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DOI: https://doi.org/10.1111/mmi.15120

Received: 3 May 2023 | Revised: 21 June 2023 | Accepted: 22 June 2023

DOI: 10.1111/mmi.15120

REVIEW ARTICLE

WILEY

Tapping lipid droplets: A rich fat diet of intracellular bacterial pathogens

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Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 310030_207826 and 31003A_175557

Abstract

Lipid droplets (LDs) are dynamic and versatile organelles present in most eukaryotic cells. LDs consist of a hydrophobic core of neutral lipids, a phospholipid monolayer coat, and a variety of associated proteins. LDs are formed at the endoplasmic reticulum and have diverse roles in lipid storage, energy metabolism, membrane trafficking, and cellular signaling. In addition to their physiological cellular functions, LDs have been implicated in the pathogenesis of several diseases, including metabolic disorders, cancer, and infections. A number of intracellular bacterial pathogens modulate and/or interact with LDs during host cell infection. Members of the genera *Mycobacterium*, *Legionella*, *Coxiella*, *Chlamydia*, and *Salmonella* exploit LDs as a source of intracellular nutrients and membrane components to establish their distinct intracellular replicative niches. In this review, we focus on the biogenesis, interactions, and functions of LDs, as well as on their role in lipid metabolism of intracellular bacterial pathogens.

KEYWORDS
amoeba, bacterial pathogenesis, *Chlamydia*, *Dictyostelium discoideum*, fatty acid transport, host–pathogen interaction, inclusion, intracellular pathogens, *Legionella*, Legionnaires' disease, lipid droplet, *Mycobacterium*, pathogen vacuole, *Salmonella*

1 | INTRODUCTION

Lipid droplets (LDs) are pivotal organelles, which integrate metabolism and cellular homeostasis, and are ubiquitously found in almost all nucleated cells and even some bacteria (Krahmer et al., 2009; Murphy, 2012; Walther & Farese, 2012). LDs comprise a hydrophobic core of neutral lipids such as triacylglycerols (TAG) and cholesterol esters (CE), and they are coated by a phospholipid monolayer and associated proteins. In addition to lipid storage and metabolism, LDs are implicated in the production of hormones, secondary messengers, and inflammatory mediators such as prostaglandins and leukotrienes (Bozza et al., 2011; Bozza & Viola, 2010; Saka & Valdivia, 2012). Furthermore, LDs modulate fatty acids (FA) induced lipotoxicity (Kohlwein, 2010), cell development (Li et al., 2012, 2014), and innate immunity (Bosch et al., 2021; Saka & Valdivia, 2012).

Due to their many functions, LDs have become the focus of intensive research. In the course of the last decade, there have been multiple excellent reviews extensively discussing the topics of LD homeostasis (Cohen, 2018; Hashemi & Goodman, 2015; Henne et al., 2018), function (Welte & Gould, 2017), motility (Kilwein &

Abbreviations: CCV, *Coxiella*-containing vacuole; CE, cholesterol esters; CERT, ceramide transfer protein; ER, endoplasmic reticulum; FA, fatty acids; FIT, fat storage-inducing transmembrane; Icm/Dot, intracellular multiplication/defective organelle trafficking; Inc, inclusion membrane; LCV, *Legionella*-containing vacuole; LD, lipid droplet; LPS, lipopolysaccharide; MCV, *Mycobacterium*-containing vacuole; PAMPs, pathogen-associated molecular patterns; PDM, peridroplet mitochondria; PI, phosphoinositide; PLIN, perilipin; PRRs, pattern recognition receptors; SE, sterol esters; SIF, *Salmonella*-induced filaments; SM, sphingomyelin; T3SS, type III secretion system; T4SS, type IV secretion system; T7SS, type VII secretion system; TAG, triacylglycerols; TLRs, Toll-like receptors; UPR, unfolded protein response.

Welte, 2019), and organelle interactions (Olzmann & Carvalho, 2019; Rakotonirina-Ricquebourg et al., 2022), as well as LD-dependent cellular signaling and innate immunity (Bosch et al., 2021; Bosch & Pol, 2022), and the role of LDs for host–pathogen interactions (Brink et al., 2021; Herker et al., 2021; Libbing et al., 2019; Welte, 2015). This review will summarize the current state of knowledge regarding the biogenesis and functions of LDs, and then focus on the modulation of lipids and LDs in the context of intracellular lipid and FA metabolism by selected pathogenic bacteria.

2 | LIPID DROPLET BIOGENESIS AT THE ENDOPLASMIC RETICULUM

LD assembly originates in the bilayer of the endoplasmic reticulum (ER) membrane, where enzymes involved in the terminal steps of neutral lipids synthesis reside (Figure 1) (Buhman et al., 2001). These neutral lipids play important functions in energy storage and are a major source of phospholipid precursors and cholesterol for cell membranes. TAG and sterol esters (SE) are generated by diacylglycerol acyltransferases (DGAT1 and DGAT2) and acyl-CoA:cholesterol O-acyltransferases (ACAT1 and ACAT2), respectively. Cells lacking DGAT1 and DGAT2 are defective for TAG biosynthesis and LD formation (Harris et al., 2011). As TAG and SE accumulate and diffuse laterally in the ER membrane bilayer, they eventually reach a critical concentration, where they coalesce and form an "oil lens" between the outer and the inner ER membrane leaflet (Thiam & Foret, 2016).

Cytosolic and ER-residing proteins play important roles in the formation and stabilization of nascent LDs (Figure 1). In fact, these proteins seem to favor the initial convex curvature of the cytosolic ER membrane leaflet, driving LD budding toward the cytosol and not the ER lumen. Perilipin 3 (PLIN3/TIP47), which binds to nascent LDs from the cytosol, is a good candidate for this function (Bulankina et al., 2009; Skinner et al., 2009). PLIN3 and other members of the perilipin family comprising PLIN1-5 bind to LDs through conserved amphipathic helices (Rowe et al., 2016). Seipin, a conserved ER membrane protein regulating protein and lipid trafficking into LDs (Fei et al., 2008; Salo et al., 2016; Szymanski et al., 2007), facilitates the deformation of the ER lipid bilayer by the growing lens (Henne et al., 2018) and stabilizes nascent LDs thus favoring their maturation (Wang et al., 2016). Growth of the nascent LD core requires enzymes for neutral lipids synthesis. Accordingly, enzymes involved in TAG biosynthesis, such as DGAT2 and glycerol-3-phosphate acyltransferase 4 (GPAT4), promote LD formation (Wilfling et al., 2013).

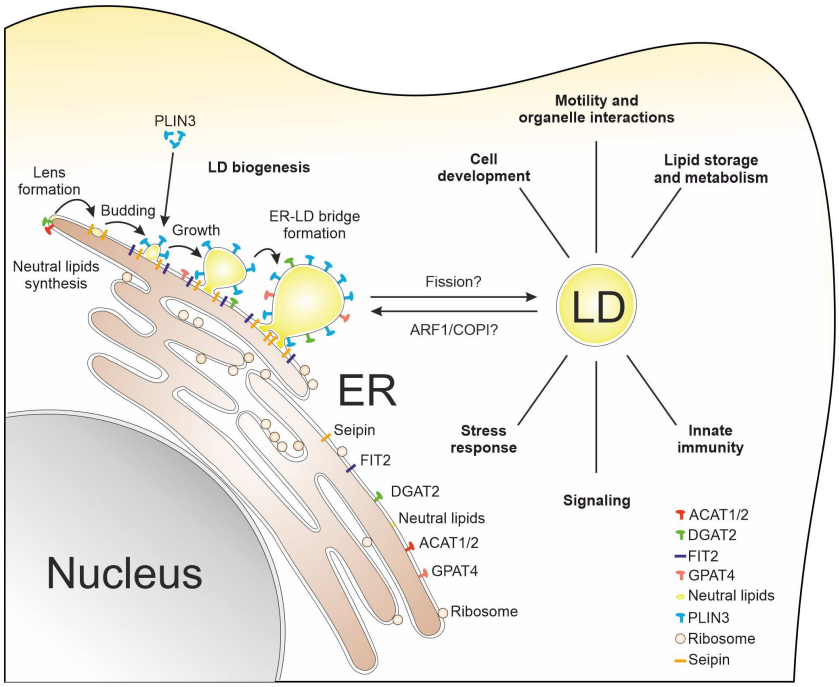

FIGURE 1 Lipid droplet biogenesis and cellular functions. LD biogenesis is based on the synthesis of neutral lipids by DGAT1/2 and ACAT1/2 in the ER membrane bilayer. The neutral lipids accumulate by lateral diffusion and form an oil lens, which gives rise to an emerging LD. Cytosolic and ER-derived proteins (PLIN3, FIT2, seipin) stabilize the nascent LD. Seipin regulates lipid and protein trafficking into the growing LD and mediates assembly of a stable ER–LD bridge allowing the transfer of, for example, DGAT2 and GPAT4 from the ER to the LD. A fraction of LDs is released via an unknown fission process to the cytosol, and the reversion of the process, that is, LD–ER reassociation depends on the small GTPase ARF1 and the COPI coat complex. Mature cytosolic and ER-bound LDs are involved in various cell processes, including cell development, motility and organelle interactions, lipid storage and metabolism, stress response, signaling, and innate immunity. ACAT1/2, acyl-CoA:cholesterol O-acyltransferase 1 and 2; COPI, coatomer complex I; DGAT2, diacylglycerol acyltransferase 2; ER, endoplasmic reticulum; FIT2, fat storage-inducing transmembrane protein 2; GPAT4, glycerol-3-phosphate acyltransferase 4; LD, lipid droplet; PLIN3, perilipin 3. For details see text.

The modulation of ER dynamics also plays a central role in LD budding. Reticulons, receptor expression-enhancing proteins (REEPs), and atlastins (ATLs), which are fundamental in maintenance of the tubular ER morphology (Hu et al., 2009; Shibata et al., 2010; Voeltz et al., 2006), have been suggested to be major players in LDs homeostasis. In fact, expression of a dominant-negative ATL mutant resulted in smaller LDs, whereas overexpression of native ATL had the opposite effect (Klemm et al., 2013). Moreover, REEP1 deletion caused lipodystrophy similar to the absence of seipin (Renvoise et al., 2016), reinforcing the idea that regulation of ER dynamics may have a strong effect on LD budding.

LDs often connect with the ER via an ER-LD lipid "bridge", which is used to transfer DGAT2, GPAT4, and possibly other enzymes and substrates, from the ER to LDs (Figure 1) (Wilfling et al., 2013, 2014). Most mature LDs remain in close contact with the ER, and only a subset of droplets is released through an unknown mechanism of membrane fission (Olzmann & Carvalho, 2019; Renne et al., 2020). In fact, as much as 94% of LDs in yeast and 85% of LDs in mammalian COS-7 cells seem to stay attached to the ER (Szymanski et al., 2007; Valm et al., 2017), and only a subpopulation of growing LDs is accumulating ER-derived GPAT4 (Wilfling et al., 2013). The conserved fat storage-inducing transmembrane (FIT) family of proteins is required for proper budding of LDs from the ER (Choudhary et al., 2015). Elimination or reduction of FIT proteins in yeast and higher eukaryotes causes LDs to remain in the ER membrane. The lipid bridge linking the bilayer of the ER to the LD monolayer is decorated by different enzymes and proteins including seipin (Cottier & Schneiter, 2022; Fei et al., 2008; Salo et al., 2016; Szymanski et al., 2007). Interestingly, cytosolic LDs reversibly associate with the ER and form new ER–LD lipid bridges in a process dependent on the small GTPase ARF1 and the coatomer complex I (COPI) (Thiam et al., 2013; Wilfling et al., 2013, 2014).

