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Review

Lipid Droplet Contact Sites in Health and Disease

Eva Herker,[1,*] Gabrielle Vieyres,[2,3,*] Mathias Beller,[4,5,*] Natalie Krahmer,[6,7,*] and Maria Bohnert[8,9,*]

After having been disregarded for a long time as inert fat drops, lipid droplets (LDs) are now recognized as ubiquitous cellular organelles with key functions in lipid biology and beyond. The identification of abundant LD contact sites, places at which LDs are physically attached to other organelles, has uncovered an unexpected level of communication between LDs and the rest of the cell. In recent years, many disease factors mutated in hereditary disorders have been recognized as LD contact site proteins. Furthermore, LD contact sites are dramatically rearranged in response to infections with intracellular pathogens, as well as under pathological metabolic conditions such as hepatic steatosis. Collectively, it is emerging that LD–organelle contacts are important players in development and progression of disease.

Highlights

Intracellular lipid droplets (LDs) extensively communicate via contact sites, places where they are physically attached to other cellular organelles.

Many LD contact site proteins are disease factors mutated in hereditary neurological and metabolic disorders.

Different types of intracellular pathogens, including viruses, bacteria, and parasites, induce drastic rearrangements of host LD contact sites and formation of novel contacts between LDs and pathogen replication organelles.

During non-alcoholic fatty liver disease (NAFLD), remodeling of LD contact sites promotes pathological lipid storage in hepatocytes.

Lipid Droplets (LDs) Communicate via Organelle Contact Sites

LDs are multifunctional organelles that are present in most eukaryotic cell types [1]. They consist of a central compartment composed of nonpolar lipids, which is shielded towards the aqueous cytoplasm by a phospholipid monolayer [2]. The phospholipid monolayer contains a set of LD surface proteins that fulfill different LD functions (Box 1). A central role of LDs is the storage of esterified, nonpolar lipids, particularly triacylglycerols (TAGs) and sterol esters (SEs). Lipids act as efficient energy storage units, as building blocks for the formation and maintenance of all cellular membrane systems, and as signaling molecules. The ability to store lipids is important to ensure continuous lipid supply independent of external nutrient availability. Furthermore, some lipids have adverse toxic effects, which can be controlled by esterifying and safely depositing them inside LDs. In addition to their roles as lipid storage and detoxification organelles, LDs house numerous lipid metabolism enzymes and thus are actively involved in the formation and/or breakdown of neutral lipids, phospholipids, and sterols. In summary, LDs are central hubs in the handling of lipids [1].

To fulfil their diverse functions, LDs need to tightly communicate with the other cellular organelles that require and provide specific lipids. One main way for interorganellar material exchange is via the trafficking of bilayer-bounded vesicles, which, however, may not be relevant for LDs due to their incompatible phospholipid monolayer-bounded architecture [3]. Instead, in recent years it has been shown that LDs extensively form organelle contact sites, which mediate an alternative way of communication [4,5]. Contact sites are places in the cell where the surfaces of two or more organelles are directly attached to each other via special tether proteins, thus positioning the partner organelles at a distance of just a few nanometers (Box 2) [6]. These structures mediate the interorganellar exchange of lipids, ions, and other components via locally enriched transport machineries such as lipid transfer proteins or ion channels. Besides material transfer, contact sites are involved in signaling, in lipid metabolism, in organelle biogenesis, dynamics, positioning, and inheritance, in autophagy, and in cellular stress responses [7]. LD contact sites to all organelles tested so far have been detected [4,5] and in recent years, more and more proteins involved in their formation and function are being

[1]Institute of Virology, Philipps-University Marburg, 35043 Marburg, Germany
[2]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
[3]Leibniz ScienceCampus InterACt, Hamburg, Germany
[4]Institute for Mathematical Modeling of Biological Systems, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany
[5]Systems Biology of Lipid Metabolism, Heinrich Heine University Düsseldorf, Germany
[6]Institute for Diabetes and Obesity, Helmholtz Zentrum München, 85764 Neuherberg, Germany
[7]German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany
[8]Institute of Cell Dynamics and Imaging, University of Münster, 48149 Münster, Germany
[9]Cells in Motion Interfaculty Centre (CiM), University of Münster, Münster, Germany

*Correspondence:
eva.herker@uni-marburg.de (E. Herker),
gabrielle.vieyres@leibniz-hpi.de
(G. Vieyres), mathias.beller@hhu.de
(M. Beller), natalie.krahmer@helmholtz-
muenchen.de (N. Krahmer), and
bohnertm@uni-muenster.de
(M. Bohnert).

Box 1. The Changing View on LDs

LDs are known as the cytosolic neutral lipid storage depots [1]. Yet, research of the past decade has strongly expanded our knowledge of the functional repertoire of LDs. For example, it has been realized that LDs can act as a safe harbor for otherwise toxic proteins and lipids and play a pivotal role in various cellular processes such as autophagy [1]. Uncovering the functional diversity of LDs was paralleled by the appreciation of their intimate interactions with other cellular organelles, thus changing the historic view that LDs are inert cytosolic inclusions. In fact, even the subcellular localization of LDs needs to be re-evaluated, as they are also found within cellular compartments other than the cytosol, such as the nucleus [96] or mitochondria [97].

The proteins attached to LDs are key to their different functions. What began with the seminal works uncovering the role of LD-associated perilipin protein family members [98,99] for lipid storage regulation is now carried on for the diverse set of LD-associated proteins identified in a number of proteomics studies. It is still unclear how complex the proteome of LDs actually is. At least in part, this question is difficult to answer as there is a constant flow of LD emergence, maturation, persistence, and degradation and a heterogeneity within the LD pool of cells. This LD diversity is tied to specific proteome compositions, resulting in a considerable plasticity. Of course, there are signature LD proteins such as structural and regulatory proteins, including the perilipins, and a range of metabolic enzymes necessary to build, edit, and remobilize the stored lipids. On top of these somewhat expected proteins, however, a number of previously uncharacterized proteins, as well as a diverse set of surprising proteins often considered contaminants are found. Especially those unexpected proteins have the potential to reveal novel LD functions. Histones, for example, which usually help package the DNA into chromatin in the nucleus, were found on LDs of *Drosophila* embryos as well as in droplet preparations of other origins [100,101]. In the end, the specific recruitment of histones to LDs as well as their regulated release turned out to be important for survival of the developing *Drosophila* embryo and to serve an antibacterial function in other systems [101,102]. Thus, a deeper analysis of the set of proteins attached to LDs harbors the promise to also discover new LD functions.

discovered [8]. It is emerging that LD contact sites likely enable the central way of LD–organelle communication.

This is reflected in latest findings that point towards links of LD contact site biology to a diverse set of human genetic, infectious, and metabolic diseases, as detailed in this review. Briefly, within the past few years, the products of a number of genes, mutations of which cause hereditary diseases, have been identified as LD contact site proteins involved in organelle tethering and material exchange. Furthermore, diverse intracellular pathogens alter LD contact sites and even generate novel contacts between LDs and membrane-bounded pathogen replication

Box 2. Organellar Communication by Contact Sites

The interior of eukaryotic cells is subdivided into distinct compartments termed organelles, which offer optimized separate spaces for different biochemical processes and thus allow for the cell to work on the basis of division of labor. Organelle biology research has for a long time strived for understanding the individual roles of each compartment, but more recently, the question of how all organelles communicate to enable coordinated cellular behavior has become a further central focus. Interorganellar exchange of material and information is achieved by diffusion, cytoskeleton-based directed transport mechanisms, vesicular trafficking, and via organelle contact sites. Contact sites are specialized regions where organellar surfaces are physically attached to each other to enable a direct mode of communication. First indications towards the existence of such structures came from electron microscopy studies that revealed incidences where organelles were positioned at a very close distance of just a few nanometers apart from each other [103]. However, it was not clear at the time whether this phenomenon was functionally relevant and it took another half century before the field of organelle contact site research really gained speed. In the past decade, dozens of proteins have been identified that act as physical tethers between organelles and thus form the molecular basis of contact sites [104], collectively yielding the notion that contact site formation is an active process. We now know that contact sites can occur between most, very likely even between all possible pairs of organelles [4,5], and that they respond dynamically to environmental cues [8]. Functionally, they are involved in material transfer, signaling, organelle positioning, dynamics, and inheritance, stress response, and autophagy. Contact sites are defined by several characteristic features: (i) they are actively formed, or in other words, they depend on the function of tethering molecules; (ii) they serve a biological function; (iii) they have a unique composition (proteome and/or lipidome) that is different from the untethered rest of the interacting organelles; and (iv) they do not alter the integrity of the interacting organelles, in other words, they do not promote organelle fusion [6]. LD contact sites largely follow this definition, with the exception of the last point. LDs are special cases in that they are surrounded by a phospholipid monolayer and fusion of this monolayer with the outer leaflet of bilayer-bounded organelles has been observed at LD contacts [2].

compartments. Finally, dramatic alterations in the abundance of LD–organelle contacts are being observed in response to pathological metabolic conditions, such as steatohepatitis. This key role of LD contact sites in human diseases is discussed in the following three sections.

