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Review

A different kind of love – lipid droplet contact sites☆

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ABSTRACT

Lipid droplets (LDs) store lipids and hence serve as energy reservoir and as a source for building-blocks for the organelle membrane systems. LD biology therefore depends on tight communication with other organelles. The unique architecture of LDs, consisting of a neutral lipid core shielded by a phospholipid-monolayer, is however an obstacle to bulk-exchange of bilayer-bounded vesicles with other organelles. In recent years, it is emerging that contact sites, places where two organelles are positioned in close proximity allowing vesicle-independent communication, are an important way to integrate LDs into the organellar landscape. However, few LD contact sites have been studied in depth and our understanding of their structure, extent and function is only starting to emerge. Here, we highlight recent findings on the functions of LD contact sites and on the proteins involved in their formation and hypothesize about the unique characteristics of the contact sites formed by these intriguing organelles. This article is part of a Special Issue entitled: Recent Advances in Lipid Droplet Biology edited by Rosalind Coleman and Matthijs Hesselink.

1. Introduction

Lipid droplets (LDs) are important metabolic hubs of cells. LDs are specialized in housing different classes of lipids and thus serve both as energy reservoirs and as storage places for key building blocks for formation of the cellular membrane system. LDs are, however, far from being mere lipid pantries. Instead, through their membrane proteome, they are actively involved in regulating cellular metabolism. They contribute enzymatically to both biosynthesis and breakdown of neutral lipids, mainly triacylglycerols (TAGs) and sterol esters, and therefore directly affect energy storage as well as neutralization of cytotoxic free fatty acids and cholesterol. Furthermore, LDs also have active roles in synthesis of diverse structural membrane lipids. Thus, they need to tightly coordinate different pathways of lipid metabolism in response to diverse cellular stimuli [1–3]. In addition, LDs mediate temporal storage of proteins [4] as well as contribute to clearance of inclusion bodies [5].

In order to fulfill their numerous functions in cellular physiology, LDs need to communicate with other organelles. Material exchange with other types of organelles via bulk flow of bilayer vesicles is however hampered by the structural peculiarities of LDs. Unlike other organelles, which contain an aqueous lumen, LDs comprise a fatty core consisting of neutral storage lipids. Furthermore, while organelles with aqueous lumens are bounded by phospholipid bilayer membranes, the hydrophobic core of LDs is shielded from the cytosol by a phospholipid monolayer, with the phospholipid fatty acid tails oriented towards the neutral lipid core and the hydrophilic head-groups pointing towards the cytosol. This unique architecture is incompatible with regular fusion/fission with bilayer bounded structures.

Organelle contact sites constitute an alternative, important, way of inter-organellar communication that bypasses the need for vesicular traffic. Contact sites occur when two organelles are actively tethered in very close proximity to each other (defined to date as 10-70 nm), thus enabling efficient and rapid transfer (by diffusion or active transport) of lipids, ions and other small molecules. Recently, it is becoming clear that contact sites also create regulatory hubs controlling organelle morphology, positioning, inheritance and fission. The last 15 years have seen an explosion in contact site research, and have brought along a basic understanding of contact site architecture and the molecular constituents that make up these specialized organellar connections.

At the heart of contact sites are proteinaceous structures termed molecular tethers [6] that keep the membranes of the interacting organelles at a defined distance and thus form the structural basis for the contact site architecture. Other contact site residents are either effectors, which fulfill the specific functional roles of the contact site such as material transfer, or regulators, which mediate adaptation of the contact site to the functional state of the cell. It is also possible that proteins will fulfill several roles at the same time such as being both tethers and

Abbreviations: LD, lipid droplet; ER, endoplasmic reticulum; NVJ, nucleus vacuole junction; TAG, triacylglycerol; DAG, diacylglycerol; PA, phosphatidic acid

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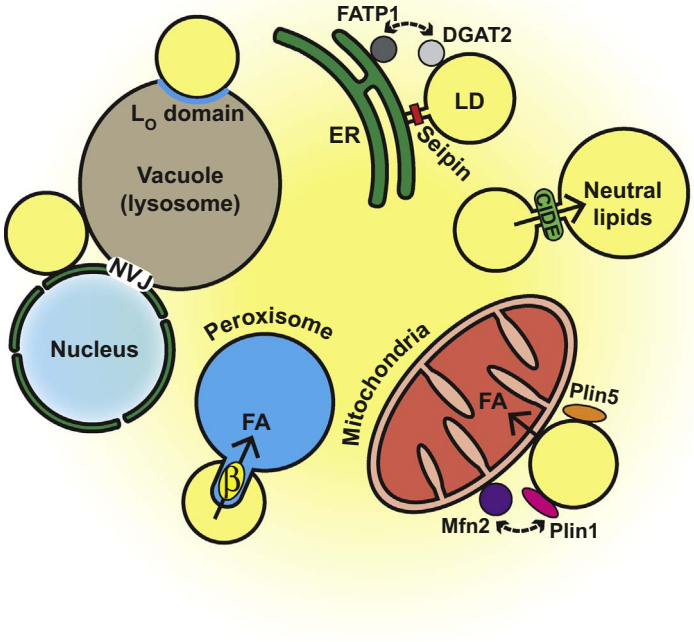