3 | LIPID DROPLET MOTILITY AND ORGANELLE INTERACTIONS

Active and directed motility of LDs has been described for several cell types, including cultured mammalian cells (Figure 1) (Welte, 2009; Welte & Gould, 2017). In some cells, LDs are transported via actin–myosin filaments (Knoblach & Rachubinski, 2015; Pfisterer et al., 2017). However, in the majority of cases, LDs transport is largely directed along microtubules by the microtubule motor proteins kinesin and dynein (Orlicky et al., 2013; Welte, 2009, 2015). This transport appears to be driven by direct binding of LD-associated proteins, like PLIN3 and ARF1, to microtubule motor proteins (Gu et al., 2019; Guo et al., 2008; Rai et al., 2017). In addition to direct motion, LDs can also be transported indirectly in several ways, for example, by "hitchhiking" on other organelles such as endosomes, which are transported along microtubules (Kilwein & Welte, 2019).

Motility is an important factor in mediating LD–organelle contact (Figure 1). LDs can interact and grow by homotypic contact and fusion with other LDs. Homotypic LD fusion is promoted by proteins implicated in cellular fusion processes: NSF (*N*-ethylmaleimide-sensitive factor), α-SNAP (soluble NSF attachment protein), and SNAREs (SNAP receptors) (Bostrom et al., 2007). Fusion is also mediated by PLIN1 and the cell death-inducing DNA fragmentation factor alpha-like effector (CIDE) family proteins (Gao et al., 2017; Grahn et al., 2013; Sun et al., 2013). In addition, LDs interact with several other compartments including the ER, Golgi, nucleus, lysosomes, mitochondria, peroxisomes, and the plasma membrane (Herker et al., 2021; Olzmann & Carvalho, 2019; Rakotonirina-Ricquebourg et al., 2022).

LDs are not only tethered to their origin compartment, the ER, but are also observed in close proximity of the Golgi (Valm et al., 2017). Candidates for Golgi–LD linkers are the golgin family of tether proteins (Krahmer & Mann, 2019; Witkos & Lowe, 2015) and the conserved vacuolar protein sorting-associated protein 13B (VPS13B), which binds to both the trans-Golgi network (TGN) membrane and the LD monolayer, thereby promoting Golgi–LD association (Du et al., 2020). LDs have also been found inside the nucleus, though the biogenesis and biological significance of this LD population remain mostly unknown (Farese & Walther, 2016; Romanauska & Kohler, 2018). Furthermore, LDs interact with and are degraded by lysosomes in a process called lipophagy (Schulze et al., 2020; Zechner et al., 2017) (see below).

LDs and mitochondria associate in different cells and tissues, especially in those with increased capacity for storage and oxidation of FA, such as brown adipose tissue, skeletal muscle, and heart (Rakotonirina-Ricquebourg et al., 2022). LD–mitochondria interactions involve the cellular fusion proteins NSF, α-SNAP, and SNAREs (syntaxin-5; SNAP23, synaptosomal-associated protein of 23 kDa; VAMP4, vesicle-associated membrane protein 4) (Bostrom et al., 2007; Jägerström et al., 2009). Other tether proteins belong to the family of perilipins, such as PLIN1 and PLIN5, which bind to LDs and provide a direct physical and metabolic link to different mitochondrial subpopulations (Benador et al., 2018, 2019; Kimmel & Sztalryd, 2014, 2016). PLIN1 is phosphorylated by protein kinase A (PKA) upon adrenergic stimulation of lipolysis and interacts with mitochondrial optic atrophy 1 (OPA1) as well as with mitofusin 1 (MFN1) and MFN2 to promote the anchoring of LDs to mitochondria (Boutant et al., 2017; Pidoux et al., 2011). Moreover, LD-bound DGAT2 associates with mitochondria, thus also causing mitochondria redistribution and interaction with LDs (Stone et al., 2009). While LD–mitochondria tethering is likely executed mainly by proteins, lipids might also facilitate this association. The contact might be mediated by fusion or hemi-fusion of the cytoplasmic leaflet of the outer mitochondrial membrane with the phospholipid monolayer of LDs, thus favoring the exchange of FA between the two organelles (Mahamid et al., 2019).

Finally, the interaction of LDs with peroxisomes has been observed in yeast (Binns et al., 2006) and mammalian cells, where tethering of LDs to peroxisomes is modulated by the peroxisomal ATPase spastin and the transporter ABCD1 (Chang et al., 2019). ABCD1 is an importer for long-chain FA, thus promoting FA trafficking between the two organelles.

4 | LIPID DROPLETS AND LIPID METABOLISM

LDs are an essential hub for lipid metabolism, as they provide FA for cellular processes such as the production of metabolic energy and the synthesis of membranes (Figure 1). Depending on the cellular energy needs, lipids stored in LDs must be mobilized quickly. FA release from TAG stored in LDs is efficiently catalyzed by cytoplasmic lipases, localizing to the LD surface, in a process called lipolysis (Zechner et al., 2017). Lipolysis is initiated by the enzymatic activity of a central triglyceride lipase, the adipose triglyceride lipase (ATGL) (Smirnova et al., 2006; Zechner et al., 2017). This is then followed by hormone-sensitive lipase (HSL), an important diacylglycerol lipase, and finally by monoacylglycerol lipase (MGL), resulting in release of fatty acids and glycerol from TAG (Zechner et al., 2017).

Intact LDs can also be metabolized through lysosome-dependent autophagy, in a process called lipophagy (Schulze et al., 2020; Zechner et al., 2017). During lipophagy, the LD is engulfed by a double-membraned autophagosome, followed by fusion and digestion by the lysosome (Singh et al., 2009). Deletion of the core autophagic protein, ATG7, impairs lipophagy and promotes the accumulation of TAG in LDs. In a process termed chaperone-mediated autophagy (Kaushik & Cuervo, 2012), cytosolic lipases and the autophagy machinery are recruited to LDs upon digestion of PLIN2 and PLIN3 at the surface of the droplets (Kaushik & Cuervo, 2016). The cellular cues required for preferential use of lipolysis or lipophagy in LD metabolism are still unclear, but likely involve metabolic signals (Cohen, 2018; Rambold et al., 2015).

Mitochondria as well as peroxisomes catalyze the β oxidation of FA and contain several enzymes involved in redox homeostasis. Nutrient-starved cells harbor increased LD numbers and LDs associated with fused mitochondria (Rambold et al., 2015). During starvation, LDs and mitochondria relocate on detyrosinated microtubules from the cell center to adopt a dispersed distribution in the cell periphery in an AMP-activated protein kinase (AMPK)-dependent way (Herms et al., 2015). Consequently, LD–mitochondria interactions increase, and LDs are efficiently tapped as source of metabolic energy by supplying FA for mitochondrial β oxidation. PLIN1 promotes LD–mitochondria contacts, lipolysis, and FA import for mitochondrial β oxidation (Pidoux et al., 2011), and the absence of PLIN5 also causes reduced LD–mitochondria contacts and mitochondrial oxidative capacity (Andersson et al., 2017; Varghese et al., 2019).

Analogously, the extensive interaction between LDs and peroxisomes promotes peroxisomal FA β oxidation, and thus, lipolysis is also coupled with peroxisomal FA oxidation (Binns et al., 2006). In fact, LDs, mitochondria, and peroxisomes are all implicated in a complex metabolic machinery regulating energy homeostasis in white and brown adipose tissue (Zhou et al., 2018). Therefore, it is plausible that LDs intimately interact with various different oxidative organelles to form a metabolic circuit for FA exchange and metabolism, thus avoiding FA spillover in the cytoplasm and cellular toxicity (Khaddaj et al., 2022; Rakotonirina-Ricquebourg et al., 2022).

A recent study highlighted the presence of a unique peridroplet mitochondria (PDM) population that does not share components with or fuse with the rest of the cytoplasmic mitochondria (Benador et al., 2018). The distinct PDM population supports TAG synthesis and LDs expansion, instead of consuming stored TAG to fulfill the metabolic demand of the cell. In comparison to cytoplasmic mitochondria, PDM show a distinct proteome, cristae organization, elongated morphology, and reduced motility. Moreover, PDM are characterized by specific bioenergetics, as they prefer pyruvate oxidation instead of lipid oxidation. In addition to binding LDs through PLIN5, the specialization into PDM may require a multitude of other factors (Benador et al., 2018).

5 | LIPID DROPLET FUNCTION IN VARIOUS CELLULAR PATHWAYS

Aside from their function in lipid supply and energy metabolism, LDs participate in many other cellular pathways, such as stress response, protein homeostasis, biosynthesis of inflammatory mediators, and innate immunity (Figure 1) (Bosch et al., 2021; Saka & Valdivia, 2012; Welte & Gould, 2017). Stress not only derives from protein misfolding in the ER due to increased protein synthesis (unfolded protein response, UPR), but also from the accumulation of saturated FA, which may perturb the composition of the ER membrane (Han & Kaufman, 2016; Volmer et al., 2013; Volmer & Ron, 2015). Upon UPR activation, a concomitant increase in LD number has been observed in many cases, suggesting a connection of LDs to ER stress. Furthermore, LDs might represent an efficient mechanism for controlling the FA concentration in the ER.

LDs are involved in the regulation of several aspects of protein homeostasis like protein maturation, storage, and turnover. Studies in yeast postulated an important role of LDs in modulation of protein folding (Moldavski et al., 2015). Protein storage on LDs has been best characterized for histones, which play a role in *Drosophila* development (Cermelli et al., 2006; Li et al., 2012) and innate immunity (Anand et al., 2012; Bosch et al., 2020). The antiviral protein viperin (virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible) localizes to LDs as well as to the ER (Hinson & Cresswell, 2009a, 2009b; Rivera-Serrano et al., 2020). Moreover, LDs are involved in protein turnover (Fujimoto & Ohsaki, 2006) and might assist in proteasome-mediated protein degradation (Ohsaki et al., 2006), or facilitate protein digestion by autophagy (Vevea et al., 2015).

The LD neutral lipid core is an appropriate storage location for hydrophobic biosynthetic precursor molecules, including ether lipids (Bartz et al., 2007; Zehmer et al., 2009) and squalene, the hydrocarbon precursor of cholesterol (Valachovic et al., 2016), as well as hydrophobic vitamins (Traber & Kayden, 1987). Intriguingly, LDs also accumulate and store hydrophobic antimicrobial drugs and toxins, thereby affecting drug bioavailability (Greenwood et al., 2019; Sandoz et al., 2014; Walther & Farese, 2019) and toxin resistance (Chang et al., 2015). The partitioning of hydrophobic compounds to

LDs might either enhance their activity (as in the case of the antibiotic bedaquiline) (Greenwood et al., 2019), or quench their activity (as in the case of antifungal compounds) (Chang et al., 2015).