LD Contact Site Proteins Are Implicated in Genetic Diseases

While LDs form extensive contacts with several organelles, contact sites to the endoplasmic reticulum (ER) are particularly abundant across cell types. This extensive link reflects the close interplay of these two organelles in lipid metabolism and the fact that the ER is the LDs' parent organelle [9]. The process of LD *de novo* biogenesis is not fully understood, but according to a widely accepted model, it starts with the synthesis of neutral lipids by enzymes localized at the ER membrane [10,11]. Subsequently, neutral lipids form lipid lenses between the leaflets of the ER phospholipid bilayer, which ultimately bud towards the cytosol [12]. LDs stay closely connected to the ER throughout their lifetime. In yeast, it has been found that lipidic bridges connect the phospholipid monolayer surrounding each LD to the outer leaflet of the ER membrane [10]. In mammalian cells, such lipidic bridges have also been observed, but cell type-specific differences appear to exist regarding their abundance [9]. Additionally, LD–ER contacts depend on proteinaceous factors [9]. Within the past years, several such LD–ER contact site proteins have been identified that are involved in hereditary diseases (Table 1).

Seipin is a conserved ER protein that is enriched at LD–ER interfaces and that has a role in formation and maintenance of regular LDs [13,14], as well as in the process of adipogenesis [15]. Loss of functional seipin results in morphologically abnormal LDs, ranging from tiny, immature LDs to clustered as well as supersized variants. Seipin forms symmetric ring-shaped oligomers [16,17] and physically interacts with LDAF1/promethin [18,19], which is also required for regular LD formation [18]. Seipin mutations result in an irregular architecture of LD–ER interfaces [20,21], suggesting that oligomeric seipin rings could have a structural role and support the lipidic LD–ER connection during or possibly also after LD formation. On a systemic level, loss of functional seipin results in a severe form of human lipodystrophy, termed Berardinelli-Seip congenital generalized lipodystrophy type 2 (BSCL2) [22]. This disorder is characterized by a virtually complete absence of adipose tissue, in combination with severe insulin resistance. In addition to this recessive disease that is caused by loss of seipin function, a subset of gain of function mutations in seipin result in a heterogeneous group of dominant motor neuropathies [23,24]. These neurological seipinopathies may be linked to ER stress and perturbed organelle function. Several cases are known where neurological defects and lipodystrophy are combined [25].

A number of further important players at the LD–ER interface are linked to neurological human diseases. One of these is sorting nexin 14 (SNX14), a member of the sorting nexin family of proteins. Mutations in SNX14 are causal for the autosomal recessive spinocerebellar ataxia 20 (SCAR20) [26]. This disease is characterized by cerebellar hypertrophy, intellectual disability, hearing loss, facial alterations, and defects in speech. On a cellular level, SNX14 is a metabolically controlled LD–ER tether [27]. SNX14 is an ER-anchored multidomain protein [28] that, upon exposure to fatty acids, relocalizes from a dispersed to a punctate pattern [27]. These puncta represent SNX14 proteins bound to the LD surface via a C terminal nexin domain. Absence of SNX14 results in defects in LD formation and morphology [27]. Loss of the yeast SNX14 homolog, mitochondrial distribution and morphology 1 (Mdm1), results in hypersensitivity to fatty acid-induced lipotoxicity [29], opening the possibility of lipotoxicity being involved in etiology of the disease.

The small GTPase Rab18 has multiple cellular locations, among them the LD surface. Rab18 overexpression enhances LD–ER association by a mechanism involving alteration of perilipin 2

Table 1. Human Diseases Associated with Alterations in LD Contact Sites

Human disease	Alterations in LD contact sites	Description	Refs
Genetic diseases			
Berardinelli-Seip congenital generalized lipodystrophy type 2 (BSCL2); OMIM 269700	Mutations in seipin, an ER–LD contact site protein	Seipin loss of function results in defective LD formation and adipogenesis	[22]
Neurological seipinopathies; OMIM 619112 and 270685	Mutations in seipin, an ER–LD contact site protein	Toxic gain of function mutations in seipin might cause ER alterations	[23,24]
Progressive encephalopathy with or without lipodystrophy (PELD); OMIM 615924	Mutations in seipin, an ER–LD contact site protein	Homozygous or compound heterozygous seipin truncation results in neurological syndrome often associated with mild lipodystrophy	[25]
Autosomal recessive spinocerebellar ataxia 20 (SCAR20); OMIM 616354	Mutations in SNX14, an ER–LD contact site protein	SNX14 is involved in LD formation and has been suggested to prevent lipotoxicity	[26,27]
Chorea acanthocytosis; OMIM 200150	Mutations in VPS13A, an ER–LD contact site protein	VPS13 proteins are bulk lipid transfer proteins. Mutations in all four human VPS13 genes result in neurological diseases	[34,35,42,43]
McLeod syndrome; OMIM 300842	Mutations in Kell blood group precursor XK	XK interacts with VPS13A, pointing towards a link between McLeod syndrome and chorea acanthocytosis	[40,41]
Early onset Parkinson's disease; OMIM 616840	Mutations in VPS13C, an ER–LD contact site protein	See earlier (description of VPS13A)	[37,42]
Warburg Micro syndrome; OMIM 614222	Mutations in the ER–LD contact site protein Rab18 and its partner proteins	Warburg Micro syndrome involves diverse nerve cell defects, including alterations in morphology and migrations. The role of LDs in its etiology is unclear	[30,31,33]
Hereditary spastic paraplegia (HSP); OMIM 182601	Mutations in spastin, an LD–peroxisome contact site protein	Spastin mutations are the most frequent cause for HSP	[44,45]
Adrenoleukodystrophy (ALD); OMIM 300100	Mutations in ABCD1, an LD–peroxisome contact site protein	ABCD1 has been identified as a partner protein of spastin	[44,46]
Infectious diseases			
Poliomyelitis	Induction of LD–replication organelle contacts	Poliovirus proteins 2B and 2C target to the LD surface	[49,50]
Dengue fever	Induction of LD–autophagosome contacts and lipophagy; induction of LD–replication organelle contacts	DENV protein NSA4 interacts with LD AUP1 and triggers lipophagy; Rab18 mediates LD–replication organelle contacts	[53,54,56]
Hepatitis C	Induction of LD contacts with the ER that contains viral replication and assembly sites	HCV NS5A interacts with Rab18	[58,63]
Tuberculosis	Induction of LD–phagosome contacts	Mtb cell wall lipids LAM and PIM and host protein Rab7 promote contacts	[67]
Chlamydia infection	Induction of contacts between LDs and bacterial inclusions	*Chlamydia* protein Lda3 localizes to host LDs and mediates association with inclusion and ultimately uptake at IncA-enriched domains	[69]
Toxoplasmosis	Induction of LD–parasitophorous vacuole contacts	Rab7-positive LDs associate with parasitophorous vacuole and, upon uptake, with an intravacuolar membrane network	[70]
Metabolic liver disease			
Non-alcoholic fatty liver disease	Increased LD contacts to mitochondria, plasma membrane, and ER, and fragmentation of the Golgi around LDs	Lipid accumulation in hepatocytes might be promoted by decreased secretory capacity and by reprogramming towards lipid storage	[92]

(PLIN2) levels on LDs [30]. Rab18 forms a complex with three factors termed *N*-acetyl-β-D glucosaminidase (NAG), RAD50-interacting protein 1 (RINT1), and centromere/kinetochore protein zw10 homolog (ZW10), and with ER-integral soluble *N*-ethylmaleimide attachment

protein receptor (SNARE) proteins, which has been proposed to act as LD–ER tether [31]. A further partner of Rab18 is double FYVE-containing protein 1 (DFCP1), loss of which results in decreased LD–ER contacts, while overexpression leads to contact expansion [32]. Mutations in Rab18, as well as in Rab18 partner proteins, causes Warburg Micro syndrome, a neurodevelopmental disorder characterized by optical atrophy, intellectual disability, microcephaly, and hypogenitalism [33]. Rab18 is also involved in the remodeling of organelle interfaces in response to infection (see next section).