Fig. 1. Overview of cellular lipid droplet contact sites. Lipid droplets (LDs) form extensive contacts with all other organelles:
LD/endoplasmic reticulum (ER) contacts: In these contacts transfer of proteins, phospholipids and possibly other molecules occurs via lipidic bridges connecting the organelles. The seipin complex might have a molecular function at these bridges. FATP1 and DGAT2 have both been suggested to act as LD-ER tethers, indicated by a dashed arrow.
LD/LD contacts: Homotypic LD interactions topologically resembling contact sites are mediated by members of the CIDE (cell death-inducing DFF45-like effector) protein family. These interactions may not be real contact sites, but instead intermediates of LD fusion, a slow process that occurs via transfer of neutral lipids from the smaller into the larger LD dependent on internal LD pressure differences.
LD/mitochondria contacts: Interactions between these two organelles mediate shuttling of fatty acids (FAs) from LDs to mitochondria. Perilipin 5 (Plin5) is important for the LD-mitochondria interplay. In brown adipocytes, LD perilipin 1 (Plin1) and mitochondrial mitofusin 2 (Mfn2) were found to directly interact, indicated by a dashed arrow.
LD/peroxisome contacts: Peroxisomes form very intimate contact sites, termed pexopodia, with LDs. Pexopodia involve fusion of their outer phospholipid bilayer leaflet with the LD phospholipid monolayer and invasion of the LD core by protrusions bordered by the inner bilayer leaflet. Pexopodia are enriched in components of the β-oxidation machinery (symbolized by β).
LD/vacuole contacts: Coupling of LDs to the vacuole depends on the nutritional state of the cell. LDs associate with the vacuolar domain adjacent to the nucleus vacuole junction (NVJ) under conditions of glucose limitation. If starvation progresses, LDs start to encircle the vacuole and form close contacts with liquid ordered (L_O), raft-like vacuolar membrane domains. In deep starvation, LDs are internalized by the vacuole in a process termed lipophagy.

effectors [6].

Close proximity between lipid droplets and numerous cellular membrane systems has phenomenologically been well documented by electron microscopy for a long time [7–14] (Fig. 1). The structural basis of these organellar interactions and their functions in cellular physiology, on the other hand, are only recently beginning to be unraveled. In this review, we describe recent findings on the extent of LD contact sites, their different emerging roles, as well as on molecular players involved in their formation, function and regulation. We also put forward a hypothesis that the unique architecture and physical properties of LDs brings along fundamental structural differences of LD contact sites as compared to contacts between two phospholipid bilayer bounded organelles.

2. Same-same, but different: the architecture of lipid droplet contact sites

The defining feature of each contact site is organelle proximity, which generally depends on molecular tethers. Intriguingly, although numerous tethers in diverse organelle contact sites have been discovered in the past, very little is currently known about tethers specifically at LD contact sites. However, filamentous, likely proteinaceous structures of unknown identity anchoring LDs onto the ER surface have been observed by electron tomography [15], indicating that just like other contact sites, LD contact sites probably rely on proteinaceous tethers (Fig. 2A). Nevertheless, since no tethering complexes have been proven to date, some other form of tethering may still be discovered.

LDs have a fundamentally different architecture compared to bilayer bound organelles, resulting in unique physical properties of the LD surface, which consists of a phospholipid monolayer. Consistently, a peculiar structural feature has been described in LD-ER contact sites that is usually not found in contacts between bilayer membranes: by electron microscopy, so called “lipidic bridges” topologically resembling hemifusion intermediates were detected. Such bridges represent continuities between the phospholipid monolayer bordering the LD and the outer leaflet of the phospholipid bilayer of the ER (Figs. 1, 2B). These structures have been observed in diverse cell types [15–19] and reported to connect virtually all cellular LDs to the ER in yeast [17]. Such lipidic connections might however not provide the rigidity required to structurally maintain contacts, suggesting that they likely exist in addition to and not instead of proteinaceous tethers (Fig. 2C).

For many years there was a notion that “lipidic bridges” of LDs to the ER are simply remnants of the LD biogenesis route. Biogenesis of LDs is believed to be initiated through neutral lipid synthesis in the ER, which leads to formation of a neutral lipid lens between the two leaflets of the ER lipid bilayer and subsequent budding of LDs from the ER surface [20,21]. Topologically, lipidic structures connecting the two organelles could thus indeed represent remainders of the LD budding process. In support of this, continuities between ER and LD membranes are required for the process of targeting of helical hairpin containing LD surface proteins from the ER. These proteins are initially inserted into the ER membrane via regular ER targeting/translocation pathways and subsequently diffuse onto LDs via the lipidic bridges connecting the two organelles [16,22–25].

However, several lines of evidence suggest that “lipidic bridges” are more than mere biogenetic remnants. First, similar lipidic structures have been reported between LDs and peroxisomes as well as between distinct LDs [13,26] (Fig. 1). And second, recent findings indicate that lipidic LD-ER bridges can form *de novo* to reconnect LDs previously separated from the ER [27,28], suggesting that these bridges are more than simple remainders from the LD biogenesis process. These findings indicate that lipidic connections might be a more general feature of LD contact sites, that could provide a unique means of LD-organelle communication.