6 | THE ROLE OF LIPID DROPLETS IN CELLULAR SIGNALING AND INNATE IMMUNITY

In addition to the organelle interactions and diverse functions outlined earlier, LDs are involved in cellular signaling and innate immunity (Figure 1). Environmental cues modulate the sequestration to and the release from LDs of transcription factors to regulate cell metabolism, and starvation regulates the partitioning of transcription factors to LDs (Bosch & Pol, 2022). FA released upon nutrient deprivation from LDs by ATGL-mediated lipolysis also activate different signaling proteins, including the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPARα), PPARγ coactivator 1-alpha (PGC1α), and the NAD-dependent lysine deacetylase sirtuin 1 (SIRT1) (Haemmerle et al., 2011; Khan et al., 2015; Najt et al., 2020). Following lipolysis, the LD–mitochondria tether PLIN5 is phosphorylated by PKA, binds to LD-derived monounsaturated FA, and translocates them to the nucleus to activate SIRT1 (Najt et al., 2020). In turn, SIRT1 deacetylates PGC1α and promotes PGC1α/PPARα signaling, which stimulates mitochondrial biogenesis and oxidative metabolism (Bonkowski & Sinclair, 2016).

Importantly, the hydrophobic LD core is also the storage compartment for precursors of lipid signaling molecules, such as CE, the precursors of steroid hormones involved in cell-to-cell signaling (Papackova & Cahova, 2015; Shen et al., 2016) and arachidonic acid, the precursor of eicosanoids involved in innate immunity (Bozza et al., 2011). Eicosanoids (prostaglandins and leukotrienes) are powerful mediators of inflammatory responses and are synthesized by LD-localizing cyclooxygenase 2 (COX2), which oxidizes arachidonic acid (Jarc & Petan, 2020).

While a number of intracellular pathogens exploit LDs (see the following chapters), it is becoming increasingly clear that stimulation of LD biogenesis represents a host response, which is an integral part of cell-autonomous innate immunity to counteract infections (Bosch et al., 2021; Bosch & Pol, 2022; Melo & Dvorak, 2012). In eukaryotic cells, pathogens are first detected by the recognition of common microbial constituents, known as pathogen-associated molecular patterns (PAMPs). PAMPs are bound by pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs), which localize to the host cell plasma membrane, endosomes, and the cytoplasm (Paludan et al., 2021). LD biogenesis is stimulated upon activation of PRRs in cells exposed to PAMPs such as lipopolysaccharide (LPS) (Nicolaou et al., 2012), lipoarabinomannan (D'Avila et al., 2006), or synthetic nucleic acids resembling viral DNA and RNA (Monson et al., 2021). Furthermore, the stimulation of different TLRs and cytokines reshape distinct macrophage lipidomes, thus enhancing inflammation (Hsieh et al., 2020). On the other hand, LPS-mediated activation markedly increases triglyceride accumulation in macrophages, which through the upregulation of the hypoxia-inducible lipid droplet-associated (HILPDA) protein downregulates ATGL, triglyceride hydrolysis, and prostaglandin E2 (PGE2) production, eventually attenuating the inflammatory response (van Dierendonck et al., 2022). Taken together, lipid metabolism and LD biogenesis significantly contribute to cellular innate immunity.

Upon infection and stimulation by PAMPs, eukaryotic cells switch from mitochondrial oxidative metabolism (oxidative phosphorylation) to glycolysis (Kelly & O'Neill, 2015), probably as a consequence of the uncoupling between LDs and mitochondria (Bosch et al., 2020; Herms et al., 2013). Hence, LDs dissociate from mitochondria and accumulate in the cytoplasm, and the separated LDs can be instrumentalized for storage and efficient usage of antimicrobial peptides, thus avoiding mitochondrial damage and restricting "LD-avid" pathogens (Bosch & Pol, 2022).

The role of LDs in innate immunity is supported by increasing evidence revealing LD-mediated defense mechanisms during infection with viruses (Monson et al., 2018, 2021; Truong et al., 2020), bacteria (Jaisinghani et al., 2018; Knight et al., 2018), and parasites (Rabhi et al., 2016). In agreement with the concept of LDs serving as innate immunity platforms, the antiviral protein viperin, histones, and the IFN-γ-induced GTPase Irgm3 are immunity-related proteins that have been localized to LDs (Anand et al., 2012; Bougneres et al., 2009; Saitoh et al., 2011). The antiviral activity of viperin includes the inhibition of protein secretion, the recruitment and activation of several signaling proteins, which promote the expression of type I IFNs (Crosse et al., 2021; Saitoh et al., 2011), and the production of a ribonucleotide that blocks synthesis of viral RNA and thus inhibits viral replication (Gizzi et al., 2018). Histones adopt antimicrobial activity by disrupting the bacterial proton gradient and chromosome organization (Doolin et al., 2020).

7 | SUBVERSION OF LIPID DROPLETS BY MICROBIAL PATHOGENS

Viruses, bacteria, and parasites subvert LDs for their own benefit. A variety of different viruses exploit LDs for energy provision during replication, for protecting viral particles from degradation, or for enhancing viral assembly via LD–microtubule transport (Boulant et al., 2008; Cloherty et al., 2020; Filipe & McLauchlan, 2015; Heaton & Randall, 2010). Intracellular bacterial pathogens take advantage of the host cell's vast cytoplasmic nutrient pool. To do so, they rewire host metabolic pathways, which often results in recruitment and interaction with host LDs (Figure 2) (Bosch et al., 2021). Likewise, intracellular protozoan parasites such as *Trypanosoma cruzi* or *Leishmania major* associate with LDs to acquire host lipids (D'Avila et al., 2011; Rabhi et al., 2012). Excellent reviews have been published on the role of LDs for interactions of host cells with viruses, bacteria, fungi, and parasites (Barisch & Soldati, 2017a; Brink et al., 2021; Herker et al., 2021; Libbing et al., 2019; Roingeard & Melo, 2017; Vallochi et al., 2018). In the following chapters, we will outline recent discoveries elucidating the role of LDs for selected

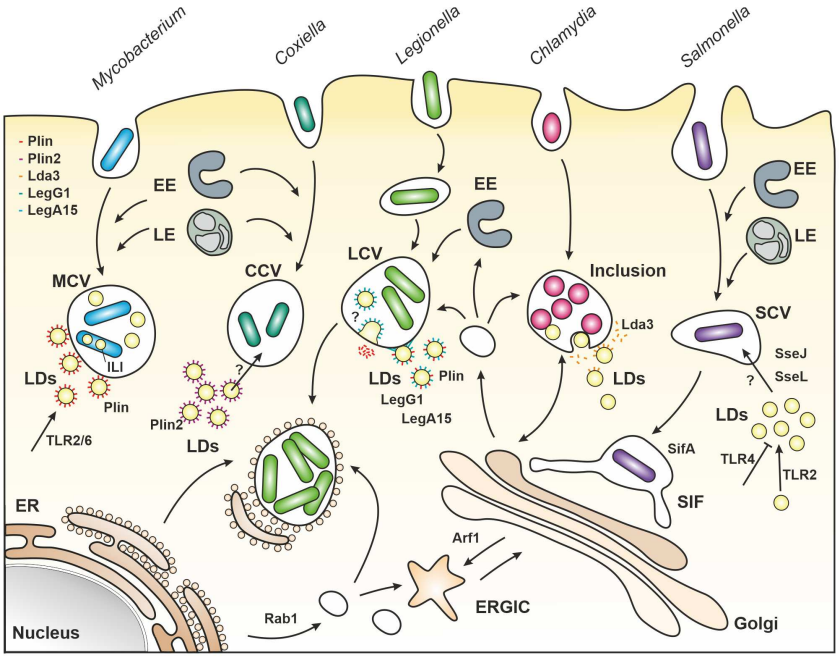

FIGURE 2 Interactions of intracellular bacterial pathogens with host lipid droplets. *Mycobacterium* spp. infect macrophages and form a distinct MCV dependent on T7SSs. LD formation in the host cell is induced through TLR2 and TLR6 signaling. LDs accumulate at and transverse the MCV membrane in a process that includes perilipin shedding. *Mycobacterium marinum* utilizes host-derived lipids to accumulate intracytosolic lipid inclusions (ILIs). Upon *Coxiella burnetii* infection of macrophages, an acidic CCV is formed by means of a T4SS, which upregulates Plin2 expression and LD formation. *Legionella pneumophila* forms a unique LCV in macrophages and amoeba. LDs intimately interact with the LCV membrane, translocate into the LCV lumen, and are implicated in FA metabolism. The large fusion GTPase Sey1 as well as the *L. pneumophila* T4SS and the Ran GTPase activator LegG1 promote LCV–LDs interactions. Moreover, the *L. pneumophila* GTPase effector LegA15 localizes to and modulates LDs. *Chlamydia trachomatis* employs a T3SS to form a membrane-bound inclusion in eukaryotic cells and releases the effector protein Lda3 into the cytoplasm upon infection. LDs may be tethered to the inclusion membrane by Lda3 leading to their "endocytic" translocation into the inclusion lumen and association with *Chlamydia* reticulate bodies. *Salmonella* Typhimurium forms the SCV and SIF in infected cells by means of two T3SSs. The SPI-2 T3SS translocates the effectors SseJ, SseL, and SifA, which promote cholesterol accumulation at the SCV. The accumulation of LDs is positively and negatively regulated by TLR2 and TLR4, respectively. CCV, *Coxiella*-containing vacuole; EE, early endosome; ILI, intracytosolic inclusions; LCV, *Legionella*-containing vacuole; LDs, lipid droplets; LE, late endosome; MCV, *Mycobacterium*-containing vacuole; Plin, perilipin; Plin2: perilipin 2; SCV, *Salmonella*-containing vacuole; SIF, *Salmonella*-induced filaments.

bacteria–host cell interactions. The best studied intracellular bacteria, which are covered in detail in this review, belong to the genera *Mycobacterium*, *Legionella*, *Coxiella*, *Chlamydia*, and *Salmonella*.

8 | MODULATION OF LIPID DROPLETS BY *MYCOBACTERIUM* SPECIES

Mycobacterium tuberculosis is the causative agent of tuberculosis, the world's most deadly infectious disease, which is still responsible for 10.6 million infected people and 1.4 million deaths in the year 2021 alone (World Health Organization, 2022). Major virulence factors of *M. tuberculosis* are several ESX type VII secretion systems (T7SSs) and their secreted "effector" proteins, which are implicated in the formation of the distinct, replication-permissive *Mycobacterium*-containing vacuole (MCV) in macrophages (Figure 2) (Bunduc et al., 2020).

A hallmark of tuberculosis is the formation of granuloma containing "foamy", LD-filled macrophages (Peyron et al., 2008; Russell et al., 2009). *Mycobacterium tuberculosis* triggers the differentiation into foamy macrophages through long-chain fatty acids, namely oxygenated mycolic acids (Peyron et al., 2008), and through the manipulation of host cell metabolism (Singh et al., 2012). Intriguingly, the *M. tuberculosis* terpene nucleoside 1-tuberculosinyladenosine has recently been shown to induce lysosomal lipid storage of TAG and CE, which matched the intralysosomal and peribacterial lipid storage patterns of foamy macrophages (Bedard et al., 2023). The induction of LDs, PGE2, and PPARγ is mediated by TLR2 (Almeida et al., 2009; Bowdish et al., 2009; D'Avila et al., 2006) and TLR6 (Mattos et al., 2011). Moreover, Wnt family member 6 (WNT6), a ligand of the evolutionarily conserved Wingless/Integrase 1 (WNT) signaling pathway, also promotes the formation of foamy macrophages and intracellular survival of *M. tuberculosis* by regulating key lipid metabolic genes such as acetyl-CoA carboxylase 2 (ACC2) (Brandenburg et al., 2021).

