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Moving out but keeping in touch: contacts between endoplasmic reticulum and lipid droplets

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The formation of neutral lipid filled and phospholipid monolayer engulfed lipid droplets (LDs) from the bilayer of the endoplasmic reticulum (ER) is an active area of investigation. This process harnesses the biophysical properties of the lipids involved and necessitates cooperation of protein machineries in both organelle membranes. Increasing evidence suggests that once formed, LDs keep close contact to the mother organelle and that this may be achieved via several, morphologically distinct and potentially functionally specialized connections. These may help LDs to dynamically respond to changes in lipid metabolic status sensed by the ER. In this review, we will discuss recent progress in understanding how LDs interact with the ER.

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Introduction

Lipid droplets (LDs) are ubiquitous intracellular storage organelles specialized in the storage of excess energy in the form of neutral lipids [1,2]. LDs are engaged in contacts with several organelles, but have the most intimate relationship with the ER [3,4]. The ER is not only the site of LD biogenesis, but may be a life-long partner of this organelle, enabling dynamic remodelling of LDs in response to cellular energy status and needs.

The neutral lipid core of LDs consists of triglycerides (TAG) and cholesterol esters, while the phospholipid monolayer is primarily composed of phosphatidylcholine (PC). The majority of both neutral lipid and PC synthesizing enzymes are not present on LDs [2], implying that contacts of LDs to the ER are needed to facilitate LD growth. The presence of a monolayer makes LDs unique as cellular organelles. Its main function is to shield the hydrophobic core from the aqueous cytosolic environment, acting as surfactant stabilizing the droplet, and its biophysical properties confer specificity to the LD proteome [5]. Here, we review some of the recent advances in understanding LD forming sites in the ER as well as the morphology, stability and protein composition of ER–LD contacts.

LD forming sites in the ER

LDs originate from the ER that harbours the majority of enzymes required for neutral lipid synthesis. In mammalian cells, the terminal step of TAG synthesis is catalysed by DGAT1 and DGAT2 enzymes that reside in the ER, although DGAT2 and its yeast homologue can also be located at the LD monolayer [6–8]. In the current model of LD biogenesis, newly synthesized neutral lipids are initially relatively freely diffusing within the ER bilayer. In the absence of LDs, TAG synthesizing machinery appears dispersed around the ER [8,9], and it is not known whether these initial steps take place in specified subdomains. However, with rising local concentrations, neutral lipids have the tendency to aggregate, which might lead to formation of lens-like structures in the ER bilayer. Such lenses have been predicted by simulations [10] and also observed in yeast by electron microscopy [11]. Subsequently, the coalescence of neutral lipids buds out towards the cytosol, forming a nascent LD.

Several candidate proteins acting at the initial steps of LD formation have been identified, including members of the perilipin family, ACLS3, FITM2, lipin, seipin and Rab18. Perilipins may aid in LD budding by accumulating at ER regions containing high contents of neutral lipids or diacylglycerol (DAG) and may thus help to stabilize nascent forming LDs [12•,13–15]. ACSL3, a key acyltransferase in the generation of TAG in mammalian cells, is enriched at the sites of LD formation [16], producing possible localized metabolic hotspots for activated fatty acid generation [17•]. Lipin mediated DAG synthesis has been shown to be important for nascent LD formation in yeast [18], while FITM2 has been proposed to shuffle TAG to the forming LD [19,20] and control local DAG levels at LD forming sites [21•]. In yeast, FITM2 is present at LD forming sites and may facilitate LD budding towards the cytosol [11,21•]. Finally, seipin and Rab18 are enriched at ER–LD contact sites, with suggested functions therein (see below).

Furthermore, increasing evidence speaks for the importance of the local ER membrane lipid composition in the LD forming sites. The intrinsic curvature of phospholipids was found to regulate LD budding [21•]. Negatively curved lipids, such as DAG, facilitated LD embedding in the ER while positively curved lipids, such as lysolipids, facilitated budding out of the LD. In another study, conducted primarily with artificial LDs in model bilayer membranes, the size of LDs could be controlled by the phospholipid composition of the bilayer through effects on surface tension. Different phospholipids were found to have differential LD budding aptitudes, and decreasing surface tension (such as accumulation of lysolipids) favoured egress of neural lipids, resulting in budding of small LDs [22••]. Indeed, in simulations LD budding can occur spontaneously, that is without proteins [23]. Overall, these observations suggest that LD formation could be a process driven by lipids, with proteins playing important regulatory roles in controlling their local concentrations.