3. Contact sites versus lipidic continuities – mission (im)possible

Formally, lipidic bridges clash with the prevailing definition of contact sites, which are generally described as places of apposition between organelles that *do not* enable fusion of the respective organelle membranes. Instead, material exchange at contact sites is usually mediated by specialized effector proteins, e.g. lipid transfer proteins that extract lipids from one membrane, protect them from the aqueous environment of the cytosol with the help of a hydrophobic lipid binding domain, and release them into the neighboring membrane. Thus, contact sites are places that mediate a limited, defined, interorganellar

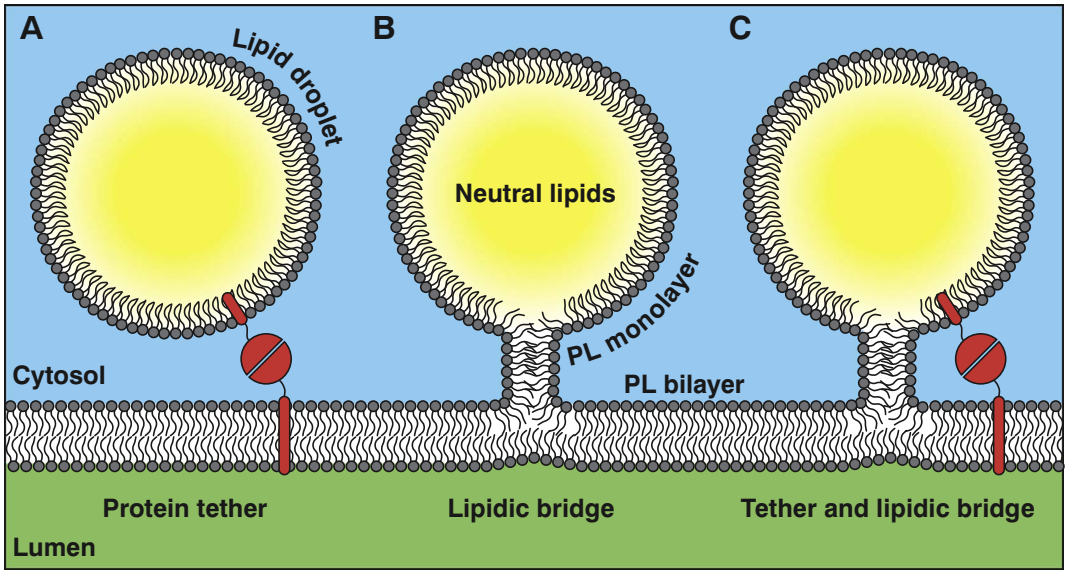

Fig. 2. Architecture of lipid droplet contact sites. (A) Contact sites are generally connected by proteinaceous tethers (red) that are firmly associated with the membranes of the interacting organelles. (B) In some lipid droplet (LD) contact sites, continuities of the LD phospholipid monolayer and the outer leaflet of the phospholipid bilayer bordering the partner organelle have been detected. These structures are termed lipidic briges. (C) Proteinaceous tethers and lipidic bridges might be present at the same time in contact sites. PL, phospholipid.

interplay that does not compromise the structural identity of the communicating organelles, enabling creation of niches with unique features.

So, are LD contacts with lipidic bridges even "real contact sites"? These lipidic continuities no doubt represent a striking difference to contact sites between two bilayer bounded organelles, that has to be kept in mind when comparing different types of contacts. However, LDs have several unique features that efficiently prevent loss of identity of the interacting organelles via these bridges, and thus only allow for limited interorganellar interplay, similar to what happens at regular contact sites between bilayer membranes.

First, although the lipidic structures observed in LD-ER contacts are reminiscent of hemifusion intermediates between bilayer membranes, they do not represent a fusion intermediate. This is because a continuity between a phospholipid monolayer and one leaflet of a bilayer is a topological arrangement that cannot progress into full fusion. It is possible that no mechanism to prevent lipid mixing of the two organelle surfaces has evolved simply because there is no need to prevent membrane fusion.

Second, while formation of a hemifusion intermediate has an energetic cost [29], lipidic bridges connecting LDs to bilayers can be energetically favorable and persistent. Stability of the lipidic bridges depends largely on the LD surface tension [30]. While the surface tension of bilayer membranes is, in general, very low, the surface tension of monolayer bounded LDs depends to a great extent on the ratio of surface phospholipids to neutral core lipids. As the volume to surface ratio of an LD (unlike for morphologically flexible organelles with aqueous lumens) is largely determined by the amount of neutral lipids in its core, a shortage of phospholipids leads to exposure of hydrophobic LD core patches to the cytosol and thus to an increase in surface tension [30]. The Arf1/COPI machinery, which is best known for its role in trafficking of bilayer vesicles from the Golgi to the ER, has a unique relationship to LD surface tension. This machinery mediates budding of nano-LDs, small LDs with an average diameter of 60 nm, from LD surfaces, leading to a phospholipid deprivation and concomitant increase in LD surface tension and a final *de novo* formation of lipidic connections to the ER [27,28]. It remains to be determined to which extent this modulation of LD surface tension regulates other LD contact sites under physiological conditions.