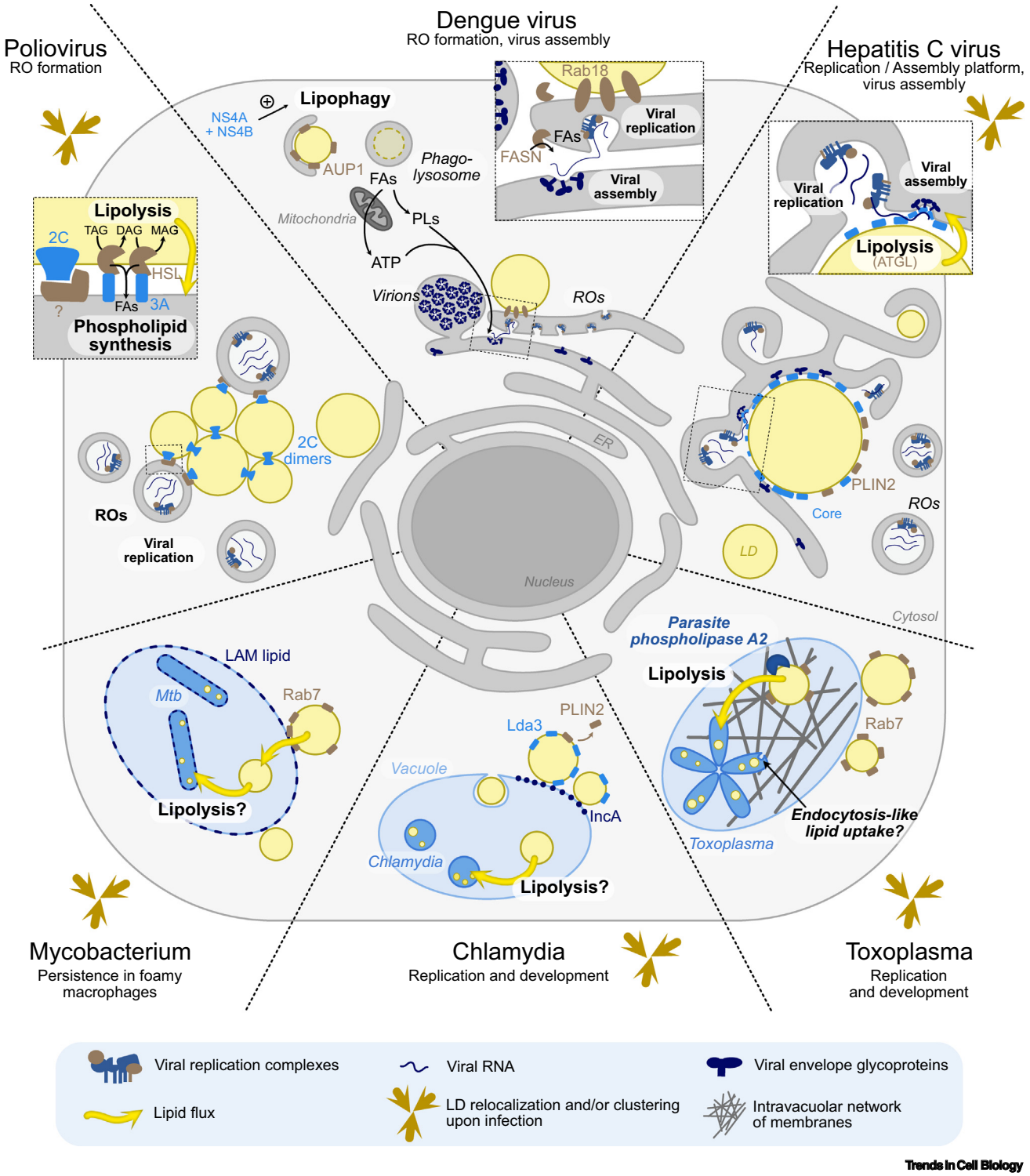
Vacuolar protein sorting-associated 13 (VPS13) proteins are a family of lipid transfer proteins with four members in human, termed VPS13A–D. Mutations in all four human VPS13 variants are linked to neurological disorders: chorea acanthocytosis (VPS13A) [34,35], a disease that involves neuronal loss from the striatum and blood cell abnormalities; Cohen syndrome (VPS13B) [36], a clinically heterogeneous disorder that typically includes microcephaly, intellectual disability, and hypotonia; early onset Parkinson's disease (VPS13C) [37]; and a form of spastic ataxia (VPS13D) [38,39]. VPS13A forms a complex with the Kell blood group precursor protein XK [40], mutations of which cause McLeod syndrome [41], a disease with similarities to chorea acanthocytosis. Several members of the conserved VPS13 family localize to multiple organelle contact sites, including LD contacts. For example, human VPS13A localizes to ER–LD as well as ER–mitochondria contacts [42,43], while human VPS13C localizes to ER–LD and ER–endosome contacts [42]. The lipid transfer domain of VPS13 proteins is unusually long and can bind multiple lipid molecules at once. The domain is rod-shaped and long enough to span an entire contact site cleft, pointing towards a possible molecular role in bulk interorganellar lipid transfer [42]. VPS13 proteins show a pronounced accumulation on LDs during metabolic alterations in the liver (see section on metabolic liver disease).

In addition to the LD–ER contact site factors, a recently identified LD–peroxisome tether complex is also linked to neurological diseases. This tether complex contains spastin [44], mutations of which are the most common cause for hereditary spastic paraplegia [45], a disease characterized by progressive spasticity, particularly of the lower extremities. The spastin protein binds to LDs via an N terminal hydrophobic helical hairpin and physically interacts with the peroxisomal fatty acid transporter ATP binding cassette subfamily D member 1 (ABCD1) [44]. ABCD1 mutations are related to X-linked adrenoleukodystrophy [46], which includes demyelination and adrenocortical insufficiency. The spastin–ABCD1 tether is involved in interorganellar fatty acid trafficking in a manner likely mediated by endosomal sorting complexes required for transport (ESCRT) III subunits and in controlling lipid peroxidation [44].

In summary, mutations in numerous LD contact site factors are associated with hereditary neurodevelopmental, neurodegenerative, and metabolic diseases. Our understanding of the molecular mechanisms underlying etiology of these diseases and of the exact roles of LD contact site alterations in disease progression is currently in its infancy. A key prerequisite for progress will be a detailed understanding of the full repertoire of contact site cargoes. As alterations in metabolic conditions, such as nutrient availability, can result in dramatic rerouting of interorganelle lipid transport [47], methodologies and probes to broadly monitor interorganelle lipid dynamics in different cell types in response to diverse metabolic cues, and ultimately in different tissues of *in vivo* disease models, will be crucial.

Intracellular Pathogens Exploit LD Contact Sites in Infectious Disease

LD–organelle interfaces have been identified as important factors in a range of infectious diseases caused by obligate intracellular pathogens. These include viral, bacterial, and parasite infections (Figure 1).



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Intracellular pathogens rely on the host cells' metabolism for multiplication. As such, it is not surprising that diverse host lipid species are utilized by pathogens either as a source of energy, as building blocks, or for formation of intracellular replication organelles (Box 3). Prominent cases are the membranous replication organelles induced by positive-stranded RNA viruses [48]. Lipid transfer to these organelles can occur via membrane contact sites and lipid transfer proteins, but recent data suggest that some of these viruses can additionally alter LD contact sites to facilitate lipid flow (Table 1).