Further advances in the field necessitate more detailed information on the lipid composition of the LD forming ER subdomains. This is a challenging task but steps forward have recently been taken. In yeast, the concomitant depletion of seipin and Pex30, a peroxisomal biogenesis factor, prevented LD formation, with accumulation of TAG in the ER membrane [24••]. Interestingly, this defect was largely rescued by modulation of the ER phospholipid composition. This prompted the authors to propose that in the absence of seipin and Pex30, LD formation was fully-dependent on the ER phospholipid composition. Remarkably, these findings also emphasize, together with other recent studies [25,26••], the intimate relationship between LD and peroxisome biogenesis sites. While LDs and peroxisomes are structurally distinct, both form at ER subdomains and play important roles in lipid metabolism.

LDs as constituents of the ER network

The overall structure of the ER network, its dynamics and subdomain organization may contribute to LD formation and ER–LD junctions. Interestingly, key ER–LD contact proteins Rab18 and FITM2 have been shown to be important for ER tubular network morphology [27,28]. The high membrane curvature in tubules or tubule regulating proteins might somehow facilitate LD formation. In support of this, a number of proteins important for ER network formation and maintenance have been shown to effect LD biogenesis [29–33]. An interesting recent proposal is that ER shaping proteins could modulate the surface tension of the ER and thus effect LD budding [34].

The ER network is continuously reshaped by the cytoskeleton, and both acto-myosin and microtubule networks have been implicated in LD trafficking and maintenance [1,35,36]. How ER–LD contacts are regulated during LD trafficking is not known, but in a recent study in Cos-7 cells ER–LD interactions were found not to be altered by disturbing the microtubule cytoskeleton [37]. However, there may be cell-type-specific differences, as the formation of ER–LD contacts was proposed to be kinesin-1-dependent in hepatocytes [38]. Given that cytoskeletal rearrangements may impose elastic stress on the membrane, they might also contribute to ER–LD contact stability, for example via alterations in ER or LD surface tension.

Studies in yeast have identified a subpopulation of LDs that form at nuclear-ER vacuole junctions and contain a specific subsets of LD proteins [39•,40]. These LDs are important during nutritional stress and can be modulated by protein factors recently linked to seipin [40,41], suggesting that spatial coordination of ER–LD contact sites may play a role in LD diversification.