And third and most importantly, recent studies indicate that at least in the LD contact site to the ER, molecular mechanisms are in place that efficiently prevent equilibration of surface proteins and phospholipids via the lipidic bridges. A key player in maintenance of LD versus ER surface identity is a protein complex localized at LD-ER contacts termed seipin (Fig. 1; see below).

In summary, while other organelles disenable fusion between membranes at contact sites, we hypothesize that due to their unique biophysical nature, LDs may not have had the need to ban such connections. If so, it may be that a unique feature of all LD contact sites is the presence of a lipidic bridge. Such a bridge would enable diffusion of proteins and small molecules without the need for active transfer proteins and may, therefore, underlie a very different contact site composition than previously described for any other organelle. This may also explain why tethering complexes and effector molecules have been difficult to uncover as they would have different, unique, characteristics.

4. Lipid droplet-endoplasmic reticulum proximity: till death do us part

The endoplasmic reticulum (ER) is the birthplace of LDs, which maintain a unique relationship to their parent organelle throughout their lifetime. Biogenesis of a large fraction of the LD surface proteins requires initial insertion into the ER membrane and only later transfer to the LD. Hence it is no surprise that LDs fulfill many of their functions in close collaboration with the ER. As a consequence, LD-ER contact sites are highly abundant in numerous cell types and the most well studied of the LD contact sites.

Mechanistic insight into how LD/ER contact sites form is still sparse. Two lipid metabolism enzymes have been suggested to create an ER-LD tether in *Caenorhabditis elegans*: The ER acyl-CoA synthetase FATP1 and the LD localized diacylglycerol (DAG) acyltransferase 2 (DGAT2). These two enzymes have been proposed to physically interact across the opposing membranes to form a TAG biosynthesis complex that could be bridging the two organelles to mediate efficient LD expansion [31].

The most studied protein by far in the context of the LD/ER contact sites is seipin (*BSCL2* in human; Sei1/Fld1 in yeast), although its exact mode of action is not yet quite worked out. Seipin is a highly conserved transmembrane protein of the ER. Seipin mutations are related to several human diseases: the autosomal-recessive congenital generalized lipodystrophy type 2 [32], and two autosomal-dominant neurological disorders, distal hereditary motor neuropathy and Silver syndrome

[33]. Generalized lipodystrophy is characterized by an intriguing combination of symptoms that point towards an underlying lipid related defect: A striking lack of adipose tissue with concomitant ectopic fat accumulation in organs and a metabolic syndrome including a strong tendency to develop diabetes [34]. While seipin is clearly required for regular LD biogenesis, its exact molecular role is still under debate. Both human and yeast seipin have been reported to form ring-shaped oligomeric complexes [35,36]. The yeast seipin homolog Sei1/Fld1 cooperates with a second crucial component Ldb16 to form the seipin complex. However human seipin can functionally complement *ldb16*Δ, indicating that the human protein covers the functions of both Sei1 and Ldb16 [37]. On a cellular level, seipin dysfunction is associated with diverse morphological alterations of LDs across species. Such alterations often manifest as clustered or supersized LDs dependent on the environmental conditions [15,37–40]. As numerous studies detected seipin at the ER-LD contact site [15,19,37,40–42], seipin could have a role in determining the composition of LDs as compared to the ER. Indeed, in the absence of functional seipin, the composition of LDs is altered in several ways: (1) In lymphoblastoid cell lines from lipodystrophy patients that encode mutant seipin, cellular TAG levels were reduced and TAG and phospholipids were found to contain an elevated fraction of saturated fatty acids [38]. (2) Detailed analysis of LD biogenesis in human and *Drosophila* cells harboring seipin mutations revealed accumulation of large amounts of small, nascent LDs on the ER surface that appeared to fail to grow into mature LDs [15]. Hence, seipin may be required for maturation of nascent LDs into mature ones, potentially through regulation of lipid transfer at LD-ER contact sites. If this is the case, it suggests that the prominent supersized LDs generally observed in seipin mutants might be aberrantly maturing LDs that prematurely acquire lipid synthesis enzymes [15]. (3) Finally, deletion of either of the two seipin complex components *SEI1* or *LDB16* in yeast results in drastic alterations of both LD surface phospholipid and protein composition, including failure of enriching LD proteins that are known to be targeted to the LD surface via the ER [19]. Furthermore, *ldb16*Δ mutants have a grossly altered LD-ER interface, suggesting a role for seipin in stability of these structures [19]. Thus, seipin has a key role in biogenesis of mature LDs by preventing an LD identity crisis, potentially by controlling transfer processes at the ER-LD lipidic bridges.