One example is the formation of direct LD–replication organelle contact sites in poliovirus-infected cells [49,50]. Poliovirus infection relocalizes LDs to replication organelle clusters [50]. The poliovirus proteins 2B and 2C target the LD phospholipid monolayer with amphipathic helices that tether LDs to the replication organelles via a currently unknown adaptor protein [49]. A third viral protein, 3A, interacts with the two major lipases adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL), directing liberated fatty acids to the replication organelle [49]. Consistently, HSL inhibition delays viral replication kinetics [49]. In parallel, the viral protease 2A triggers the recruitment of cellular CTP:phosphocholine cytidylyltransferase α (CCTα) from the nucleus to the replication organelle and its concomitant activation [51]. CCTα catalyzes the rate-limiting step in phosphatidylcholine synthesis, with TAG-derived fatty acids being the main supply for membrane lipid synthesis [52]. Increased phospholipid synthesis sustains the formation of poliovirus replication organelles, protecting the virus against recognition by the host immune defenses and thereby facilitating virus spread [51].

In contrast, dengue virus (DENV) morphogenesis, but not replication organelle formation or RNA replication, depends on a different mechanism of TAG breakdown, which is the lipophagic release of fatty acids stored in LDs [53,54]. This process depends on viral nonstructural (NS) proteins 4A and 4B. NS4A directly interacts with LD-resident ancient ubiquitous protein 1 (AUP1) to initiate LD–autophagosome contacts and to trigger lipophagy [54]. The released fatty acids are used as energy source and for morphogenesis of progeny virions [53,54]. DENV capsid protein C also localizes to LDs and facilitates LD accumulation in DENV-infected cells [55]. Whether these differing results, LD accumulation versus LD consumption in DENV infection,

Figure 1. LD Contact Sites in Infection. Schematic representation of several known and putative LD contact sites induced or hijacked by intracellular pathogens for their replication and persistence. Infection can induce the accumulation of LDs [55,64,65,70], their relocalization towards the pathogen inclusion or replication site, and their clustering (group of yellow arrows) [49,69,70]. Whether these changes of the LD population are directly induced by the infection or secondarily via activation of the host cell's innate immune response or cell stress pathways is not always clear [64–66]. In any case, they are likely to facilitate formation of heterologous (e.g., LD–replication organelle or LD–parasitophorous vacuole) as well as homologous LD contact sites (LD–LD contacts upon clustering, see poliovirus). The LD contacts might serve to extract lipids as an energy source for pathogen replication or assembly (e.g., DENV [53]) or as structural lipids for the pathogen itself or its replication organelle (e.g., poliovirus [49]). The lipolytic machinery, including the ATGL and HSL lipases, is instrumental for this lipid transfer in the case of poliovirus and *Toxoplasma* [49,70], but also for HCV assembly [62]. On the opposite, DENV hijacks the lipophagy machinery to extract fatty acids from LDs [53,54]. In some cases of bacterial or parasite infections, LDs are directly taken up into the pathogen vacuole [69,70], where lipid transfer to the pathogen occurs [64,65,69,70]. The molecular tethers between the LDs and the apposed organelles often remain unknown. The best described example is likely the poliovirus 2C protein, targeted to the LD surface and anchoring LDs to an unknown replication organelle-embedded partner. In DENV-infected cells, the LD protein AUP1 remains mostly nonubiquitylated and redistributes towards autophagosomes, where it is involved in virus-induced lipophagy and virus production. Among the effectors secreted by *Chlamydia* into the host cytosol and targeted to the LD surface, Lda3 competes with PLIN2 and directs the LD recruitment to the inclusion, initiating their engulfment at sites enriched in the bacterial IncA, a mimic of the eukaryotic SNARE proteins [69]. As for *Mycobacterium*-infected cells, Rab7 localizes to LDs and binds the bacterial lipids LAM and PIM that are incorporated in the phagocytic vacuole. While Rab7 was also evidenced at the surface of vacuolar LDs in *Toxoplasma*-infected cells, its role in the LD–vacuole contact in this context is unclear. Note that contact sites might involve not only the LDs and pathogen-induced organelles, but also a third partner, for instance, the ER in case of poliovirus [49] or HCV [63]. To which extent pathogen infections also modulate existing contacts between LDs and other cellular organelles is not yet well understood. Also, as a *mise en abyme*, the intrabacterial and intraparasitic LDs might themselves maintain contacts with other organelles or organelle-like structures inside the pathogen, a question that to our knowledge has not been studied so far. Abbreviations: ATGL, adipose triglyceride lipase; AUP1, ancient ubiquitous protein 1; DAG, diacylglycerol; DENV, dengue virus; ER, endoplasmic reticulum; Fas, fatty acids; FASN, fatty acid synthase; HCV, hepatitis C virus; HSL, hormone sensitive lipase; IncA, inclusion membrane protein A; LAM, lipoarabinomannan; LD, lipid droplet; Lda3, LD-associated protein 3; MAG, monoacylglycerol; Mtb, *Mycobacterium tuberculosis*; NS4A/B, nonstructural protein 4A/B; PIM, diacylglycerophosphoinositolmonomannosides; PLIN2, perilipin 2; PLs, phospholipids; RO, replication organelle; SNARE, soluble *N*-ethylmaleimide attachment protein receptor; TAG, triacylglycerol.

Box 3. Rearrangement of Host Cell Membranes by Intracellular Pathogens to Sustain Their Replication

Despite their broad genetic diversity, it is a common feature of plus-strand RNA viruses to rearrange host cell membranes to form replication organelles [48]. This virus-induced niche harbors the replication complexes formed by host and viral factors that replicate the viral genome. It also provides a compartment that is inaccessible to receptors and effectors of the host innate immune responses. The replication organelles in HCV-, DENV-, and poliovirus-infected cells all originate from the host ER or secretory pathway. Intracellular bacteria and parasites are also typically internalized in plasma membrane-derived compartments (parasitophorous vacuole, or inclusion) that resemble phagosomes [105]. However, these pathogens actively suppress phagosome maturation and fusion to lysosomes. Owing to the secretion of pathogen effectors into the host cell's cytosol, these vacuoles act as a magnet for host organelles, for instance, they selectively attract LDs, which are often consumed by the pathogens (see Figure 1 in main text). All these pathogen-induced compartments are tightly linked to host cell metabolic pathways involving active crosstalk. We are only starting to uncover the functional role of LD contact sites to supply them with lipids and to decipher the molecular machinery involved.

are due to cell type differences or depend on viral replication kinetics, and thus energy and lipid demand, still needs to be determined. Importantly, the small GTPase Rab18, a protein that induces close apposition of LDs with ER cisternae [30], facilitates DENV infection by targeting fatty acid synthase to sites of viral replication and thus stimulating local fatty acid biosynthesis [56].

Rab18 was also described to mediate the transfer of hepatitis C virus (HCV) capsid protein core to LDs, likely through direct contact sites [57]. Rab18 but also PLIN3 interact with NS5A and contribute to HCV RNA replication, possibly by promoting LD–replication organelle contacts [58,59]. Mechanistically, the tight interactions of LDs with ER-membranes in cells overexpressing Rab18 were linked to decreased PLIN2 levels at LDs [30]. However, sole PLIN2 depletion causes tight wrapping of LDs in ER-derived membranes, but prevents HCV core trafficking to LDs [60]. These data indicate that Rab18 establishes LD–ER contacts not only via displacing PLIN2 and that intact LD-ER contact sites are required for HCV core translocation to LDs, a prerequisite for HCV morphogenesis [61]. LDs are indeed primarily involved in HCV assembly and maturation [61] and their ATGL-driven lipolysis facilitates formation of infectious low-density lipoviroparticles [62]. Still, HCV RNA replication was observed in isolated LD fractions [61]. Moreover, latest correlative light and electron microscopy and electron tomography studies revealed double-membrane vesicles that represent HCV replication organelles connected to ER membranes, themselves tightly wrapped around LDs [63]. This indicates that LDs may indeed represent a lipid source for both replication organelle formation and virion morphogenesis in HCV-infected cells and that both replication steps occur in close apposition to LDs.