Morphological features of ER–LD contacts

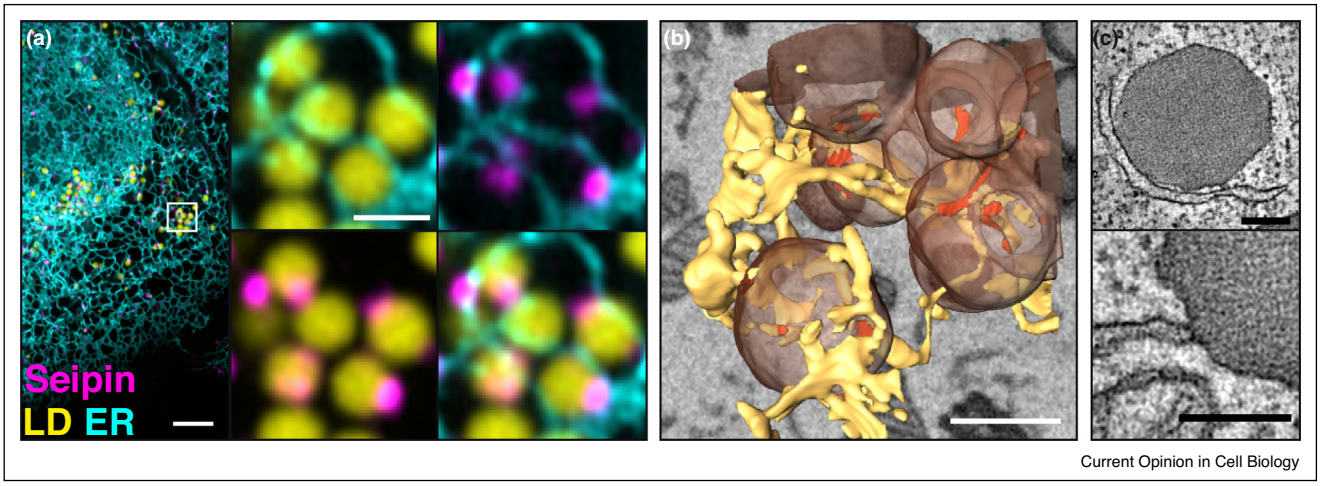
ER–LD contacts with variable morphology have been reported. These include lipidic bridges, where the LD monolayer appears to be directly continuous with the cytosolic leaflet of the ER bilayer [4]. Such discrete, relatively well-defined and small-sized contacts are visible in electron microscopic images in numerous cell types [7,42•,43,44,45•,46–51] (see also Figure 1). This also seems to hold for the recently characterized intranuclear LDs [52,53]. These connections often appear to have a finger-like protrusion extending from the ER. More extensive ER–LD contacts, where the LD appears to lie like an egg in a cup, have also been observed (for example in [8,54,55] and Figure 1). Finally, proteinaceous tethers between the ER and LDs have been noted [42•]. While it is likely that contacts with variable appearance are functionally distinct, direct evidence for this is still lacking.

Extent and stability of ER–LD contacts

Mounting evidence suggests that the formed LDs remain associated with the ER [8,45•,46,54] and in fact, there is little evidence for total dissociation of LDs from the ER. By quantitative 3D-EM in human A431 cells, we found all LDs to be associated with ER membranes both during early and later stages of LD biogenesis [45•], and LDs without ER connections were only observed in seipin-deficient cells. High resolution live cell imaging of Cos-7 cells also indicated that virtually all LDs are in contact with ER membranes [37]. However, mature LDs in HeLa cells may not be fully associated with the ER, based on FRAP experiments [47]. So far, machineries facilitating ER–LD detachment have not been identified.

How long-lasting individual ER–LD contacts are, is at present not well understood. Studies in *Drosophila* cells suggest that they may be dynamic and form in response to

Figure 1



Examples of ER–LD contacts in human cells. **(a)** Live cell Airyscan microscopy of a human A431 cell grown in complete medium and treated with oleic acid for 2 h, expressing BFP-KDEL (ER, cyan), endogenously tagged seipin (magenta) and stained with a lipid droplet dye (LD540, yellow). Scale bars 5 and 1 μm. **(b)**. 3D models of LDs and surrounding ER profiles shown on top of a block-face image from a serial block-face scanning EM dataset of a A431 cell treated with oleic acid for 20 h. Yellow indicates ER, brown LDs and red membrane proximities between ER and LDs. Scale bar 1 μm. **(c).** Exemplary snapshots of ER–LD contacts from EM tomogram slices. Scale bars 200 nm. For detailed methodology, see Ref. [45•].

alterations in the properties of the phospholipid monolayer [7,56]. In these studies, bridges were concluded to be important for the trafficking of lipogenic enzymes from the ER to a subset of LDs to allow subsequent LD expansion [7] and this trafficking was dependent on COPI machinery [56]. As COPI machinery can modulate the LD surface tension by decreasing the monolayer PC levels (through budding out of small nano-LDs), the authors suggested that such decrease in surface tension may facilitate the bridge formation [5,56]. In addition, the LD monolayer phospholipid composition may be modulated by resident LPCAT enzymes [57] and localized