However, an additional function of seipin seems to be modulation of lipid metabolism at LDs. The rate-limiting enzyme in phosphatidic acid (PA) synthesis, glycerol 3-phosphate acyltransferase (GPAT), has been found to bind to seipin in several organisms. Seipin binding results in a reduced activity of GPAT and thus in decreased PA production [43]. Intriguingly, increased levels of the fusogenic lipid PA have previously been found to result in formation of supersized LDs, a morphology phenotype also associated with seipin deficiency [44]. Seipin-GPAT binding is decreased in a seipin mutant (T78A) identified in lipodystrophy patients [36,43]. Furthermore, seipin has also been found to simultaneously bind to the PA synthesizing enzyme 1-acylglycerol 3-phosphate acyltransferase 2 (AGPAT2) and the PA phosphatase lipin-1, a connection that might influence the dynamics of PA turnover by presenting it to a PA utilizing enzyme directly upon synthesis [45,46].

Knowledge about further molecular players in ER-LD contact sites is still limited. However, at least one function of the contact site is quite clear as several lipid metabolism pathways comprise both LD and ER localized enzymes, indicating that the LD/ER interface might be a hotspot for lipid synthesis and breakdown. Indeed, on one hand, lipid metabolism enzymes have been found to concentrate at LD-ER contact sites dependent on seipin (see above). On the other hand, dynamic enzyme re-localization between the two organelles under different physiological conditions has been observed, as outlined below.

The major pathway of synthesis of the storage fat TAG generally starts in the ER, where membrane bound GPAT mediates formation of lysophosphatidic acid from glycerol 3-phosphate and acyl-CoA, which is further acylated to PA by ER integral AGPAT. PA marks a key branching point between lipid storage and membrane biosynthesis, as it can either be dephosphorylated by phosphatidate phosphatases/lipin proteins to form diacylglycerol (DAG), a precursor of storage TAG, or converted by CDP-diacylglycerol synthase (CDS) into cytidine diphosphate diacylglycerol (CDP-DAG), a precursor of several phospholipids. In the former case, DAG is converted into TAG by diacylglycerol acyltransferase (DGAT). DAG can also enter an alternative pathway of phospholipid biosynthesis, the Kennedy pathway. Intriguingly, under conditions of pronounced LD biogenesis, components of the TAG biosynthesis pathway have been found to dynamically re-localize to the surface of LDs, for example DGAT2 in mammals, an isoform of the enzyme catalyzing the final step in TAG synthesis [47], as well as its yeast homolog Dga1 [17]. In *Drosophila* cells, a specific population of large LDs was identified in the presence of high amounts of the fatty acid oleate, that contained at least one isoenzyme for every single step in TAG synthesis. These enzymes likely move onto LDs under conditions requiring TAG synthesis to support enlargement of specific LDs [16].

Growth of LDs by neutral lipid incorporation requires balanced supply of surface phospholipids, particularly of phosphatidylcholine (PC), which acts as the main LD surfactant and prevents LD coalescence. Phospholipid synthesis directly on LDs is usually limited, however, LD growth results in LD targeting of CTP-phosphocholine cytidyltransferase (CCT), the rate-limiting enzyme of the Kennedy pathway. This leads to activation of the enzyme and concomitantly to increased production of PC, which stabilizes the surface of the growing LD [48]. However, the enzyme catalyzing the final step in PC synthesis is not present on LDs [48,49], indicating that material exchange between LDs and the ER is required that likely takes place at the contact sites.

5. Lipid droplet-vacuole proximity: an intriguing LD dance on membranes

The contact sites between LDs and the late endomembrane system have been most extensively studied in yeast. In *Saccharomyces cerevisiae* (*S. cerevisiae*), LDs and the vacuole (the yeast lysosome) show a dynamic interplay that is dependent on the nutritional state of the cell (Fig. 3). During logarithmic growth when glucose is replete and division is rapid, the association between LDs and the vacuole is not prominent. During the diauxic shift however, when cells run out of glucose and slow down their cell cycle to adapt to utilization of other carbon sources, LDs start to concentrate in a unique niche in the cell, directly adjacent to the contact site between the outer nuclear membrane and the vacuole, the nucleus vacuole junction (NVJ) [50,51]. The mechanisms mediating this relocation as well as tethering of the LDs to the vacuole are currently unknown. Interestingly, the NVJ houses numerous factors involved in lipid metabolism and trafficking [52–58], and the size of the NVJ has been reported to expand dramatically once cells run out of glucose [59]. Furthermore, LD accumulation at the NVJ temporarily overlaps with concentration of the PA phosphatase Pah1/lipin in the same area on the nuclear surface thus serving to co-incide the two events. Pah1 mediates conversion of PA, a key precursor for biosynthesis of phospholipids, to DAG, a precursor of TAG synthesis (see above), and thus acts at the crossroads of lipid storage and membrane lipid biosynthesis. The functional role of LD accumulation at the NVJ is unclear, but the presence of Pah1 suggests a connection to lipid storage. Whatever the exact function of this intimate association of organelles might be, it is of transient nature and coupled to the metabolic reprogramming occurring during the diauxic shift. Upon progression into stationary phase, which is characterized by very slow division rates due to carbon source restriction, LDs move away from the NVJ and start to encircle the vacuole [50,51]. Intriguing, this relocation on the vacuolar surface coincides with a striking structural transformation of the vacuolar membrane. In stationary phase, this membrane partitions into liquid ordered (Lo), likely sterol rich domains, and liquid disordered domains, which are each marked by specific sets of proteins [51,60]. LDs localize specifically to the liquid ordered, sterol rich part