Some obligate intracellular bacteria that are major human pathogens also depend on LD–replication center contacts for replication or persistence. In tuberculosis patients, *Mycobacterium tuberculosis* (Mtb) persists in granulomas, which are organized aggregates of immune cells containing plenty of LD-rich foamy macrophages [64]. In fact, Mtb itself, its induction of innate immune response, and the granuloma hypoxic conditions might all cause LD accumulation in macrophages [64–66], which is thought to induce a switch to pathogen latency and provide a nutritional source for bacterial persistence. In infected cells, LDs surround and eventually fuse with bacterial phagosomes [64], thereby providing fatty acids for LD formation within the bacterium [64,65]. The bacterial cell wall lipids lipoarabinomannan (LAM) and glycerophosphoinositolmannoside (PIM), as well as the host protein Rab7, promote the LD–phagosome contacts [67]. Rab7 controls lipophagy in hepatocytes [68]; whether lipid mobilization from LDs in Mtb-infected macrophages depends on the lipophagic machinery of the host is currently unknown.

Another bacterial pathogen, *Chlamydia*, also ingests host LDs into the bacterial inclusions. *Chlamydia* encodes three LD-associated (Lda) proteins that localize to host LDs. Lda3 directs LD transport to the bacterial inclusion and mediates their uptake, at vacuole subdomains

enriched in the bacterial inclusion membrane protein A (IncA), and concomitantly with a loss in PLIN2 [69].

A final example where LD contact sites are implicated in infection is *Toxoplasma*: this obligate intracellular parasite also depends on host LDs as a nutritional source for efficient replication and development. Upon infection, the number of host LDs increases and they tightly surround the parasitophorous vacuole, which represents the intracellular replication and development organelle [70]. Some of these Rab7-positive LDs are transported into the parasitophorous vacuole and form tight contacts with an intravacuolar network of membranes [70]. These membranes contain a parasitic phospholipase that liberates fatty acids [70]. After lipolysis, *Toxoplasma* incorporates these lipids into its own membranes and LDs for intracellular development [70].

While these examples demonstrate that LD–organelle contacts favor pathogen replication, LDs are also involved in the innate immune response towards infection, highlighted, for example, by the LD localization of the interferon-inducible broadly acting antiviral protein viperin to LDs [71]. More recently, an additional involvement of LD organelle contacts became evident. In cells stimulated with lipopolysaccharides (LPS), the LD proteome is dramatically altered, with interferon-inducible proteins like viperin, guanosine triphosphatases, and cathelicidin forming protein clusters at LDs [72]. On the opposite, PLIN5 is depleted from LDs by LPS treatment; this leads to reduced LD–mitochondria contacts impairing β-oxidation while simultaneously increasing the contacts between bacterial inclusions and LDs [72]. If LD contacts are more directly involved in the innate immune response (i.e., by interacting with innate immune signaling platforms on mitochondria or peroxisomes) is currently not known.

In summary, while a functional role of LDs in the replication cycles of diverse pathogens and in the host cell's response to infection is evident, the molecular characterization of LD contacts to other organelles in infectious diseases is still rather limited but as the provided examples highlight, certainly warrants in-depth examination. The utilization of advanced methods to define LD proteome alterations induced by infection (i.e. through proximity labeling strategies) [73], will help to characterize LD contacts in infectious diseases. Likewise, advances in microscopy methods will aid to visualize dynamic interactions between LDs and other organelles during the infectious cycle. How this affects the cellular lipid flux, especially of pro- and anti-inflammatory lipid mediators and their precursors, remains to be investigated and will require more sensitive methods to track and follow lipids through the cellular compartments.

LD Contact Sites Are Remodeled in Metabolic Liver Disease

Modulation of organelle contact sites is a hallmark of metabolic rewiring [8]. The contact sites of LDs respond drastically to metabolic alterations occurring during non-alcoholic fatty liver disease (NAFLD) (Table 1). Characteristic for NAFLD is the accumulation of large LDs in hepatocytes, the most abundant cell type in the liver fulfilling most of the organ's metabolic functions. These cells tightly regulate the uptake, synthesis, storage, and secretion of lipids in order to accomplish the liver's major role in regulating the distribution of lipids within the body [74]. Dependent on substrate availability and metabolic signals, hepatocytes dynamically store fatty acids in the form of TAGs and SEs in LDs or secrete them in the form of lipoproteins and bile acids into the circulation to maintain whole-body lipid homeostasis [74]. Under normal conditions, only small amounts of lipids are stored in the liver, but any imbalance between external fatty acid uptake, internal *de novo* synthesis, and fatty acid degradation or secretion results in pathologic lipid accumulation. This is manifested in diverse forms of fatty liver disease, ranging from asymptomatic, to mild steatosis, to severe steatohepatitis [75]. Indeed, due to the epidemic of obesity, the

prevalence of NAFLD has reached 25% of people worldwide [76] and has become a major health problem as it can lead to liver failure and increases the risk for hepatocellular carcinoma.

Considering the extreme dynamics of lipid metabolism and the high lipid flux rates in hepatocytes, LDs extensively cooperate and interact with each other and other cellular compartments by contact sites (Figure 2). As an example, lipid transfer at LD–LD contacts is facilitated by members of the family of cell death-inducing DFFA-like effector (CIDE) proteins. These proteins localize to homotypic LD interfaces and mediate directional lipid transfer from smaller to larger LDs in order to promote LD growth [77,78]. In lean mice, hepatocytes express only CIDEB, and *cideb–/–* mice are resistant to high-fat diet-induced liver steatosis [79]. CIDEC and CIDEA, which are normally only expressed in adipose tissue, are highly upregulated during high-fat diet feeding, but also during fasting, when adipose tissue-derived lipids are channeled to the liver [80]. CIDEC upregulation is also observed in humans with NAFLD [81] and might contribute to the formation of the characteristic large LDs in steatosis similar to what is observed in adipocytes.

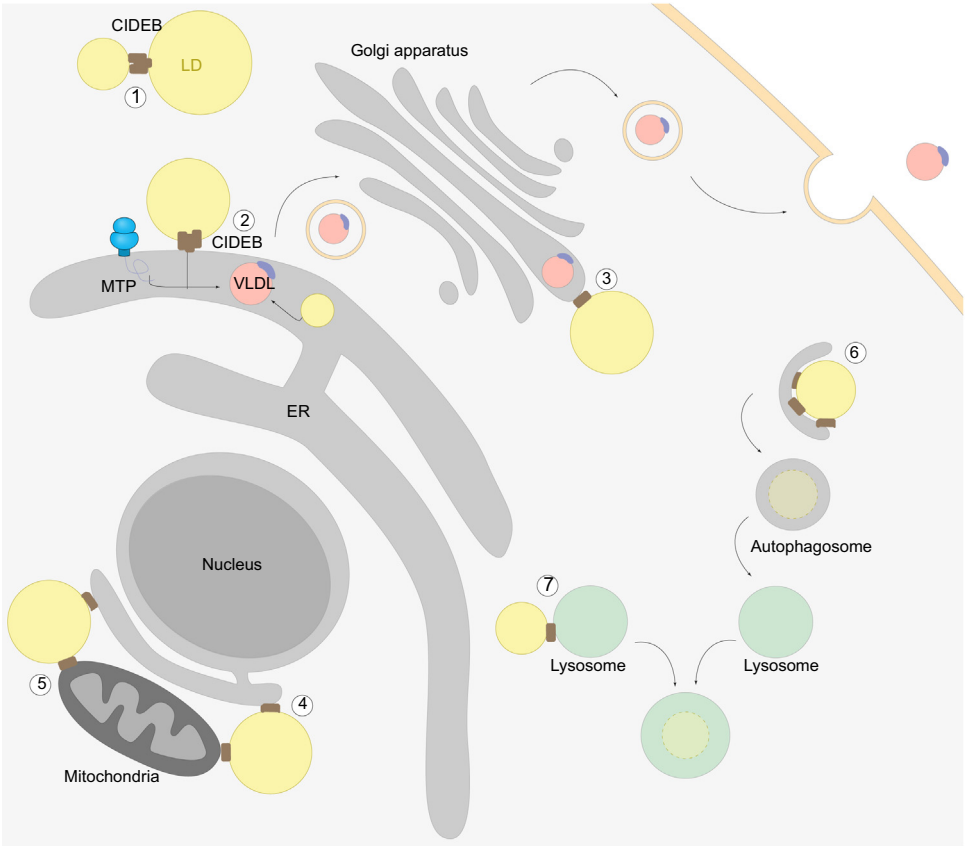

Figure 2. LD Contact Sites in Hepatocytes. Hepatocyte LDs form multiple contact sites to orchestrate lipid metabolism. (1) Inter-LD contacts are mediated by CIDEB that functions in lipid transfer for LD growth. (2) LDs form contacts with the ER and CIDEB was speculated to transfer lipids to nascent VLDL particles. (3) Especially in steatotic conditions, LDs form contacts with the Golgi apparatus. Partial fragmentation of the Golgi apparatus and a reduction of the secretory capacity were observed under lipid load. The function of the contact sites for VLDL lipidation is unclear. (4,5) In steatotic liver contacts between LDs and mitochondria and the ER are increased. Contact site proteins regulating these interactions are still under investigation. (6,7) Contacts with autophagosomes and lysosomes are required for lipophagy. Abbreviations: CIDEB, cell death-inducing DFFA-like effector B; LD, lipid droplet; MTP, microsomal transfer protein; VLDL, very-low density lipoprotein.