Table 1

Proteins proposed to function at ER–LD contacts

Name	Proposed function at ER–LD contacts	Localization[a]	References
Seipin	Regulating phosphatidic acid levels, functioning as a scaffold for lipogenic enzymes, as a diffusion barrier, or as a structural component facilitating LD growth	ER, enriched at ER–LD contacts	[42•,45•,60,61,64,65]
FITM2	Shuffling TAG to forming LDs, decreasing DAG at LD-forming sites to allow for LD emergence, regulating LD-budding to cytosol	ER, in yeast localised to ER–LD contacts	[11,19,20,21•]
Rab18	Inducing apposition of LDs to ER, interacts with NRZ-SNARE proteins at ER–LD interface, COPI-TRAPPII complex also regulates its LD localisation	ER and LD monolayer	[68, 70•,72]
DGAT2-FATP1	Forms a complex to facilitate LD expansion	ER (FATP1) and LD (DGAT2) in *C. elegans*	[74]
ORP2-VAPA	May regulate hydrolysis, ORP2 depletion increases LD proximity to ER	ER (VAPA), multiple localizations including LD (ORP2)	[75,76]
VMP1	May modulate SERCA activity, knockdown increases LD proximity to ER	ER, overexpressed protein localized at multiple ER contacts, including ER–LD contacts.	[50,77]
ICE2	Channelling DAG from LDs to ER	ER and ER–LD contacts in yeast.	[78]

[a] Localization in multiple cell types unless specified.

lipid transport. Interestingly, recent observations suggest that VPS13 proteins localize at ER contact sites, including those between ER and LDs, and may act as phospholipid transporters [58].

ER–LD contact proteins

The molecular machineries bridging the ER to LDs are becoming unravelled, with an increasing number of proteins localized and proposed to function at ER–LD contacts (Table 1).

The most studied of these proteins is seipin/BSCL2, a homo-oligomeric ER transmembrane protein of unknown function, mutated in severe lipodystrophy [59–61]. Seipin has been localised at ER–LD contacts in both yeast and human cells [42•,45•,60,61] and it plays an important role in LD biogenesis. In the absence of seipin, cells generate supersized and tiny, growth abortive LDs and exhibit functional defects in ER–LD contacts [42•,44,45•,52]. It is unclear whether the LD abnormalities arise from defective LD biogenesis or if seipin plays a role also in ER–LD contact maintenance. However, both nascent and more mature LDs harbour seipin at the ER–LD contact site and by live cell imaging these associations appear stable at least in minutes time-scale [42•,45•].

At ER–LD contacts seipin may regulate the local lipid environment, particularly phosphatidic acid levels [62–64], or act as a diffusion barrier between these organelles [44]. Seipin has also been suggested to act as a scaffold for lipid processing enzymes [65], or as a structural component of the ER–LD contacts facilitating LD growth [42•,45•]. Recently, the structures of the ER luminal regions of human and *drosophila* seipin oligomers have been solved using cryo-electron microscopy [66•,67•]. These structures indicate a ring-shaped oligomer of ~15–20 nm in diameter, with 11 (human) and 12 (*drosophila*) monomers. The luminal region harbours a β sandwich fold with structural similarity to lipid binding proteins, such as NPC2 [66•,67•]. These data suggest that seipin may function as a lipid transporter, but the putative lipid substrate(s) *in vivo* await further characterization.

Another protein implicated in ER–LD contacts is Rab18. Rab18 is localized to the ER and LDs [27,68,69,70•], and its LD localization is regulated by multiple factors including cellular metabolic status [71], COP1-TRAPPII complex [72] and Rab3GAP1/2 [70•]. Rab18 has been linked to both lipolysis and lipogenesis [71], and its overexpression can induce close apposition of ER and LDs [68]. Recently, Rab18 was shown to bring together ER and LDs through an interaction with the NRZ complex and associated SNARE proteins, and depletion of this machinery resulted in reduced LD growth and maturation [70•]. These data suggest that the Rab18-NRZ complex might function as a tethering protein between ER and LDs, although this function is apparently not important for LD growth in all cell types [70•,73].

Concluding remarks

In the past couple of years, we have witnessed substantial progress in understanding the connections between the ER and the lipid storage organelle it can give rise to, the LD. This has prompted a number of exciting avenues for further research. For instance, it will be interesting to dissect the functions of ER–LD contact proteins beyond their roles in initial LD formation, including LD maintenance and lipolysis. Furthermore, combining high resolution techniques, especially EM, with dynamic measurements and spatially resolved lipidomics will be instrumental for understanding the functional specifications of the various kinds of ER–LD contacts identified.

Conflict of interest statement

Nothing declared.

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