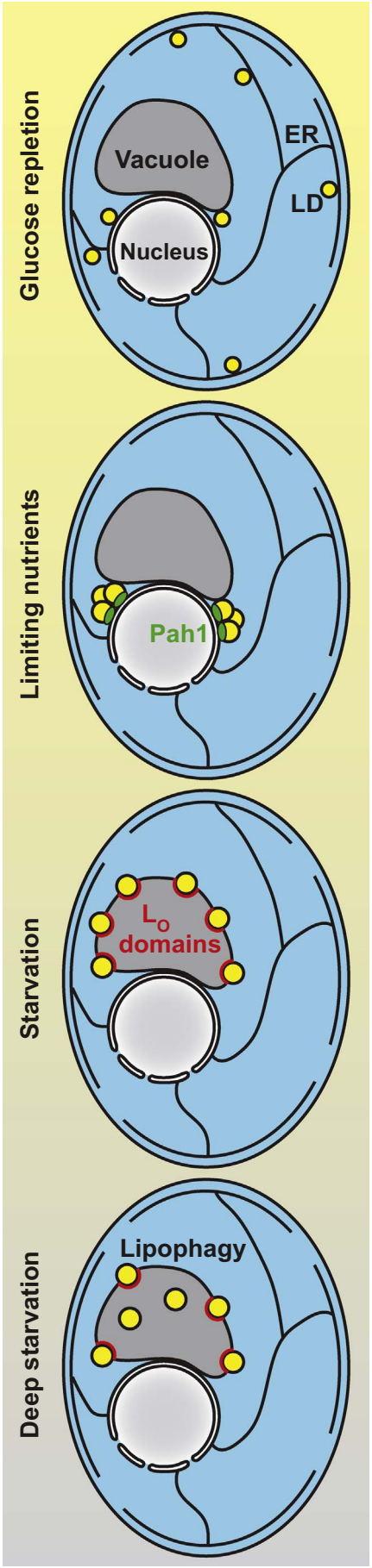

Fig. 3. Lipid droplet contacts to vacuolar and endoplasmic reticulum membranes in the yeast *Saccharomyces cerevisiae* depend on the nutritional state. In yeast cells, LDs form contact sites with all regions of the ER (nucleus, peripheral ER, and cytoplasmic ER) under conditions, where plenty of nutrients are present. During the diauxic shift, when cells run out of fermentable sugars and halt growth to adapt to utilization of non-fermentable carbon sources, LDs grow in size and cluster in close proximity to the contact site between the nucleus and the vacuole (yeast lysosome). The lipin Pah1 accumulates on the nuclear membrane adjacent to the LD clusters. If starvation progresses, LDs move away from the nucleus and encircle the vacuole. At the same time, sterol rich, liquid ordered (L_O) domains form in the vacuolar membrane. LDs specifically associate with these domains. If starvation conditions persist, LDs become internalized into the vacuole by lipophagy.

of the membrane, which forms numerous lipid raft like structures surrounded by liquid disordered regions. LDs stay associated with these rafts for several days. If the condition of nutrient deprivation persists, LDs sink into the liquid ordered patches and ultimately get engulfed by the vacuole through a microautophagy mechanism termed microlipophagy [61]. Mutants affected in lipophagy, including mutants of the core autophagy machinery as well as *atg21* and *atg32*, show defects in vacuolar membrane domain formation, and mutants affected in domain formation, like *fab1*, *vps4*, and *nem1*, conversely display lipophagy defects, indicating that the two processes stimulate each other, resulting in a feed forward loop [51]. Lipophagy also happens in mammals, where it depends on a macroautophagy mechanism [62].

Although the LD dance on vacuolar membranes in stationary phase yeast ultimately results in lipophagy, this is a late event occurring only after several days of close LD-vacuole apposition. It is likely that during progression through stationary phase, long before lipophagy occurs, contact between LDs and vacuolar membrane subdomains serves a physiological role. The exact functions of the different stages of the LD-vacuole interplay remain to be determined. Furthermore, potential functions of the liquid ordered and disordered vacuolar membrane domains beyond lipophagy and the exact role of associated LDs are topics for future research.

Interestingly, LD/vacuole interactions are required for efficient prospore membrane biogenesis during yeast sporulation [63]. Sporulation is a developmental program initiated in diploid yeast cells in response to lack of nitrogen and sugar that results in formation of four haploid spores (gametes) via meiosis. In sporulating cells, LDs start out in contact sites with the vacuolar membrane, but then lose contact to cluster around the nucleus and form contact sites with the prospore membranes, specialized intracellular plasma membranes that are newly formed to package the four spores.

Since the late endomembrane system is the destiny for endocytosis and autophagy, processes that recycle large amounts of lipids, it is clear that contacts to LDs should be conserved in mammals. However, how exactly lysosome/LD contacts occur in mammalian cells remains to be described.