Mycobacterium tuberculosis and other pathogenic *Mycobacterium* species such as *M. leprae* or *M. marinum* induce the formation and association of LDs with MCVs in different host cells, such as macrophages (Peyron et al., 2008; Roque et al., 2020), Schwann cells (*M. leprae*) (Jin et al., 2017; Mattos et al., 2011), and the amoeba *Dictyostelium*

discoideum (*M. marinum*) (Barisch et al., 2015). The growth of *M. marinum* is impaired in *D. discoideum* lacking the perilipin homolog PlnA (Barisch et al., 2015), but not in an amoeba mutant strain lacking Dgat1 and Dgat2 (Barisch & Soldati, 2017b). Since both mutant strains are defective for LD formation, LDs per se do not seem to be essential for intracellular growth. Moreover, hypoxia—through the IFN-γ-dependent transcription factor hypoxia-inducible factor-1α (HIF-1α)—promotes LD formation, eicosanoid production, and a metabolic shift to aerobic glycolysis (Braverman et al., 2016; Knight et al., 2018).

In foamy macrophages, LDs accumulate at MCVs, but the bacteria no longer grow and adopt a dormant, nonreplicative state (Peyron et al., 2008). *Mycobacterium tuberculosis* uses host TAG to accumulate LDs, and inside LD-laden macrophages nearly half the bacterial population develops a dormant/persistent phenotype (Daniel et al., 2011). Furthermore, mycobacterial persistence requires the utilization of host cholesterol (Pandey & Sassetti, 2008). The inhibition of oxygen-dependent respiration by low, nontoxic nitric oxide concentrations also induced dormancy in *M. tuberculosis* (Voskuil et al., 2003). The hypoxic response of *M. tuberculosis* is mediated by the bacterial transcription factor DosR (Park et al., 2003).

Mycobacterium tuberculosis factors implicated in intrabacterial TAG synthesis are the acyl-CoA synthetase FacL6 (Daniel et al., 2014) and the perilipin-like protein PPE15 (Daniel et al., 2016). Among the 24 putative *M. tuberculosis* lipases, only LipY shows significant activity toward long-chain TAG, and the corresponding Δ*lipY* mutant strain is impaired for TAG utilization (Deb et al., 2006). Still, FA rather than carbohydrates are a major energy source of *M. tuberculosis* during infection and dormancy in mammalian cells (Rodriguez et al., 2014). Intriguingly, intrabacterial accumulation of LDs (intracytosolic lipid inclusions, ILI) by *M. marinum* in *D. discoideum* does not lead to dormancy (Barisch et al., 2015; Barisch & Soldati, 2017b).

In the context of *Mycobacterium* infection, LDs are not only a source of metabolic energy, but also accumulate mycobactins (Chao et al., 2019), a class of siderophores (low-molecular-weight Fe^{3+} chelators) of *Mycobacterium* species including *M. tuberculosis* (Luo et al., 2005) and *M. marinum* (Knobloch et al., 2020). Water-soluble carboxy-mycobactin and lipophilic mycobactin efficiently scavenge intracellular iron in macrophages, and the iron–mycobactin complex accumulates with high selectivity in LDs, which directly contact MCVs. Finally, the antimycobacterial compound bedaquiline accumulates primarily in LDs in *M. tuberculosis*-infected macrophages (Greenwood et al., 2019) as well as in foamy macrophages in *M. tuberculosis*-infected lungs in mice (Fearns et al., 2020). Intriguingly, the LDs do not sequester the antibiotic, but constitute a drug reservoir that enhances antibacterial efficacy (Walther & Farese, 2019).

9 | LIPID DROPLET-MEDIATED FATTY ACID METABOLISM OF *LEGIONELLA PNEUMOPHILA*

Legionella pneumophila is a Gram-negative environmental bacterium, which upon inhalation of contaminated aerosols replicates in lung macrophages and causes a severe pneumonia termed Legionnaires' disease (Hilbi et al., 2011; Mondino et al., 2020; Newton et al., 2010). *Legionella pneumophila* is flagellated, grows strictly aerobic, and replicates in free-living protozoa (Boamah et al., 2017; Hoffmann et al., 2014). Although likely not a natural host, the genetically tractable social soil amoeba *D. discoideum* is a powerful and versatile model to analyze *L. pneumophila*–phagocyte interactions (Steinert & Heuner, 2005; Swart et al., 2018). In *D. discoideum*, LDs accumulate upon feeding the cells with fatty acids (in particular palmitate) or bacteria. *D. discoideum* LDs have a similar composition as mammalian LDs, including TAG, SE, free FA, and perilipin (Du et al., 2013), and they are coated by a polar phospholipid monolayer and distinct proteins (Du et al., 2013; Miura et al., 2002).

In mammalian and protozoan host cells, *L. pneumophila* employs a conserved mechanism to establish a unique compartment, the *Legionella*-containing vacuole (LCV) (Figure 2) (Asrat et al., 2014; Hilbi & Buchrieser, 2022; Hubber & Roy, 2010). To govern LCV formation and other pathogen–host interactions, *L. pneumophila* employs the Icm/Dot (intracellular multiplication/defective organelle transport) type IV secretion system (T4SS) (Kubori & Nagai, 2016). The Icm/Dot T4SS translocates the astonishing number of more than 300 "effector" proteins into host cells, where they undermine trafficking pathways, cytoskeleton dynamics, signal transduction, and metabolism (Lockwood et al., 2022; Manske & Hilbi, 2014; Personnic et al., 2016; Qiu & Luo, 2017; Swart et al., 2020). The effectors target small GTPases, phosphoinositide (PI) lipids, trafficking components, and many other cellular factors.

Key steps during LCV maturation are (i) the PI conversion from PtdIns(3)*P* to PtdIns(4)*P*, which re-routes the pathogen vacuole from the endocytic to the secretory pathway (Swart & Hilbi, 2020; Weber et al., 2006, 2014), (ii) the subversion of small GTPases of the Arf, Rab, Ran, and Rap families (Asrat et al., 2014; Hubber & Roy, 2010; Steiner, Weber, & Hilbi, 2018; Swart et al., 2020), (iii) the interception of and fusion with ER-derived vesicles, and (iv) the tight association and membrane contact site (MCS) formation with the ER (Asrat et al., 2014; Hilbi & Buchrieser, 2022; Hubber & Roy, 2010; Steiner, Weber, & Hilbi, 2018; Vormittag et al., 2023).

The ER-residing large fusion GTPase Sey1/atlastin 3 promotes LCV expansion and intracellular replication of *L. pneumophila* (Hüsler et al., 2021; Steiner et al., 2017; Steiner, Weber, Kaech, et al., 2018). *Dictyostelium discoideum* Δ*sey1* mutant amoeba show pleiotropic defects, including aberrant ER architecture and dynamics, impaired LCV–ER interactions, and intracellular bacterial replication (Hüsler et al., 2021), as well as reduced LCV–LD interactions (Hüsler et al., 2023).

Once the replication-permissive LCV is formed, *L. pneumophila* starts to grow intracellularly (Molofsky & Swanson, 2004). *Legionella pneumophila* grows strictly aerobic, and not only metabolizes amino acids, but also carbohydrates and fatty acids (Abu Kwaik & Bumann, 2013; Manske & Hilbi, 2014). Indeed, isotopolog profiling studies with stable ^{13}C isotopes indicated that serine, glucose, inositol, glycerol, and palmitate are metabolized by *L. pneumophila* (Eylert

et al., 2010; Harada et al., 2010; Häuslein et al., 2016, 2017; Manske et al., 2016).

A recent study revealed that LCVs intimately interact with palmitate-induced LDs in *D. discoideum*, and the large GTPase Sey1 as well as the *L. pneumophila* Icm/Dot T4SS and the effector LegG1 promote LCV–LD interactions (Hüsler et al., 2023). In vitro reconstitution of LCV–LD interactions using purified LCVs and LDs from *D. discoideum* Ax3 or Δ*sey1* indicated that Sey1 and GTP promote this process. Moreover, exogenously added palmitate enhances the growth of *L. pneumophila* wild-type in *D. discoideum* Ax3 but not in Δ*sey1* mutant amoeba. Palmitate does also not promote the growth of an *L. pneumophila* Δ*fadL* mutant strain lacking a homolog of the *Escherichia coli* fatty acid transporter FadL, and the intracellular catabolism of ^{13}C palmitate is reduced in *D. discoideum* Δ*sey1* and upon infection with *L. pneumophila* Δ*fadL*.

Legionella pneumophila also produces an Icm/Dot-translocated effector, LegA15, which localizes to LDs (Chen et al., 2022). LegA15 is an ankyrin repeat-containing protein that shows GTPase activity, binds to the Golgi tethering factor p115 and—depending on its GTPase and p115-binding activity—modulates LD–LCV interactions in macrophages. In summary, LCVs intimately associate with LDs depending on the large fusion GTPase Sey1, and *L. pneumophila* produces effectors (LegG1, LegA15), which modulate LD–LCV interactions, as well as the putative FA transporter FadL, which promotes intracellular fatty acid metabolism.

10 | LIPID DROPLET HOMEOSTASIS AFFECTS INTRACELLULAR GROWTH OF *COXIELLA BURNETII*

Coxiella burnetii is a Gram-negative obligate intracellular bacterium and causes the zoonosis Q fever, a severe form of culture-negative endocarditis (Delsing et al., 2011). *Coxiella burnetii* initially infects alveolar macrophages, but the pathogen has also been identified in LD-containing foamy macrophages in the cardiac valves of endocarditis patients. In macrophages as well as in nonphagocytic cells, *C. burnetii* forms a replication-permissive compartment, the *Coxiella*-containing vacuole (CCV) (Figure 2) (Heinzen et al., 1999; Voth & Heinzen, 2007). The nascent CCV interacts with the endosomal pathway, fuses with early and late endosomes, lysosomes, and autophagosomes, and adopts an acidic pH of *ca.* 4.5 (Newton et al., 2013; Voth & Heinzen, 2007). The acidic pH activates the *C. burnetii* Icm/Dot T4SS and the translocation of more than 130 effector proteins into the host cytosol (Beare et al., 2011; Qiu & Luo, 2017; Segal et al., 2005). Only a few of the *C. burnetii* effectors have been characterized to date. The gene encoding the effector ElpA (ER-localizing protein A) is present in most *C. burnetii* strains, and the effector disrupts ER structure and function during infection (Graham et al., 2015). However, it has not been assessed whether ElpA also affects LD biogenesis and/or function.

Coxiella burnetii-infected macrophages upregulate the expression of *PLIN2* and, dependent on the *C. burnetii* Icm/Dot T4SS, accumulate twice as many LDs as mock-infected macrophages (Mulye et al., 2018). Unexpectedly, blocking the LD formation with pharmacological inhibitors, or deleting ACAT1 in macrophages, increases *C. burnetii* growth at least twofold. Conversely, preventing LD lipolysis by blocking ATGL activity with the inhibitor atglistatin almost completely abolishes bacterial growth. Taken together, these findings suggest that LD breakdown is essential for *C. burnetii*, and maintenance of LD homeostasis, possibly via the *C. burnetii* T4SS, is critical for intracellular growth of the pathogen (Mulye et al., 2018).

