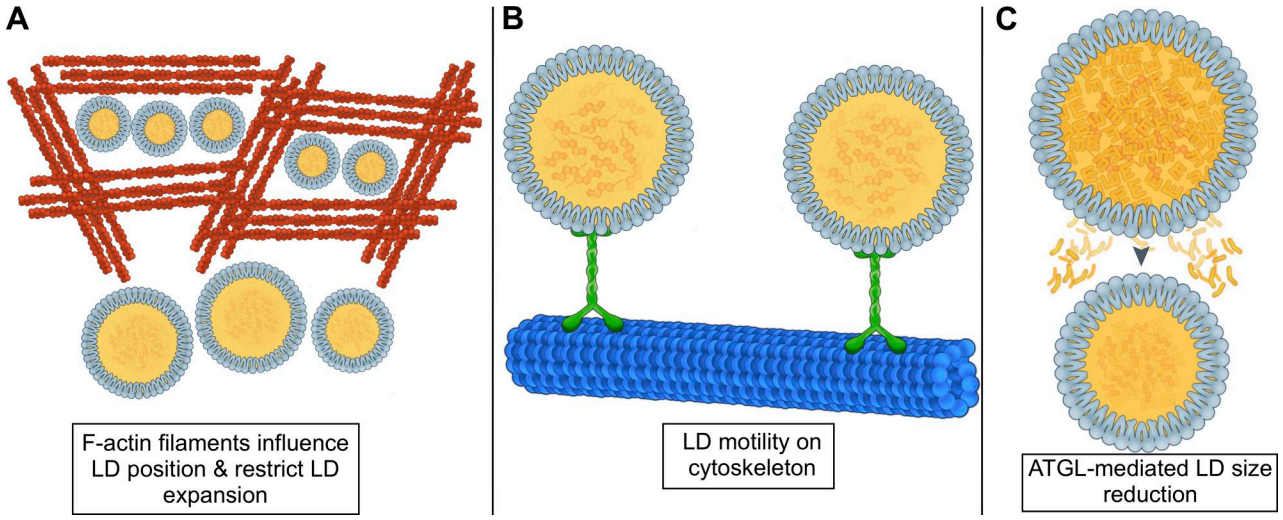

Figure 6. **Further mechanisms of LD size control and spatial organization. (A)** LD size and spatial positioning controlled by surrounding LDs with an actin meshwork, which restricts LD movement and growth. **(B)** LD movement from actin-myosin–based translocation. **(C)** LD size reduction from lipolysis.

et al., 2018). The large unilocular LD of a white adipocyte by contrast provides a more long-term fat storage depot.

LDs in specific tissues can exhibit remarkable size consistency, implying LD size is highly regulated at the cell and tissue levels. In one unusual example, extremely uniform LDs accumulate in *Drosophila* embryos and serve as docking platforms for histones prior to the cellularization of the early embryo (Stephenson et al., 2021). We are only beginning to understand the mechanisms by which this size consistency is regulated, but it likely involves tight control on TG biosynthesis and channeling into LDs. Additional work on the factors that regulate LD growth and fusion will provide further answers to these pressing questions.

How do cells create and maintain compositionally and functionally unique LD populations?

It has long been noted that even in the context of single cells, there are distinct LD pools that are decorated by specific proteins. A classic example are 3T3-L1 preadipocytes that exhibits LD populations that decorated primarily with PLIN1, PLIN2, or PLIN3 (Wolins et al., 2006). This LD heterogeneity was initially thought to be dictated simply by LD age, but recent studies reveal proteins target to LD subsets for many reasons. Other factors such as LD size may also influence the LD proteome. *Drosophila* encode two PLINs termed LSD1/PLIN1 and LSD2/PLIN2, and these localize to differently sized LDs in the larval FB, suggesting LD size can be a determinant of LD targeting bias (Bi et al., 2012). Biochemical fractionation of small and large LDs from human cells also reveals that they contain distinct proteomes (Zhang et al., 2016). The mechanism of this size-dependent protein targeting is unclear but may be due to changes in the LD monolayer as well as the molecular crowding and protein–protein competition for access to the LD surface.

LDs also exhibit distinct phospholipid and neutral lipid compositions. LDs generally contain both TG and SE, and these can be present at different ratios within the hydrophobic LD core. Notably, the TG:SE ratio influences not only the overall LD lipid composition but also the biophysical phase of the SEs within the LD. This is due to the biophysical properties of SE, which spontaneously arranges into smectic liquid-crystalline lattices at physiological temperatures (Kroon, 1981) (Fig. 7).