6. Lipid droplet-mitochondria proximity: get close to your clients

Fats stored in LDs are energy rich molecules that can be utilized for ATP production under conditions of glucose restriction. Breakdown of the main storage fat, TAG, requires release of fatty acids from LDs either through TAG hydrolysis by cytosolic lipases, a process termed lipolysis, or through lipophagy (see above). Subsequently, the free fatty acids are metabolized by β-oxidation, citric acid cycle and oxidative phosphorylation (OXPHOS), yielding large amounts of ATP. Mitochondria are key players in this process, as they house the machinery required for either several (in yeast and plants) or all (in mammals) of the steps for fatty acid break down. Indeed, it was shown that in mammalian cells, under acute starvation, fatty acids reach mitochondria directly from LDs instead of from a free cytosolic pool (Fig. 1). Efficient fatty acid delivery required lipolysis, replenishment of LD content by autophagy, as well as a functional mitochondrial fusion machinery [64]. As a direct handover of fatty acids between storage and oxidation organelles might

be an efficient way to prevent high concentrations of cytotoxic fatty acids in the cytosol, it is conceivable that these observations reflect a general phenomenon, and indeed, close apposition of mitochondria and LDs has been observed in various cell types [10,12,65,66]. In skeletal muscle, a tissue with high energy demands, mitochondria and LDs have a particularly intimate physical relationship, displaying rows of alternating LDs and mitochondria [10]. Intriguingly, exercise training, a condition that requires pronounced fat dependent ATP production, augments this phenotype [10,66].

Several proteins have been reported to have a role in LD-mitochondria contact site formation. Depletion of the SNARE protein SNAP23 in mouse fibroblasts results in decreased LD-mitochondria interaction and β-oxidation through a yet unclear mechanism [67]. The most well-studied effector of the LD-mitochondria interaction is perilipin 5, a member of the perilipin family of structural LD proteins, that appears to have a key role in coordinating LDs with fatty acid oxidation in mitochondria. In accordance with such a role, this protein is mainly expressed in tissues with high lipid oxidative metabolism: cardiomyocytes, brown and beige fat, liver and skeletal muscle [68–70]. Furthermore, perilipin 5 is upregulated in muscle tissue in response to exercise [71–73]. Although perilipin 5 is a *bona fide* LD protein, it has also been found associated with mitochondria [74,75]. Perilipin 5 overexpression, unlike overexpression of other perilipins, has been reported to induce recruitment of mitochondria and LDs towards each other. This recruitment critically depends on the C-terminal 20 amino acids of the protein [75]. It remains to be determined whether perilipin 5 directly acts as a mitochondria-LD tether, or whether it affects collaboration between the two organelles by different means. In brown adipose tissue, a different member of the perilipin family, perilipin 1, has been recently reported to be involved in mitochondria-LD interactions. Perilipin 1 was found to interact with the mitochondrial outer membrane fusion GTPase Mfn2, but not with its homolog Mfn1. Mfn2 depletion indeed resulted in a reduction of LD-mitochondria interactions and in alterations of lipid metabolism [76]. It remains to be determined whether these effects are related to a direct role of Mfn2 in LD-mitochondria tethering or whether they stem from alterations in mitochondrial fusion.

Association of LDs with mitochondria likely goes beyond shuttling of fatty acids, and the functional scope of these contact sites remains to be determined. In addition to transfer of energy-rich lipids for breakdown, it might potentially also involve the transfer of phospholipids for lipid biosynthetic purposes. Mitochondria have a role in phosphatidylethanolamine synthesis, an LD surface component that can be further transformed into the main LD phospholipid, PC. Furthermore, several lipid metabolism enzymes, Ayr1, Hfd1 and Pgc1, have been suggested to reside both in LDs and mitochondria in yeast, opening the possibility that protein transfer could occur also via these contacts. A recent study reported on proteins involved in apoptosis shuttling between mitochondria and LDs dependent on the V-domain, a particular protein domain consisting of two α-helices. This process might have a role in modulation of cellular responses to stress [77]. How molecules are transferred between mitochondria and LDs and what regulates contact formation all remain to be explored.

7. Lipid droplet-peroxisome proximity: brothers in arms

While citric acid cycle and OXPHOS are restricted to mitochondria, the first step in fatty acid catabolism, β-oxidation, also happens in peroxisomes. In fact, in some yeasts, like the model organism *S. cerevisiae*, and in plants, peroxisomes are the sole organelles that perform β-oxidation. Hence it would be expected that LDs would also have an interface with peroxisomes. Indeed, close proximity between LDs and peroxisomes has been observed in mammals, yeast, and plants [13,78,79].