11 | LIPID DROPLETS ARE TRANSPORTED INTO THE *CHLAMYDIA* INCLUSION

Chlamydia pneumoniae and *Chlamydia trachomatis* are Gram-negative, obligate intracellular pathogens that causes a range of diseases in humans, including respiratory ailments, sexually transmitted infections, and trachoma, the leading cause of preventable blindness worldwide (Cheong et al., 2019; Wright et al., 2008). Upon infecting mucosal epithelial cells, *C. trachomatis* resides within a pathogen vacuole termed the inclusion (Figure 2) (Abdelrahman & Belland, 2005; Elwell et al., 2016). *Chlamydia* species employ a type III secretion system (T3SS) to govern their interactions with eukaryotic host cells (Ferrell & Fields, 2016; Lara-Tejero & Galan, 2019). Some T3SS-translocated effectors termed inclusion membrane (Inc) proteins are embedded in the inclusion membrane and play a role in the direct interaction of the inclusion membrane with the ER (Bugalhao & Mota, 2019; Derré, 2017; Dumoux et al., 2015). The *C. trachomatis* Inc protein CT006 associates with LDs and the ER when expressed ectopically in yeast and mammalian cells, suggesting that the protein might participate in the interaction of LDs with the inclusion (Bugalhao et al., 2022).

Chlamydia trachomatis inclusions acquire host sphingomyelin (SM), a process that is essential for inclusion biogenesis as well as for bacterial replication (Elwell & Engel, 2012). *Chlamydia trachomatis* hijacks components of both vesicular and nonvesicular lipid trafficking pathways to acquire SM, which is then utilized by the pathogen either catabolically as an energy source for replication or anabolically for inclusion membrane growth and stability (Elwell et al., 2011). For vesicle-mediated SM acquisition and inclusion membrane growth, *C. trachomatis* selectively subverts the brefeldin A target, GBF1, a regulator of Arf1-dependent vesicular trafficking within the early secretory pathway. In contrast, for nonvesicular lipid trafficking and bacterial replication, *C. trachomatis* subverts the ceramide transfer protein (CERT), which is a key component in nonvesicular ER to TGN trafficking of ceramide (the precursor of SM) (Elwell et al., 2011). *Chlamydia trachomatis* recruits CERT and its ER binding partner VAP-A, as well as the SM synthases, SMS1 and SMS2, to the inclusion. Hence, a SM biosynthetic factory at or near the inclusion is likely formed (Elwell et al., 2011).

Acquisition of host lipids by the *C. trachomatis* inclusion occurs through LDs (Cocchiaro et al., 2008). Interestingly, LDs are

translocated from the host cytosol into the inclusion lumen and tightly associate with reticulate bodies, the replicative form of *Chlamydia*. The chlamydial effector protein Lda3 promotes the uptake of LDs into the inclusions through the formation of LD-inclusion membrane contact sites, and an "endocytic" entry leading to membrane-coated, intraluminal LDs (Cocchiaro et al., 2008). However, the metabolism of FA by *C. trachomatis* does not necessarily need to involve LDs, since upon LD depletion even more bacterial progeny is produced (Sharma et al., 2018).

The *C. trachomatis* effector IncA cofractionates with LDs and accumulates in the inclusion lumen (Cocchiaro et al., 2008). Accordingly, the IncA-enriched inclusion subdomains might constitute the LD translocation sites. The effector IncD interacts with CERT, which in turn binds VAP on the ER (Agaisse & Derré, 2014; Derré et al., 2011). Finally, IncV directly interacts with VAP through two FFAT motifs and is enriched at ER-inclusion MCS (Stanhope et al., 2017). Depletion of CERT or VAP results in a significant decrease in inclusion size and infectious progeny production (Derré et al., 2011; Elwell et al., 2011). Taken together, IncA, the IncD–CERT–VAP complex, and the IncV–VAP complex contribute to ER-inclusion MCS and are proposed to function in the nonvesicular trafficking of host lipids to the *Chlamydia* inclusion, a process essential for intracellular growth (Agaisse & Derré, 2014; Derré et al., 2011; Elwell et al., 2011). So far, it has not been explored whether IncA, IncD, and IncV contribute to the formation of LD-inclusion MCS.

12 | *SALMONELLA ENTERICA* TYPHIMURIUM INFECTION TRIGGERS LIPID DROPLET FORMATION

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a Gram-negative, facultative intracellular bacterium, which can cause gastroenteritis and diarrhea (Hohmann, 2001). *Salmonella* Typhimurium forms a replication-permissive compartment, the *Salmonella*-containing vacuole (SCV) in phagocytic and epithelial cells (Figure 2) (LaRock et al., 2015). SCV formation is mediated by two T3SSs, located on the genomic *Salmonella* pathogenicity island (SPI)-1 and SPI-2, respectively (Galan & Waksman, 2018; Jennings et al., 2017). SPI-1 is required for the initial invasion and SPI-2 is produced after internalization.

Dependent on the SPI-1 T3SS, *S.* Typhimurium induces a rapid stimulation of LD formation in macrophages (Kiarely Souza et al., 2022). LD accumulation occurs through TLR2-dependent signaling and is negatively regulated by TLR4. Pharmacological prevention of LD formation by inhibiting DGAT1 or phospholipase A2 (PLA2) reduces intracellular bacterial growth and impairs PGE2 synthesis (Kiarely Souza et al., 2022).

The SCV accumulates cholesterol as a consequence of host sterol biosynthesis perturbation due to the bacterial infection (Catron et al., 2002). The SPI-2 T3SS-translocated effectors SifA, SseJ, and SseL are necessary for cholesterol accumulation at the SCV (Greene et al., 2021; McEwan et al., 2015; Walch et al., 2021). SseJ has deacylase activity (Ohlson et al., 2005), esterifies cholesterol in vitro, in host cells and during infection, and promotes the formation of LDs (Nawabi et al., 2008). Compared to *S.* Typhimurium wild-type, an infection with the Δ*sseJ* mutant strain shows reduced levels of cholesterol ester production in HeLa cells and RAW 264.7 macrophages and a decreased number of LDs per cell. Accordingly, the ectopic production of SseJ reduces cholesterol levels in cellular membranes. On the other hand, SseJ also downregulates the production of the host cholesterol transporter ABCA1 in macrophages, leading to an increase in cellular cholesterol (Greene et al., 2021). SseL is another SPI-2-encoded *S.* Typhimurium effector implicated in lipid metabolism (Arena et al., 2011). Infection with the Δ*sseL* mutant strain causes dramatic changes in host cell lipid metabolism and leads to the massive accumulation of LDs in infected cells. This phenotype is caused by the deubiquitinase activity of SseL, as a *S.* Typhimurium strain carrying a single point mutation in the catalytic cysteine phenocopied the Δ*sseL* strain. The lipid accumulation due to the absence of a functional *sseL* gene is also observed in murine livers during *S.* Typhimurium infection. These results suggest that SseL alters host lipid metabolism in infected cells by modifying their ubiquitination pattern (Arena et al., 2011). Collectively, the studies on SPI-1- and SPI-2-translocated T3SS effectors indicate a role of LDs, lipid metabolism, and cholesterol for *S.* Typhimurium intracellular survival and replication.

13 | CONCLUSIONS

LDs are formed at the ER and comprise a hydrophobic core of neutral lipids, a phospholipid monolayer coat, and a variety of associated proteins. In addition to their roles in cell development, organelle interactions, lipid storage, energy metabolism, stress response, cellular signaling, and innate immunity, LDs have emerged as key players in the pathogenesis of several human diseases, and they play a crucial role in lipid metabolism of bacterial pathogens. The interaction of intracellular bacterial pathogens with LDs represents an intricate survival strategy and highlights the complex interplay between host lipids and pathogen factors. Intracellular pathogens of the genera *Mycobacterium*, *Legionella*, *Coxiella*, *Chlamydia*, and *Salmonella* modulate LD formation in infected host cells and exploit this compartment to acquire lipids for catabolic as well as anabolic reactions. The bacterial effector proteins promoting the modulation of and interaction with LDs are mostly unknown. Another intriguing and largely unresolved question is how LDs exchange material with pathogens across the pathogen vacuole barrier. Specifically, it is unclear how LDs covered by a lipid monolayer interact with and cross the lipid bilayer of the pathogen vacuole membrane. Further studies are needed to elucidate the mechanisms underlying pathogen vacuole–LD interactions and possibly identify new targets for therapeutic intervention.

AUTHOR CONTRIBUTIONS

Hubert Hilbi: Conceptualization; funding acquisition; writing – original draft. **Dario Hüsler:** Writing – original draft; visualization. **Pia Stauffer:** Writing – editing; visualization.

ACKNOWLEDGMENTS

Work in the group of H.H. was supported by the Swiss National Science Foundation (SNF; 31003A_175557, 310030_207826). Open access funding provided by Universitat Zurich.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Pubmed at https://pubmed.ncbi.nlm.nih.gov/.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals.

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REFERENCES

Abdelrahman, Y.M. & Belland, R.J. (2005) The chlamydial developmental cycle. *FEMS Microbiology Reviews*, *29*, 949–959.

Abu Kwaik, Y. & Bumann, D. (2013) Microbial quest for food in vivo: 'nutritional virulence' as an emerging paradigm. *Cellular Microbiology*, *15*, 882–890.

Agaisse, H. & Derré, I. (2014) Expression of the effector protein IncD in *Chlamydia trachomatis* mediates recruitment of the lipid transfer protein CERT and the endoplasmic reticulum-resident protein VAPB to the inclusion membrane. *Infection and Immunity*, *82*, 2037–2047.

Almeida, P.E., Silva, A.R., Maya-Monteiro, C.M., Torocsik, D., D'Avila, H., Dezso, B. et al. (2009) *Mycobacterium bovis* bacillus Calmette-Guerin infection induces TLR2-dependent peroxisome proliferator-activated receptor gamma expression and activation: functions in inflammation, lipid metabolism, and pathogenesis. *Journal of Immunology*, *183*, 1337–1345.

Anand, P., Cermelli, S., Li, Z., Kassan, A., Bosch, M., Sigua, R. et al. (2012) A novel role for lipid droplets in the organismal antibacterial response. *eLife*, *1*, e00003.

Andersson, L., Drevinge, C., Mardani, I., Dalen, K.T., Stahlman, M., Klevstig, M. et al. (2017) Deficiency in perilipin 5 reduces mitochondrial function and membrane depolarization in mouse hearts. *The International Journal of Biochemistry & Cell Biology*, *91*, 9–13.

Arena, E.T., Auweter, S.D., Antunes, L.C., Vogl, A.W., Han, J., Guttman, J.A. et al. (2011) The deubiquitinase activity of the *Salmonella* pathogenicity island 2 effector, SseL, prevents accumulation of cellular lipid droplets. *Infection and Immunity*, *79*, 4392–4400.

Asrat, S., de Jesus, D.A., Hempstead, A.D., Ramabhadran, V. & Isberg, R.R. (2014) Bacterial pathogen manipulation of host membrane trafficking. *Annual Review of Cell and Developmental Biology*, *30*, 79–109.