Besides CIDE proteins, perilipins are major LD proteins involved in LD contact site formation. Although perilipins were the first proteins discovered on the LDs [82], their multiple functions and roles for LD homeostasis are still not fully explored. In mammals, the perilipin protein family consists of five scaffolding proteins (PLIN1–5) that regulate access of proteins such as lipases to the LD surface [83]. In addition to their function in lipolysis, different perilipins play important roles in LD contact site formation by so far poorly understood mechanisms. PLIN5 and a Mitofusin2–PLIN1 complex are involved in formation of contacts between LDs and mitochondria [84,85]. PLIN5 acts as tethering factor since its carboxy terminus mediates the interaction with mitochondria and overexpression of PLIN5 leads to the recruitment of mitochondria to LDs [86]. Similar to other oxidative tissues, PLIN5 is highly expressed in the liver and depletion of PLIN5 leads to increased hepatic lipid accumulation. PLIN5 is also downregulated in chronic steatosis in humans [87]. However, how those phenotypes correlate with impaired organelle interactions has not been investigated yet. Another process in hepatocytes, where contact sites come into play, is lipid degradation. In the liver, lipophagy contributes substantially to lipid mobilization in addition to conventional lipase-dependent lipolysis [88]. Recently, a novel lipid degradation pathway was discovered that depends on direct LD–lysosomal interactions. At sites of LD–lysosome contacts, direct transfer of proteins and lipids into lysosomes for subsequent degradation was described, particularly under starvation conditions. The lipid transfer was independent of the canonical autophagy machinery and did not require an engulfment within autophagosomes before delivery into lysosomes [89]. How much this pathway contributes to lipid degradation in the liver and whether it also occurs in other tissues will be of interest for future research.

A key liver-specific process, for which contact sites play an important role, is the formation and assembly of very-low density lipoprotein (VLDL), the main lipoprotein derived from hepatocytes. This process requires dynamic interactions of LDs with the secretory apparatus since lipids for VLDL synthesis are derived primarily from stored lipids and less from *de novo* lipogenesis [90]. How lipids are mobilized from LDs and transferred to nascent VLDL remains enigmatic. CIDEB localizes to LD–ER contact sites, influences VLDL maturation, and regulates apolipoprotein B lipidation. This protein was proposed to facilitate transfer of TAG from the cytosol across the ER [91]. HCV highjacks the LD and lipoprotein production machinery to generate infectious lipoprotein-like viral particles. To which extent VLDL and HCV lipidation occur in the ER or during the trafficking through the secretory apparatus is not clear. In steatotic liver in mice, a fragmentation and accumulation of the Golgi apparatus around LDs was observed [92]. This organelle association could function in lipid transfer for VLDL lipidation, or could alternatively be a pathological process. The concurrent reduction of the secretory capacity might explain why hepatic lipid accumulation cannot be compensated by increased TAG secretion. In patients with non-alcoholic steatohepatitis (NASH), the increase in VLDL secretion does not reach the extent of TAG synthesis [93] and intrahepatic lipid levels are negatively correlated with VLDL-TAG secretion rates [94]. This feedback loop might be a factor driving the progression of steatosis [95].

In addition to the rearrangements of the secretory apparatus, contact sites between all major organelles involved in lipid metabolism are strengthened in steatotic liver. In the liver of mice fed a high-fat diet, increased contact sites between LDs and mitochondria, plasma membrane, and ER were reported [92]. These contact sites might help to reprogram the cells towards increased lipid storage by channeling of fatty acids after their uptake through the plasma membrane and conversion into TAG in the ER to form LDs. Increased mitochondrial contacts might favor efficient trafficking of fatty acids released from LDs by lipases for β-oxidation that is upregulated in high-fat diet. Of note, in steatotic liver, the

organelle tether proteins extended synaptotagmin 2 (ESYT2), VPS13A, and VPS13D were detected in the LD proteome [92], pointing towards a potential functional role in regulating LD contact sites in the liver.

It becomes evident that pathological and adaptive changes in steatosis affect not only protein expression and organelle composition, but also LD contact sites and dynamics. The underlying mechanisms, the involved protein and lipid factors, and the pathological relevance for disease progression are completely unexplored, but might be aspects that need to be taken into consideration for understanding cellular processes in NAFLD and NASH progression. Increasing our understanding of which proteins are involved in the contact site formation and how contact site formation is regulated by the activity of signaling pathways or post-translational modifications might offer possibilities for pharmacological interventions for promoting lipid mobilization and degradation in order to prevent the progression of NAFLD/NASH.

Concluding Remarks

The recent recognition of LD contact sites and of their association with a range of genetic, metabolic, and infectious diseases has the potential to lead towards new therapeutic and diagnostic avenues. While the relevance of LD contacts in health and disease is clear, a key challenge is our current lack of mechanistic insights into physiological as well as pathological processes at LD interfaces. A basic yet fundamental prerequisite is the identification of the full repertoire of LD contact site components in different cell types. Development of new contact site probes for high-content genetic approaches as well as advanced bioinformatics will be required. Additionally, identifying all cargoes that are exchanged at the different LD contact sites (specific lipid species and proteins) will depend on new sensitive and specific probes adapted for live cell imaging and flux analysis. The recent advances in resolution of lipidomics and proteomics approaches suggest that correlative mass spectrometry imaging at the subcellular resolution may help to tackle this question in an unbiased manner. The detection of lipids with high spatial resolution is technically challenging, particularly for low abundant lipid species. As those, however, are often involved in signaling, pushing the detection limits will be of high importance. A further challenge is the plasticity of LD contacts, which is clearly illustrated by the dramatic contact rearrangements induced by infection and nutrient overload. Therefore, it will be crucial to broadly investigate diverse cell types and metabolic conditions and to ultimately study LD contacts in intact tissues and whole organisms (see Outstanding Questions). A long-term goal of this emerging research path is the development of molecular tools to restore LD contact site functions, lipid homeostasis, and cellular functionality altered in disease.

Outstanding Questions

How can we better detect LD contact sites and how can we address their dynamic behavior?

What is the full repertoire of proteins and lipids involved in the formation of LD contact sites? Can we identify novel contact site components by techniques that are less prone to compromising cellular structure than conventional techniques, such as proximity labeling or bimolecular complementation assays?

How can we improve the monitoring of the flow of different cargoes through contact sites, *in vitro* as well as *in vivo*? What is the full spectrum of molecules that are transferred across LD contact sites? Are LD contact sites involved in the transfer of non-lipid cargoes, such as proteins?

How can we more specifically target LD contact site functions instead of knocking-down/knocking-out contact site proteins, which are often involved in additional processes besides their role in contacts?

Are LD contact sites structurally and/or functionally linked to intracellular LD diversification? Are the distinct pools of LDs often observed within a single cell engaged in particular organelle contact sites?

What is the mechanistic basis for the link between LD contact sites and the development and progression of neurological disorders?

To which extent are LD contact sites remodeled under metabolically challenging conditions in the different tissues of the body?

Can we, in the future, use contact site constituents as novel targets for disease intervention in hereditary, infectious, and metabolic diseases?

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Declaration of Interests

The authors declare no competing interests.