Yeast cells grown on the fatty acid oleate dramatically expand their peroxisome population and form extensive, long lasting contact sites between LDs and peroxisomes. Intriguingly, at these contacts, peroxisomal protrusions termed pexopodia were found that extended into the LD core. These protrusions likely represent places of hemi-fusion (see above) where the outer leaflet of the peroxisomal membrane is continuous with the LD phospholipid monolayer, while the inner leaflet invades the LD core (Fig. 1). Pexopodia are enriched in proteins involved in β-oxidation, indicating that they might be places where fatty acids are shuttled from LDs to peroxisomes. Consistently, the number of pexopodia was found to increase under growth conditions that reinforce utilization of fatty acids as a carbon source [13]. Evidence for lipidic connections between LDs and peroxisomes have also been obtained in plants. These structures have been suggested to play a role in transfer of the lipase SDP1 from peroxisomes to LDs [26]. These high-resolution studies again lend support to the theory that LDs form contacts that are structurally different than other contacts, enabling a much more intimate involvement of the two organelles in metabolic decisions.

More generally, it is clear that LDs send out lipids for breakdown to peroxisomes; however they might also be the acceptor side of the contact by receiving hydrophobic compounds, like peroxisome derived ether linked lipids [14].

8. Lipid droplet-lipid droplet proximity: contact site or fusion intermediate?

Sites of close apposition amongst LDs have been detected under numerous conditions. As for all homotypic organelle contacts, it is however not trivial to distinguish between fusion intermediates and contact sites that should form long lasting functional interactions. In yeast cells cultured on oleate, extensive contacts between LDs have been observed that displayed valve-like lipidic connections of enigmatic composition [13]. Furthermore, several conditions have been identified that result in a strong tendency to form clusters of tightly packed LDs, for example alterations of the LD biogenesis factor seipin [37,39,40], the Pah1/lipin activators Nem1 and Spo7 [80], the structural LD proteins of the perilipin family [81,82], the ancient ubiquitous protein, AUP 1 [83], and brain specific GRAF1a [84], as well as infection with Hepatitis C virus [85].

Very little is however known about the protein machinery directly involved in LD-LD association. Exceptions are the members of the family of cell death-inducing DFF45-like effector (CIDE) proteins in mammals: CIDEA, CIDEB, and CIDEC/Fsp27. These proteins have distinct tissue specific expression patterns, with CIDEA and CIDEC being expressed in white adipose tissue, CIDEA in brown adipose tissue, and CIDEB in liver [86]. Interestingly, CIDEC has been found highly enriched at foci where two LDs interact, and to closely tether LDs [87] (Fig. 1). A detailed structure-function analysis of the CIDE family member, CIDEA, revealed an amphipathic α-helix required for LD targeting and two domains that mediate homotypic interaction and hence docking of LD pairs [88]. However, contacts mediated by CIDE proteins ultimately result in LD fusion. CIDE protein-dependent fusion differs from fusion of bilayer bound organelles particularly in its timing, as it has been reported to take several hours to fully fuse two LDs. CIDEC mediated fusion depends on directional transfer of neutral lipids from the smaller to the larger partner of an LD pair connected by CIDEC foci via lipidic connections. This is therefore a slow process that is likely driven by internal pressure differences between differently sized LDs [87].

It remains to be determined whether homotypic LD contact sites exist that do not ultimately result in fusion of the tethered LDs. Theoretically, LD clustering and dissociation might be an efficient mechanism to dynamically modulate lipolysis, as it reversibly alters the total LD surface area accessible to lipases. Indeed, LDs have been found to be highly dynamic. Non-muscle myosin IIa (NMIIa) and actin are recruited to LD-LD interfaces by formin-like 1 (FMNL1) and mediate LD dissociation. Interestingly, impairment of these LD dynamics result in

increased cellular lipid storage [89].

9. Perspectives

It is emerging that contact sites are a key way of communication between LDs and other organelles and thus significantly contribute to the function of these important players in cellular metabolism [14,90–92]. LDs need to tightly communicate with the ER to coordinate lipid storage and synthesis of cellular membranes. Furthermore, LDs interact with different partner organelles to optimize utilization of the stored lipids under conditions of nutrient stress. However, the metabolic decisions leading to LD association with mitochondria, peroxisomes, or induction of lipophagy are not fully understood, and might rely on the exact type of nutrient stress [64].

More generally, more LD contact sites might be discovered in the future in specific cell types, and it is likely that most or even all cellular membrane systems interact with LDs, depending on cell type or metabolic condition. Importantly, while the protein machineries underlying formation and function of many organelle contact sites in the cell are known, the contact site machineries of LDs are currently largely enigmatic. Most intriguingly, little is known about the tethers that mediate attachment of LDs to other organelles. Identifying these components will be of invaluable benefit to the LD community, as it will allow experimental manipulation of LD contact sites and thus, greater understanding of their exact biological functions.

Dysfunction of LDs is associated with numerous pathological conditions, including obesity, diabetes, lipodystrophy and steatohepatitis. A detailed understanding of the function of the LD contact site network might help to elucidate lipid associated pathological conditions. Indeed, of the few proteins known to be involved in LD contact sites, several are related to human diseases, e.g. seipin, which is a causal gene for congenital generalized lipodystrophy [32], and perilipin 5, which has been related to hepatosteatitis [93].

The unique architecture of LDs and initial evidence about structural and functional differences of LD contact sites compared to bilayer-bilayer contacts may underlie the difficulty that the field has had until now to determine the protein machinery required for these contacts. Moreover, it implies that the contact site field should expect novel concepts of interorganellar communication from the LD.

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