Barisch, C., Paschke, P., Hagedorn, M., Maniak, M. & Soldati, T. (2015) Lipid droplet dynamics at early stages of *Mycobacterium marinum* infection in *Dictyostelium*. *Cellular Microbiology*, *17*, 1332–1349.

Barisch, C. & Soldati, T. (2017a) Breaking fat! How mycobacteria and other intracellular pathogens manipulate host lipid droplets. *Biochimie*, *141*, 54–61.

Barisch, C. & Soldati, T. (2017b) *Mycobacterium marinum* degrades both triacylglycerols and phospholipids from its *Dictyostelium* host to synthesise its own triacylglycerols and generate lipid inclusions. *PLoS Pathogens*, *13*, e1006095.

Bartz, R., Li, W.H., Venables, B., Zehmer, J.K., Roth, M.R., Welti, R. et al. (2007) Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic. *Journal of Lipid Research*, *48*, 837–847.

Beare, P.A., Gilk, S.D., Larson, C.L., Hill, J., Stead, C.M., Omsland, A. et al. (2011) Dot/Icm type IVB secretion system requirements for *Coxiella burnetii* growth in human macrophages. *mBio*, *2*, e00175-11.

Bedard, M., van der Niet, S., Bernard, E.M., Babunovic, G., Cheng, T.Y., Aylan, B. et al. (2023) A terpene nucleoside from *M. tuberculosis* induces lysosomal lipid storage in foamy macrophages. *The Journal of Clinical Investigation*, *133*, e161944.

Benador, I.Y., Veliova, M., Liesa, M. & Shirihai, O.S. (2019) Mitochondria bound to lipid droplets: where mitochondrial dynamics regulate lipid storage and utilization. *Cell Metabolism*, *29*, 827–835.

Benador, I.Y., Veliova, M., Mahdaviani, K., Petcherski, A., Wikstrom, J.D., Assali, E.A. et al. (2018) Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion. *Cell Metabolism*, *27*, 869–885.

Binns, D., Januszewski, T., Chen, Y., Hill, J., Markin, V.S., Zhao, Y. et al. (2006) An intimate collaboration between peroxisomes and lipid bodies. *The Journal of Cell Biology*, *173*, 719–731.

Boamah, D.K., Zhou, G., Ensminger, A.W. & O'Connor, T.J. (2017) From many hosts, one accidental pathogen: the diverse protozoan hosts of *Legionella*. *Frontiers in Cellular and Infection Microbiology*, *7*, 477.

Bonkowski, M.S. & Sinclair, D.A. (2016) Slowing ageing by design: the rise of NAD(+) and sirtuin-activating compounds. *Nature Reviews Molecular Cell Biology*, *17*, 679–690.

Bosch, M. & Pol, A. (2022) Eukaryotic lipid droplets: metabolic hubs, and immune first responders. *Trends in Endocrinology and Metabolism*, *33*, 218–229.

Bosch, M., Sanchez-Alvarez, M., Fajardo, A., Kapetanovic, R., Steiner, B., Dutra, F. et al. (2020) Mammalian lipid droplets are innate immune hubs integrating cell metabolism and host defense. *Science*, *370*, eaay8085.

Bosch, M., Sweet, M.J., Parton, R.G. & Pol, A. (2021) Lipid droplets and the host-pathogen dynamic: FATal attraction? *The Journal of Cell Biology*, *220*, e202104005.

Bostrom, P., Andersson, L., Rutberg, M., Perman, J., Lidberg, U., Johansson, B.R. et al. (2007) SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity. *Nature Cell Biology*, *9*, 1286–1293.

Bougneres, L., Helft, J., Tiwari, S., Vargas, P., Chang, B.H., Chan, L. et al. (2009) A role for lipid bodies in the cross-presentation of phagocytosed antigens by MHC class I in dendritic cells. *Immunity*, *31*, 232–244.

Boulant, S., Douglas, M.W., Moody, L., Budkowska, A., Targett-Adams, P. & McLauchlan, J. (2008) Hepatitis C virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner. *Traffic*, *9*, 1268–1282.

Boutant, M., Kulkarni, S.S., Joffraud, M., Ratajczak, J., Valera-Alberni, M., Combe, R. et al. (2017) Mfn2 is critical for brown adipose tissue thermogenic function. *The EMBO Journal*, *36*, 1543–1558.

Bowdish, D.M., Sakamoto, K., Kim, M.J., Kroos, M., Mukhopadhyay, S., Leifer, C.A. et al. (2009) MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and *Mycobacterium tuberculosis*. *PLoS Pathogens*, *5*, e1000474.

Bozza, P.T., Bakker-Abreu, I., Navarro-Xavier, R.A. & Bandeira-Melo, C. (2011) Lipid body function in eicosanoid synthesis: an update. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *85*, 205–213.

Bozza, P.T. & Viola, J.P. (2010) Lipid droplets in inflammation and cancer. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *82*, 243–250.

Brandenburg, J., Marwitz, S., Tazoll, S.C., Waldow, F., Kalsdorf, B., Vierbuchen, T. et al. (2021) WNT6/ACC2-induced storage of triacylglycerols in macrophages is exploited by *Mycobacterium tuberculosis*. *The Journal of Clinical Investigation*, *131*, e141833.

Braverman, J., Sogi, K.M., Benjamin, D., Nomura, D.K. & Stanley, S.A. (2016) HIF-1α is an essential mediator of IFN-γ-dependent immunity to *Mycobacterium tuberculosis*. *Journal of Immunology*, *197*, 1287–1297.

Brink, J.T.R., Fourie, R., Sebolai, O., Albertyn, J. & Pohl, C.H. (2021) The role of lipid droplets in microbial pathogenesis. *Journal of Medical Microbiology*, *70*, e001383. Available from: https://doi.org/10.1099/jmm.0.001383

Bugalhao, J.N., Luis, M.P., Pereira, I.S., da Cunha, M., Pais, S.V. & Mota, L.J. (2022) The *Chlamydia trachomatis* inclusion membrane protein CT006 associates with lipid droplets in eukaryotic cells. *PLoS ONE*, *17*, e0264292.

Bugalhao, J.N. & Mota, L.J. (2019) The multiple functions of the numerous *Chlamydia trachomatis* secreted proteins: the tip of the iceberg. *Microbial Cell*, *6*, 414–449.

Buhman, K.K., Chen, H.C. & Farese, R.V., Jr. (2001) The enzymes of neutral lipid synthesis. *The Journal of Biological Chemistry*, *276*, 40369–40372.

Bulankina, A.V., Deggerich, A., Wenzel, D., Mutenda, K., Wittmann, J.G., Rudolph, M.G. et al. (2009) TIP47 functions in the biogenesis of lipid droplets. *The Journal of Cell Biology*, *185*, 641–655.

Bunduc, C.M., Bitter, W. & Houben, E.N.G. (2020) Structure and function of the mycobacterial type VII secretion systems. *Annual Review of Microbiology*, *74*, 315–335.

Catron, D.M., Sylvester, M.D., Lange, Y., Kadekoppala, M., Jones, B.D., Monack, D.M. et al. (2002) The *Salmonella*-containing vacuole is a major site of intracellular cholesterol accumulation and recruits the GPI-anchored protein CD55. *Cellular Microbiology*, *4*, 315–328.

Cermelli, S., Guo, Y., Gross, S.P. & Welte, M.A. (2006) The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Current Biology*, *16*, 1783–1795.

Chang, C.L., Weigel, A.V., Ioannou, M.S., Pasolli, H.A., Xu, C.S., Peale, D.R. et al. (2019) Spastin tethers lipid droplets to peroxisomes and directs fatty acid trafficking through ESCRT-III. *The Journal of Cell Biology*, *218*, 2583–2599.

Chang, W., Zhang, M., Zheng, S., Li, Y., Li, X., Li, W. et al. (2015) Trapping toxins within lipid droplets is a resistance mechanism in fungi. *Scientific Reports*, *5*, 15133.

Chao, A., Sieminski, P.J., Owens, C.P. & Goulding, C.W. (2019) Iron acquisition in *Mycobacterium tuberculosis*. *Chemical Reviews*, *119*, 1193–1220.

Chen, T.T., Lin, Y., Zhang, S., Liu, S., Song, L., Zhong, W. et al. (2022) Atypical *Legionella* GTPase effector hijacks host vesicular transport factor p115 to regulate host lipid droplet. *Science Advances*, *8*, eadd7945.

Cheong, H.C., Lee, C.Y.Q., Cheok, Y.Y., Tan, G.M.Y., Looi, C.Y. & Wong, W.F. (2019) Chlamydiaceae: diseases in primary hosts and zoonosis. *Microorganisms*, *7*, 146.

Choudhary, V., Ojha, N., Golden, A. & Prinz, W.A. (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *The Journal of Cell Biology*, *211*, 261–271.

Cloherty, A.P.M., Olmstead, A.D., Ribeiro, C.M.S. & Jean, F. (2020) Hijacking of lipid droplets by hepatitis C, dengue and Zika viruses-from viral protein moonlighting to extracellular release. *International Journal of Molecular Sciences*, *21*, 7901.

Cocchiaro, J.L., Kumar, Y., Fischer, E.R., Hackstadt, T. & Valdivia, R.H. (2008) Cytoplasmic lipid droplets are translocated into the lumen of the *Chlamydia trachomatis* parasitophorous vacuole. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 9379–9384.

Cohen, S. (2018) Lipid droplets as organelles. *International Review of Cell and Molecular Biology*, *337*, 83–110.

Cottier, S. & Schneiter, R. (2022) Lipid droplets form a network interconnected by the endoplasmic reticulum through which their proteins equilibrate. *Journal of Cell Science*, *135*, jcs258819.

Crosse, K.M., Monson, E.A., Dumbrepatil, A.B., Smith, M., Tseng, Y.Y., Van der Hoek, K.H. et al. (2021) Viperin binds STING and enhances the type-I interferon response following dsDNA detection. *Immunology and Cell Biology*, *99*, 373–391.

Daniel, J., Kapoor, N., Sirakova, T., Sinha, R. & Kolattukudy, P. (2016) The perilipin-like PPE15 protein in *Mycobacterium tuberculosis* is required for triacylglycerol accumulation under dormancy-inducing conditions. *Molecular Microbiology*, *101*, 784–794.

Daniel, J., Maamar, H., Deb, C., Sirakova, T.D. & Kolattukudy, P.E. (2011) *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. *PLoS Pathogens*, *7*, e1002093.

Daniel, J., Sirakova, T. & Kolattukudy, P. (2014) An acyl-CoA synthetase in *Mycobacterium tuberculosis* involved in triacylglycerol accumulation during dormancy. *PLoS ONE*, *9*, e114877.

D'Avila, H., Freire-de-Lima, C.G., Roque, N.R., Teixeira, L., Barja-Fidalgo, C., Silva, A.R. et al. (2011) Host cell lipid bodies triggered by *Trypanosoma cruzi* infection and enhanced by the uptake of apoptotic cells are associated with prostaglandin E(2) generation and increased parasite growth. *The Journal of Infectious Diseases*, *204*, 951–961.

D'Avila, H., Melo, R.C., Parreira, G.G., Werneck-Barroso, E., Castro-Faria-Neto, H.C. & Bozza, P.T. (2006) *Mycobacterium bovis* bacillus Calmette-Guerin induces TLR2-mediated formation of lipid bodies: intracellular domains for eicosanoid synthesis in vivo. *Journal of Immunology*, *176*, 3087–3097.