References

1. Olzmann, J.A. and Carvalho, P. (2019) Dynamics and functions of lipid droplets. *Nat. Rev. Mol. Cell Biol.* 20, 137–155
2. Thiam, A.R. *et al.* (2013) The biophysics and cell biology of lipid droplets. *Nat. Rev. Mol. Cell Biol.* 14, 775–786

3. Schuldiner, M. and Bohnert, M. (2017) A different kind of love - lipid droplet contact sites. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862, 1188–1196
4. Valm, A.M. *et al.* (2017) Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546, 162–167
5. Shai, N. *et al.* (2018) Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nat. Commun.* 9, 1761
6. Scorrano, L. *et al.* (2019) Coming together to define membrane contact sites. *Nat. Commun.* 10, 1287
7. Prinz, W.A. *et al.* (2020) The functional universe of membrane contact sites. *Nat. Rev. Mol. Cell Biol.* 21, 7–24
8. Bohnert, M. (2020) Tether me, tether me not-dynamic organelle contact sites in metabolic rewiring. *Dev. Cell* 54, 212–225
9. Hugenroth, M. and Bohnert, M. (2019) Come a little bit closer! Lipid droplet-ER contact sites are getting crowded. *Biochim. Biophys. Acta. Mol. Cell Res.* 1867, 118603
10. Jacquier, N. *et al.* (2011) Lipid droplets are functionally connected to the endoplasmic reticulum in *Saccharomyces cerevisiae*. *J. Cell Sci.* 124, 2424–2437
11. Kassan, A. *et al.* (2013) Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. *J. Cell Biol.* 203, 985–1001
12. Choudhary, V. *et al.* (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J. Cell Biol.* 211, 261–271
13. Szymanski, K.M. *et al.* (2007) The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proc. Natl. Acad. Sci. U. S. A.* 104, 20890–20895
14. Fei, W. *et al.* (2008) Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *J. Cell Biol.* 180, 473–482
15. Pagac, M. *et al.* (2016) SEIPIN regulates lipid droplet expansion and adipocyte development by modulating the activity of glycerol-3-phosphate acyltransferase. *Cell Rep.* 17, 1546–1559
16. Sui, X. *et al.* (2018) Cryo-electron microscopy structure of the lipid droplet-formation protein seipin. *J. Cell Biol.* 217, 4080–4091
17. Yan, R. *et al.* (2018) Human SEIPIN binds anionic phospholipids. *Dev. Cell* 47, 248–256
18. Chung, J. *et al.* (2019) LDAF1 and seipin form a lipid droplet assembly complex. *Dev. Cell* 51, 551–563
19. Castro, I.G. *et al.* (2019) Promethin is a conserved seipin partner protein. *Cells* 8, 268
20. Grippa, A. *et al.* (2015) The seipin complex Fld1/Ldb16 stabilizes ER-lipid droplet contact sites. *J. Cell Biol.* 211, 829–844
21. Salo, V.T. *et al.* (2016) Seipin regulates ER-lipid droplet contacts and cargo delivery. *EMBO J.* 35, 2699–2716
22. Magré, J. *et al.* (2001) Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat. Genet.* 28, 365–370
23. Patel, H. *et al.* (2001) The Silver syndrome variant of hereditary spastic paraplegia maps to chromosome 11q12-q14, with evidence for genetic heterogeneity within this subtype. *Am. J. Hum. Genet.* 69, 209–215
24. Windpassinger, C. *et al.* (2004) Heterozygous missense mutations in BSCL2 are associated with distal hereditary motor neuropathy and Silver syndrome. *Nat. Genet.* 36, 271–276
25. Guillén-Navarro, E. *et al.* (2013) A new seipin-associated neurodegenerative syndrome. *J. Med. Genet.* 50, 401–409
26. Thomas, A.C. *et al.* (2014) Mutations in SNX14 cause a distinctive autosomal-recessive cerebellar ataxia and intellectual disability syndrome. *Am. J. Hum. Genet.* 95, 611–621
27. Datta, S. *et al.* (2019) Cerebellar ataxia disease–associated Snx14 promotes lipid droplet growth at ER–droplet contacts. *J. Cell Biol.* 218, 1335–1351
28. Bryant, D. *et al.* (2018) SNX14 mutations affect endoplasmic reticulum-associated neutral lipid metabolism in autosomal recessive spinocerebellar ataxia 20. *Hum. Mol. Genet.* 27, 1927–1940
29. Hariri, H. *et al.* (2019) Mdm1 maintains endoplasmic reticulum homeostasis by spatially regulating lipid droplet biogenesis. *J. Cell Biol.* 218, 1319–1334
30. Ozeki, S. *et al.* (2005) Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. *J. Cell Sci.* 118, 2601–2611
31. Xu, D. *et al.* (2018) Rab18 promotes lipid droplet (LD) growth by tethering the ER to LDs through SNARE and NRZ interactions. *J. Cell Biol.* 217, 975–995
32. Li, D. *et al.* (2019) The ER-localized protein DFCP1 modulates ER-lipid droplet contact formation. *Cell Rep.* 27, 343–358
33. Bem, D. *et al.* (2011) Loss-of-function mutations in RAB18 cause Warburg micro syndrome. *Am. J. Hum. Genet.* 88, 499–507
34. Rampoldi, L. *et al.* (2001) A conserved sorting-associated protein is mutant in chorea-acanthocytosis. *Nat. Genet.* 28, 119–120
35. Ueno, S. *et al.* (2001) The gene encoding a newly discovered protein, chorein, is mutated in chorea-acanthocytosis. *Nat. Genet.* 28, 121–122
36. Kolehmainen, J. *et al.* (2003) Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am. J. Hum. Genet.* 72, 1359–1369
37. Lesage, S. *et al.* (2016) Loss of VPS13C function in autosomal-recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. *Am. J. Hum. Genet.* 98, 500–513
38. Gauthier, J. *et al.* (2018) Recessive mutations in VPS13D cause childhood onset movement disorders. *Ann. Neurol.* 83, 1089–1095
39. Seong, E. *et al.* (2018) Mutations in VPS13D lead to a new recessive ataxia with spasticity and mitochondrial defects. *Ann. Neurol.* 83, 1075–1088
40. Park, J.-S. and Neiman, A.M. (2020) XK is a partner for VPS13A: a molecular link between chorea-acanthocytosis and McLeod syndrome. *Mol. Biol. Cell* 31, 2425–2436
41. Ho, M. *et al.* (1994) Isolation of the gene for McLeod syndrome that encodes a novel membrane transport protein. *Cell* 77, 869–880
42. Kumar, N. *et al.* (2018) VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. *J. Cell Biol.* 217, 3625–3639
43. Yeshaw, W.M. *et al.* (2019) Human VPS13A is associated with multiple organelles and influences mitochondrial morphology and lipid droplet motility. *eLife* 8, e43561
44. Chang, C.-L. *et al.* (2019) Spastin tethers lipid droplets to peroxisomes and directs fatty acid trafficking through ESCRT-III. *J. Cell Biol.* 218, 2583–2599
45. Hazan, J. *et al.* (1999) Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat. Genet.* 23, 296–303
46. Mosser, J. *et al.* (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 361, 726–730
47. Rambold, A.S. *et al.* (2015) Fatty acid trafficking in starved cells: regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. *Dev. Cell* 32, 678–692
48. Nagy, P.D. *et al.* (2016) Building viral replication organelles: close encounters of the membrane types. *PLoS Pathog.* 12, e1005912
49. Laufman, O. *et al.* (2019) Viral generated inter-organelle contacts redirect lipid flux for genome replication. *Cell* 178, 275–289
50. Melia, C.E. *et al.* (2019) Origins of enterovirus replication organelles established by whole-cell electron microscopy. *MBio* 10, e00951-19
51. Viktorova, E.G. *et al.* (2018) Phospholipid synthesis fueled by lipid droplets drives the structural development of poliovirus replication organelles. *PLoS Pathog.* 14, e1007280
52. Kent, C. (1997) CTP:phosphocholine cytidylyltransferase. *Biochim. Biophys. Acta Lipids Lipid Metab.* 1348, 79–90
53. Heaton, N.S. and Randall, G. (2010) Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe* 8, 422–432
54. Zhang, J. *et al.* (2018) Flaviviruses exploit the lipid droplet protein AUP1 to trigger lipophagy and drive virus production. *Cell Host Microbe* 23, 819–831