Liquid-crystalline deposits of SE can be directly observed in LDs through several imaging technologies. In human HeLa cells, stresses such as mitotic arrest or arsenite treatment resulted in populations of LDs containing liquid-crystalline deposits of SEs in the LD core, which were visible as onion-like layers with a regular spacing of ∼3.4 nm by cryoET (Mahamid et al., 2019). Subsequent studies using yeast and human cells indicate that the ratio of SE and TG within the LD interior dictates whether SEs form a liquid-crystalline phase. In essence, the accumulation of SE beyond a critical threshold causes it to phase transition, indicating TG within the LD core essentially maintains SE in its soluble phase (Rogers et al., 2022; Dumesnil et al., 2023). Thus individual cells can contain mixtures of amorphous and

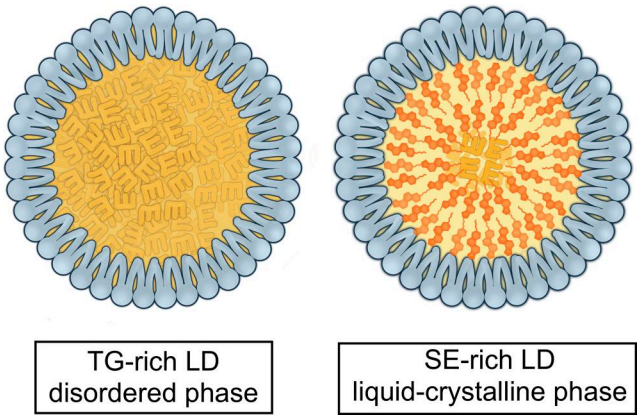

Figure 7. **Compositionally distinct LDs by neutral lipid composition.** Left: TG-rich LD with a disordered neutral lipid core. Right: SE-rich LD with a core of smectic liquid-crystalline SEs arranged in a lattice inside the LD core.

liquid-crystalline LDs, and this could be modulated by fine-tuning SE biosynthesis or TG lipolysis. Intriguingly, liquid-crystalline LDs in yeast exhibit distinct surface proteomes compared with amorphous LDs, suggesting different roles for these LDs in cell physiology (Rogers et al., 2022). Human cells can exhibit SE-rich LDs that are closely associated with the nuclear envelope and appear to play roles in inflammatory signaling (Szkalisity et al., 2025). Anecdotally, SE-rich LDs are generally smaller than TG-rich LDs (Dumesnil et al., 2023; Rogers et al., 2022), suggesting neutral lipid composition influences LD size and potentially abundance.

LD subpopulations can also be distinguished by their spatial arrangements within cells, and this positioning appears to influence LD function. Budding yeast exhibit a LD subset clustered near the yeast vacuole/lysosome, and this LD pool is decorated by specific proteins that influence its turnover through microlipophagy, the process of direct LD envelopment into the yeast vacuole. A genome-wide screen revealed LD-organizing (Ldo) proteins that specifically enrich on these LDs situated in close proximity to the nucleus-vacuole junction (NVJ), an inter-organelle contact site involved in lipid metabolism (Eisenberg-Bord et al., 2018). Parallel work characterized the Ldo proteins as metabolically linked LD-associated proteins (Teixeira et al., 2018). Ldo proteins Ldo45 and Ldo16 decorate NVJ-associated LDs and appear to function as LD adaptors to promote their engulfment into the vacuole by binding to Vac8 (Diep et al., 2024; Álvarez-Guerra et al., 2024). Notably, other LD subset-localizing proteins also regulate LD turnover. The yeast protein Tld1 targets LD subsets, and this targeting is dependent on the neutral lipid composition of the LD. Tld1 LD targeting requires TG, and Tld1 fails to localize to LDs in yeast that do not synthesize TG (Speer et al., 2024). In line with this, Tld1 depletion elevates TG lipolysis, suggesting Tld1 decorates and potentially "protects" specific LDs from lipolysis. Other proteins that decorate TG-rich LDs have been identified in yeast, such as the perilipin Pln1, indicating that LD composition can govern the proteomic landscape of the LD subsets (Gao et al., 2017).

Closing

Since they were initially observed in the late 19th and early 20th centuries and regarded as inert fat inclusions, our perception of LDs has undergone a dramatic transformation. The identification of proteins like PLINs and seipin that associate with LDs and regulate their formation, combined with the diverse roles they are appreciated to play, have elevated these inclusions into bona fide organelles with active roles in metabolism and human diseases. In the 21st century, as the world struggles with obesity and metabolic diseases as major health challenges, LDs have ascended to key players in clinical studies of metabolism. Advances in imaging and biochemical technologies like cryoET and lipidomics continue to reveal the structural and compositional diversity of LDs even within single cells. These discoveries highlight that, like other organelles, LDs serve context dependent and varied roles in physiology. Understanding these nuances and the fundamental mechanisms that dictate LD organization, composition, and networking with other organelles promises further discoveries into how these once-orphaned organelles secretly govern important aspects of cell and organismal homeostasis.

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