Deb, C., Daniel, J., Sirakova, T.D., Abomoelak, B., Dubey, V.S. & Kolattukudy, P.E. (2006) A novel lipase belonging to the hormone-sensitive lipase family induced under starvation to utilize stored triacylglycerol in *Mycobacterium tuberculosis*. *The Journal of Biological Chemistry*, *281*, 3866–3875.

Delsing, C.E., Warris, A. & Bleeker-Rovers, C.P. (2011) Q fever: still more queries than answers. *Advances in Experimental Medicine and Biology*, *719*, 133–143.

Derré, I. (2017) Hijacking of membrane contact sites by intracellular bacterial pathogens. *Advances in Experimental Medicine and Biology*, *997*, 211–223.

Derré, I., Swiss, R. & Agaisse, H. (2011) The lipid transfer protein CERT interacts with the *Chlamydia* inclusion protein IncD and participates to ER-*Chlamydia* inclusion membrane contact sites. *PLoS Pathogens*, *7*, e1002092.

Doolin, T., Amir, H.M., Duong, L., Rosenzweig, R., Urban, L.A., Bosch, M. et al. (2020) Mammalian histones facilitate antimicrobial synergy by disrupting the bacterial proton gradient and chromosome organization. *Nature Communications*, *11*, 3888.

Du, X., Barisch, C., Paschke, P., Herrfurth, C., Bertinetti, O., Pawolleck, N. et al. (2013) *Dictyostelium* lipid droplets host novel proteins. *Eukaryotic Cell*, *12*, 1517–1529.

Du, Y., Xiong, J. & Ji, W. (2020) Trans-Golgi network-lipid droplet contacts maintain the TGN integrity and function via lipid transfer activities of VPS13B. *bioRxiv: 2020.2012.2016.423147*.

Dumoux, M., Nans, A., Saibil, H.R. & Hayward, R.D. (2015) Making connections: snapshots of chlamydial type III secretion systems in contact with host membranes. *Current Opinion in Microbiology*, *23*, 1–7.

Elwell, C., Mirrashidi, K. & Engel, J. (2016) *Chlamydia* cell biology and pathogenesis. *Nature Reviews Microbiology*, *14*, 385–400.

Elwell, C.A. & Engel, J.N. (2012) Lipid acquisition by intracellular Chlamydiae. *Cellular Microbiology*, *14*, 1010–1018.

Elwell, C.A., Jiang, S., Kim, J.H., Lee, A., Wittmann, T., Hanada, K. et al. (2011) *Chlamydia trachomatis* co-opts GBF1 and CERT to acquire host sphingomyelin for distinct roles during intracellular development. *PLoS Pathogens*, *7*, e1002198.

Eylert, E., Herrmann, V., Jules, M., Gillmaier, N., Lautner, M., Buchrieser, C. et al. (2010) Isotopologue profiling of *Legionella pneumophila*: role of serine and glucose as carbon substrates. *The Journal of Biological Chemistry*, *285*, 22232–22243.

Farese, R.V., Jr. & Walther, T.C. (2016) Lipid droplets go nuclear. *The Journal of Cell Biology*, *212*, 7–8.

Fearns, A., Greenwood, D.J., Rodgers, A., Jiang, H. & Gutierrez, M.G. (2020) Correlative light electron ion microscopy reveals in vivo localisation of bedaquiline in *Mycobacterium tuberculosis*-infected lungs. *PLoS Biology*, *18*, e3000879.

Fei, W., Shui, G., Gaeta, B., Du, X., Kuerschner, L., Li, P. et al. (2008) Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast. *The Journal of Cell Biology*, *180*, 473–482.

Ferrell, J.C. & Fields, K.A. (2016) A working model for the type III secretion mechanism in *Chlamydia*. *Microbes and Infection*, *18*, 84–92.

Filipe, A. & McLauchlan, J. (2015) Hepatitis C virus and lipid droplets: finding a niche. *Trends in Molecular Medicine*, *21*, 34–42.

Fujimoto, T. & Ohsaki, Y. (2006) Proteasomal and autophagic pathways converge on lipid droplets. *Autophagy*, *2*, 299–301.

Galan, J.E. & Waksman, G. (2018) Protein-injection machines in bacteria. *Cell*, *172*, 1306–1318.

Gao, G., Chen, F.J., Zhou, L., Su, L., Xu, D., Xu, L. et al. (2017) Control of lipid droplet fusion and growth by CIDE family proteins. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1862*, 1197–1204.

Gizzi, A.S., Grove, T.L., Arnold, J.J., Jose, J., Jangra, R.K., Garforth, S.J. et al. (2018) A naturally occurring antiviral ribonucleotide encoded by the human genome. *Nature*, *558*, 610–614.

Graham, J.G., Winchell, C.G., Sharma, U.M. & Voth, D.E. (2015) Identification of ElpA, a *Coxiella burnetii* pathotype-specific Dot/Icm type IV secretion system substrate. *Infection and Immunity*, *83*, 1190–1198.

Grahn, T.H., Zhang, Y., Lee, M.J., Sommer, A.G., Mostoslavsky, G., Fried, S.K. et al. (2013) FSP27 and PLIN1 interaction promotes the formation of large lipid droplets in human adipocytes. *Biochemical and Biophysical Research Communications*, *432*, 296–301.

Greene, A.R., Owen, K.A. & Casanova, J.E. (2021) *Salmonella* Typhimurium manipulates macrophage cholesterol homeostasis through the SseJ-mediated suppression of the host cholesterol transport protein ABCA1. *Cellular Microbiology*, *23*, e13329.

Greenwood, D.J., Dos Santos, M.S., Huang, S., Russell, M.R.G., Collinson, L.M., MacRae, J.I. et al. (2019) Subcellular antibiotic visualization reveals a dynamic drug reservoir in infected macrophages. *Science*, *364*, 1279–1282.

Gu, Y., Yang, Y., Cao, X., Zhao, Y., Gao, X., Sun, C. et al. (2019) Plin3 protects against alcoholic liver injury by facilitating lipid export from the endoplasmic reticulum. *Journal of Cellular Biochemistry*, *120*, 16075–16087.

Guo, Y., Walther, T.C., Rao, M., Stuurman, N., Goshima, G., Terayama, K. et al. (2008) Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature*, *453*, 657–661.

Haemmerle, G., Moustafa, T., Woelkart, G., Buttner, S., Schmidt, A., van de Weijer, T. et al. (2011) ATGL-mediated fat catabolism regulates cardiac mitochondrial function via PPAR-α and PGC-1. *Nature Medicine*, *17*, 1076–1085.

Han, J. & Kaufman, R.J. (2016) The role of ER stress in lipid metabolism and lipotoxicity. *Journal of Lipid Research*, *57*, 1329–1338.

Harada, E., Iida, K., Shiota, S., Nakayama, H. & Yoshida, S. (2010) Glucose metabolism in *Legionella pneumophila*: dependence on the Entner-Doudoroff pathway and connection with intracellular bacterial growth. *Journal of Bacteriology*, *192*, 2892–2899.

Harris, C.A., Haas, J.T., Streeper, R.S., Stone, S.J., Kumari, M., Yang, K. et al. (2011) DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *Journal of Lipid Research*, *52*, 657–667.

Hashemi, H.F. & Goodman, J.M. (2015) The life cycle of lipid droplets. *Current Opinion in Cell Biology*, *33*, 119–124.

Häuslein, I., Manske, C., Goebel, W., Eisenreich, W. & Hilbi, H. (2016) Pathway analysis using (13)C-glycerol and other carbon tracers reveals a bipartite metabolism of *Legionella pneumophila*. *Molecular Microbiology*, *100*, 229–246.

Häuslein, I., Sahr, T., Escoll, P., Klausner, N., Eisenreich, W. & Buchrieser, C. (2017) *Legionella pneumophila* CsrA regulates a metabolic switch from amino acid to glycerolipid metabolism. *Open Biology*, *7*, e170149.

Heaton, N.S. & Randall, G. (2010) Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host & Microbe*, *8*, 422–432.

Heinzen, R.A., Hackstadt, T. & Samuel, J.E. (1999) Developmental biology of *Coxiella burnetii*. *Trends in Microbiology*, *7*, 149–154.

Henne, W.M., Reese, M.L. & Goodman, J.M. (2018) The assembly of lipid droplets and their roles in challenged cells. *The EMBO Journal*, *37*, e98947.

Herker, E., Vieyres, G., Beller, M., Krahmer, N. & Bohnert, M. (2021) Lipid droplet contact sites in health and disease. *Trends in Cell Biology*, *31*, 345–358.

Herms, A., Bosch, M., Ariotti, N., Reddy, B.J., Fajardo, A., Fernandez-Vidal, A. et al. (2013) Cell-to-cell heterogeneity in lipid droplets suggests a mechanism to reduce lipotoxicity. *Current Biology*, *23*, 1489–1496.

Herms, A., Bosch, M., Reddy, B.J., Schieber, N.L., Fajardo, A., Ruperez, C. et al. (2015) AMPK activation promotes lipid droplet dispersion on detyrosinated microtubules to increase mitochondrial fatty acid oxidation. *Nature Communications*, *6*, 7176.

Hilbi, H. & Buchrieser, C. (2022) Microbe profile: *Legionella pneumophila*—a copycat eukaryote. *Microbiology*, *168*, e001142. Available from: https://doi.org/10.1099/mic.0.001142

Hilbi, H., Hoffmann, C. & Harrison, C.F. (2011) *Legionella* spp. outdoors: colonization, communication and persistence. *Environmental Microbiology Reports*, *3*, 286–296.

Hinson, E.R. & Cresswell, P. (2009a) The antiviral protein, viperin, localizes to lipid droplets via its N-terminal amphipathic alpha-helix. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 20452–20457.

Hinson, E.R. & Cresswell, P. (2009b) The N-terminal amphipathic alpha-helix of viperin mediates localization to the cytosolic face of the endoplasmic reticulum and inhibits protein secretion. *The Journal of Biological Chemistry*, *284*, 4705–4712.

Hoffmann, C., Harrison, C.F. & Hilbi, H. (2014) The natural alternative: protozoa as cellular models for *Legionella* infection. *Cellular Microbiology*, *16*, 15–26.

Hohmann, E.L. (2001) Nontyphoidal salmonellosis. *Clinical Infectious Diseases*, *32*, 263–269.

Hsieh, W.Y., Zhou, Q.D., York, A.G., Williams, K.J., Scumpia, P.O., Kronenberger, E.B. et al. (2020) Toll-like receptors induce signal-specific reprogramming of the macrophage lipidome. *Cell Metabolism*, *32*, 128–143.e125.

Hu, J., Shibata, Y., Zhu, P.P., Voss, C., Rismanchi, N., Prinz, W.A. et al. (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell*, *138*, 549–561.

Hubber, A. & Roy, C.R. (2010) Modulation of host cell function by *Legionella pneumophila* type IV effectors. *Annual Review of Cell and Developmental Biology*, *26*, 261–283.

Hüsler, D., Stauffer, P., Keller, B., Böck, D., Steiner, T., Kroniger, T. et al. (2023) The large GTPase Sey1/atlastin mediates lipid droplet- and FadL-dependent intracellular fatty acid metabolism of *Legionella pneumophila*. *eLife*, *12*, e85142.