55. Samsa, M.M. *et al.* (2009) Dengue virus capsid protein usurps lipid droplets for viral particle formation. *PLoS Pathog.* 5, e1000632
56. Tang, W.-C. *et al.* (2014) Rab18 facilitates dengue virus infection by targeting fatty acid synthase to sites of viral replication. *J. Virol.* 88, 6793–6804
57. Dansako, H. *et al.* (2014) Rab18 is required for viral assembly of hepatitis C virus through trafficking of the core protein to lipid droplets. *Virology* 462–463, 166–174
58. Salloum, S. *et al.* (2013) Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets. *PLoS Pathog.* 9, e1003513
59. Vogt, D.A. *et al.* (2013) Lipid droplet-binding protein TIP47 regulates hepatitis C Virus RNA replication through interaction with the viral NS5A protein. *PLoS Pathog.* 9, e1003302
60. Lassen, S. *et al.* (2019) Perilipin-2 is critical for efficient lipoprotein and hepatitis C virus particle production. *J. Cell Sci.* 132, jcs217042
61. Miyanari, Y. *et al.* (2007) The lipid droplet is an important organelle for hepatitis C virus production. *Nat. Cell Biol.* 9, 1089–1097
62. Vieyres, G. *et al.* (2020) The ATGL lipase cooperates with ABHD5 to mobilize lipids for hepatitis C virus assembly. *PLoS Pathog.* 16, e1008554
63. Lee, J.-Y. *et al.* (2019) Spatiotemporal coupling of the hepatitis C virus replication cycle by creating a lipid droplet- proximal membranous replication compartment. *Cell Rep.* 27, 3602–3617
64. Peyron, P. *et al.* (2008) Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. *PLoS Pathog.* 4, e1000204
65. Daniel, J. *et al.* (2011) *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. *PLoS Pathog.* 7, e1002093
66. Knight, M. *et al.* (2018) Lipid droplet formation in *Mycobacterium tuberculosis* infected macrophages requires IFN-γ/HIF-1α signaling and supports host defense. *PLoS Pathog.* 14, e1006874
67. Roque, N.R. *et al.* (2020) Rab7 controls lipid droplet-phagosome association during mycobacterial infection. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids* 1865, 158703
68. Schroeder, B. *et al.* (2015) The small GTPase Rab7 as a central regulator of hepatocellular lipophagy. *Hepatology* 61, 1896–1907
69. Cocchiaro, J.L. *et al.* (2008) Cytoplasmic lipid droplets are translocated into the lumen of the *Chlamydia trachomatis* parasitophorous vacuole. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9379–9384
70. Nolan, S.J. *et al.* (2017) Host lipid droplets: an important source of lipids salvaged by the intracellular parasite *Toxoplasma gondii*. *PLoS Pathog.* 13, e1006362
71. Hinson, E.R. and Cresswell, P. (2009) The antiviral protein, viperin, localizes to lipid droplets via its N-terminal amphipathic -helix. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20452–20457
72. Bosch, M. *et al.* (2020) Mammalian lipid droplets are innate immune hubs integrating cell metabolism and host defense. *Science* 370, eaay8085
73. Bersuker, K. *et al.* (2018) A proximity labeling strategy provides insights into the composition and dynamics of lipid droplet proteomes. *Dev. Cell* 44, 97–112
74. Gluchowski, N.L. *et al.* (2017) Lipid droplets and liver disease: from basic biology to clinical implications. *Nat. Rev. Gastroenterol. Hepatol.* 14, 343–355
75. Younossi, Z. *et al.* (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15, 11–20
76. Cotter, T.G. and Rinella, M. (2020) Nonalcoholic fatty liver disease 2020: the state of the disease. *Gastroenterology* 158, 1851–1864
77. Gong, J. *et al.* (2011) Fsp27 promotes lipid droplet growth by lipid exchange and transfer at lipid droplet contact sites. *J. Cell Biol.* 195, 953–963
78. Xu, W. *et al.* (2016) Differential roles of cell death-inducing DNA fragmentation factor-α-like effector (CIDE) proteins in promoting lipid droplet fusion and growth in subpopulations of hepatocytes. *J. Biol. Chem.* 291, 4282–4293
79. Li, J.Z. *et al.* (2007) Cideb regulates diet-induced obesity, liver steatosis, and insulin sensitivity by controlling lipogenesis and fatty acid oxidation. *Diabetes* 56, 2523–2532
80. Vilà-Brau, A. *et al.* (2013) Fsp27/CIDEC is a CREB target gene induced during early fasting in liver and regulated by FA oxidation rate. *J. Lipid Res.* 54, 592–601
81. Sans, A. *et al.* (2019) The differential expression of cide family members is associated with NAFLD progression from steatosis to steatohepatitis. *Sci. Rep.* 9, 7501
82. Brasaemle, D.L. *et al.* (2000) Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. *J. Biol. Chem.* 275, 38486–38493
83. Bickel, P.E. *et al.* (2009) PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1791, 419–440
84. Benador, I.Y. *et al.* (2018) Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion. *Cell Metab.* 27, 869–885
85. Boutant, M. *et al.* (2017) Mfn2 is critical for brown adipose tissue thermogenic function. *EMBO J.* 36, 1543–1558
86. Wang, H. *et al.* (2011) Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria. *J. Lipid Res.* 52, 2159–2168
87. Pawella, L.M. *et al.* (2014) Perilipin discerns chronic from acute hepatocellular steatosis. *J. Hepatol.* 60, 633–642
88. Carotti, S. *et al.* (2020) Lipophagy impairment is associated with disease progression in NAFLD. *Front. Physiol.* 11, 850
89. Schulze, R.J. *et al.* (2020) Direct lysosome-based autophagy of lipid droplets in hepatocytes. *Proc. Natl. Acad. Sci. U. S. A.* 117, 32443–32452
90. Yang, L.Y. *et al.* (1995) Origin of triacylglycerol moiety of plasma very low density lipoproteins in the rat: structural studies. *J. Lipid Res.* 36, 125–136
91. Ye, J. *et al.* (2009) Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. *Cell Metab.* 9, 177–190
92. Krahmer, N. *et al.* (2018) Organellar proteomics and phosphoproteomics reveal subcellular reorganization in diet-induced hepatic steatosis. *Dev. Cell* 47, 205–221
93. Choi, S.H. and Ginsberg, H.N. (2011) Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends Endocrinol. Metab.* 22, 353–363
94. Lytle, K.A. *et al.* (2019) Hepatic fatty acid balance and hepatic fat content in humans with severe obesity. *J. Clin. Endocrinol. Metab.* 104, 6171–6181
95. Minehira, K. *et al.* (2008) Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. *J. Lipid Res.* 49, 2038–2044
96. Sołtysik, K. *et al.* (2019) Nuclear lipid droplets derive from a lipoprotein precursor and regulate phosphatidylcholine synthesis. *Nat. Commun.* 10, 473
97. Liao, Y. *et al.* (2019) Mitochondrial lipid droplet formation as a detoxification mechanism to sequester and degrade excessive urothelial membranes. *Mol. Biol. Cell* 30, 2969–2984
98. Greenberg, A.S. *et al.* (1991) Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J. Biol. Chem.* 266, 11341–11346
99. Sztalryd, C. and Brasaemle, D.L. (2017) The perilipin family of lipid droplet proteins: gatekeepers of intracellular lipolysis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862, 1221–1232
100. Cermelli, S. *et al.* (2006) The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr. Biol.* 16, 1783–1795
101. Anand, P. *et al.* (2012) A novel role for lipid droplets in the organismal antibacterial response. *eLife* 1, e00003
102. Li, Z. *et al.* (2012) Lipid droplets control the maternal histone supply of *Drosophila* embryos. *Curr. Biol.* 22, 2104–2113
103. Bernhard, W. and Rouiller, C. (1956) Close topographical relationship between mitochondria and ergastoplasm of liver cells in a definite phase of cellular activity. *J. Biophys. Biochem. Cytol.* 2, 73–78
104. Eisenberg-Bord, M. *et al.* (2016) A tether is a tether is a tether: tethering at membrane contact sites. *Dev. Cell* 39, 395–409
105. Kumar, Y. and Valdivia, R.H. (2009) Leading a sheltered life: intracellular pathogens and maintenance of vacuolar compartments. *Cell Host Microbe* 5, 593–601