Hüsler, D., Steiner, B., Welin, A., Striednig, B., Swart, A.L., Molle, V. et al. (2021) *Dictyostelium* lacking the single atlastin homolog Sey1 shows aberrant ER architecture, proteolytic processes and expansion of the *Legionella*-containing vacuole. *Cellular Microbiology*, *23*, e13318.

Jägerström, S., Polesie, S., Wickström, Y., Johansson, B.R., Schröder, H.D., Hojlund, K. et al. (2009) Lipid droplets interact with mitochondria using SNAP23. *Cell Biology International*, *33*, 934–940.

Jaisinghani, N., Dawa, S., Singh, K., Nandy, A., Menon, D., Bhandari, P.D. et al. (2018) Necrosis driven triglyceride synthesis primes macrophages for inflammation during *Mycobacterium tuberculosis* infection. *Frontiers in Immunology*, *9*, 1490.

Jarc, E. & Petan, T. (2020) A twist of FATe: lipid droplets and inflammatory lipid mediators. *Biochimie*, *169*, 69–87.

Jennings, E., Thurston, T.L.M. & Holden, D.W. (2017) *Salmonella* SPI-2 type III secretion system effectors: molecular mechanisms and physiological consequences. *Cell Host & Microbe*, *22*, 217–231.

Jin, S.H., An, S.K. & Lee, S.B. (2017) The formation of lipid droplets favors intracellular *Mycobacterium leprae* survival in SW-10, non-myelinating Schwann cells. *PLoS Neglected Tropical Diseases*, *11*, e0005687.

Kaushik, S. & Cuervo, A.M. (2012) Chaperones in autophagy. *Pharmacological Research*, *66*, 484–493.

Kaushik, S. & Cuervo, A.M. (2016) AMPK-dependent phosphorylation of lipid droplet protein PLIN2 triggers its degradation by CMA. *Autophagy*, *12*, 432–438.

Kelly, B. & O'Neill, L.A. (2015) Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Research*, *25*, 771–784.

Khaddaj, R., Mari, M., Cottier, S., Reggiori, F. & Schneiter, R. (2022) The surface of lipid droplets constitutes a barrier for endoplasmic reticulum-resident integral membrane proteins. *Journal of Cell Science*, *135*, jcs256206.

Khan, S.A., Sathyanarayan, A., Mashek, M.T., Ong, K.T., Wollaston-Hayden, E.E. & Mashek, D.G. (2015) ATGL-catalyzed lipolysis regulates SIRT1 to control PGC-1α/PPAR-α signaling. *Diabetes*, *64*, 418–426.

Kiarely Souza, E., Pereira-Dutra, F.S., Rajao, M.A., Ferraro-Moreira, F., Goltara-Gomes, T.C., Cunha-Fernandes, T. et al. (2022) Lipid droplet accumulation occurs early following *Salmonella* infection and contributes to intracellular bacterial survival and replication. *Molecular Microbiology*, *117*, 293–306.

Kilwein, M.D. & Welte, M.A. (2019) Lipid droplet motility and organelle contacts. *Contact*, *2*, 251525641989568. Available from: https://doi.org/10.1177/2515256419895688

Kimmel, A.R. & Sztalryd, C. (2014) Perilipin 5, a lipid droplet protein adapted to mitochondrial energy utilization. *Current Opinion in Lipidology*, *25*, 110–117.

Kimmel, A.R. & Sztalryd, C. (2016) The perilipins: major cytosolic lipid droplet-associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. *Annual Review of Nutrition*, *36*, 471–509.

Klemm, R.W., Norton, J.P., Cole, R.A., Li, C.S., Park, S.H., Crane, M.M. et al. (2013) A conserved role for atlastin GTPases in regulating lipid droplet size. *Cell Reports*, *3*, 1465–1475.

Knight, M., Braverman, J., Asfaha, K., Gronert, K. & Stanley, S. (2018) Lipid droplet formation in *Mycobacterium tuberculosis* infected macrophages requires IFN-γ /HIF-1α signaling and supports host defense. *PLoS Pathogens*, *14*, e1006874.

Knoblach, B. & Rachubinski, R.A. (2015) Transport and retention mechanisms govern lipid droplet inheritance in *Saccharomyces cerevisiae*. *Traffic*, *16*, 298–309.

Knobloch, P., Koliwer-Brandl, H., Arnold, F.M., Hanna, N., Gonda, I., Adenau, S. et al. (2020) *Mycobacterium marinum* produces distinct mycobactin and carboxymycobactin siderophores to promote growth in broth and phagocytes. *Cellular Microbiology*, *22*, e13163.

Kohlwein, S.D. (2010) Obese and anorexic yeasts: experimental models to understand the metabolic syndrome and lipotoxicity. *Biochimica et Biophysica Acta*, *1801*, 222–229.

Krahmer, N., Guo, Y., Farese, R.V., Jr. & Walther, T.C. (2009) SnapShot: lipid droplets. *Cell*, *139*, 1024–1024.e1021.

Krahmer, N. & Mann, M. (2019) Catching lipid droplet contacts by proteomics. *Contact*, *2*, 2515256419859186.

Kubori, T. & Nagai, H. (2016) The type IVB secretion system: an enigmatic chimera. *Current Opinion in Microbiology*, *29*, 22–29.

Lara-Tejero, M. & Galan, J.E. (2019) The injectisome, a complex nanomachine for protein injection into mammalian cells. *EcoSal Plus*, *8*, 1128.

LaRock, D.L., Chaudhary, A. & Miller, S.I. (2015) Salmonellae interactions with host processes. *Nature Reviews Microbiology*, *13*, 191–205.

Li, Z., Johnson, M.R., Ke, Z., Chen, L. & Welte, M.A. (2014) *Drosophila* lipid droplets buffer the H2Av supply to protect early embryonic development. *Current Biology*, *24*, 1485–1491.

Li, Z., Thiel, K., Thul, P.J., Beller, M., Kuhnlein, R.P. & Welte, M.A. (2012) Lipid droplets control the maternal histone supply of *Drosophila* embryos. *Current Biology*, *22*, 2104–2113.

Libbing, C.L., McDevitt, A.R., Azcueta, R.P., Ahila, A. & Mulye, M. (2019) Lipid droplets: a significant but understudied contributor of host-bacterial interactions. *Cells*, *8*, 354.

Lockwood, D.C., Amin, H., Costa, T.R.D. & Schroeder, G.N. (2022) The *Legionella pneumophila* Dot/Icm type IV secretion system and its effectors. *Microbiology*, *168*, e001187. Available from: https://doi.org/10.1099/mic.0.001187

Luo, M., Fadeev, E.A. & Groves, J.T. (2005) Mycobactin-mediated iron acquisition within macrophages. *Nature Chemical Biology*, *1*, 149–153.

Mahamid, J., Tegunov, D., Maiser, A., Arnold, J., Leonhardt, H., Plitzko, J.M. et al. (2019) Liquid-crystalline phase transitions in lipid droplets are related to cellular states and specific organelle association. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 16866–16871.

Manske, C. & Hilbi, H. (2014) Metabolism of the vacuolar pathogen *Legionella* and implications for virulence. *Frontiers in Cellular and Infection Microbiology*, *4*, 125.

Manske, C., Schell, U. & Hilbi, H. (2016) Metabolism of *myo*-inositol by *Legionella pneumophila* promotes infection of amoebae and macrophages. *Applied and Environmental Microbiology*, *82*, 5000–5014.

Mattos, K.A., Lara, F.A., Oliveira, V.G., Rodrigues, L.S., D'Avila, H., Melo, R.C. et al. (2011) Modulation of lipid droplets by *Mycobacterium leprae* in Schwann cells: a putative mechanism for host lipid acquisition and bacterial survival in phagosomes. *Cellular Microbiology*, *13*, 259–273.

McEwan, D.G., Richter, B., Claudi, B., Wigge, C., Wild, P., Farhan, H. et al. (2015) PLEKHM1 regulates *Salmonella*-containing vacuole biogenesis and infection. *Cell Host & Microbe*, *17*, 58–71.

Melo, R.C. & Dvorak, A.M. (2012) Lipid body-phagosome interaction in macrophages during infectious diseases: host defense or pathogen survival strategy? *PLoS Pathogens*, *8*, e1002729.

Miura, S., Gan, J.W., Brzostowski, J., Parisi, M.J., Schultz, C.J., Londos, C. et al. (2002) Functional conservation for lipid storage droplet association among Perilipin, ADRP, and TIP47 (PAT)-related proteins in mammals, *Drosophila*, and *Dictyostelium*. *The Journal of Biological Chemistry*, *277*, 32253–32257.

Moldavski, O., Amen, T., Levin-Zaidman, S., Eisenstein, M., Rogachev, I., Brandis, A. et al. (2015) Lipid droplets are essential for efficient clearance of cytosolic inclusion bodies. *Developmental Cell*, *33*, 603–610.

Molofsky, A.B. & Swanson, M.S. (2004) Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. *Molecular Microbiology*, *53*, 29–40.

Mondino, S., Schmidt, S., Rolando, M., Escoll, P., Gomez-Valero, L. & Buchrieser, C. (2020) Legionnaires' disease: state of the art

knowledge of pathogenesis mechanisms of *Legionella*. *Annual Review of Pathology*, *15*, 439–466.
Monson, E.A., Crosse, K.M., Das, M. & Helbig, K.J. (2018) Lipid droplet density alters the early innate immune response to viral infection. *PLoS ONE*, *13*, e0190597.
Monson, E.A., Crosse, K.M., Duan, M., Chen, W., O'Shea, R.D., Wakim, L.M. et al. (2021) Intracellular lipid droplet accumulation occurs early following viral infection and is required for an efficient interferon response. *Nature Communications*, *12*, 4303.
Mulye, M., Zapata, B. & Gilk, S.D. (2018) Altering lipid droplet homeostasis affects *Coxiella burnetii* intracellular growth. *PLoS ONE*, *13*, e0192215.
Murphy, D.J. (2012) The dynamic roles of intracellular lipid droplets: from archaea to mammals. *Protoplasma*, *249*, 541–585.
Najt, C.P., Khan, S.A., Heden, T.D., Witthuhn, B.A., Perez, M., Heier, J.L. et al. (2020) Lipid droplet-derived monounsaturated fatty acids traffic via PLIN5 to allosterically activate SIRT1. *Molecular Cell*, *77*, 810–824.
Nawabi, P., Catron, D.M. & Haldar, K. (2008) Esterification of cholesterol by a type III secretion effector during intracellular *Salmonella* infection. *Molecular Microbiology*, *68*, 173–185.
Newton, H.J., Ang, D.K., van Driel, I.R. & Hartland, E.L. (2010) Molecular pathogenesis of infections caused by *Legionella pneumophila*. *Clinical Microbiology Reviews*, *23*, 274–298.
Newton, H.J., McDonough, J.A. & Roy, C.R. (2013) Effector protein translocation by the *Coxiella burnetii* Dot/Icm type IV secretion system requires endocytic maturation of the pathogen-occupied vacuole. *PLoS ONE*, *8*, e54566.
Nicolaou, G., Goodall, A.H. & Erridge, C. (2012) Diverse bacteria promote macrophage foam cell formation via toll-like receptor-dependent lipid body biosynthesis. *Journal of Atherosclerosis and Thrombosis*, *19*, 137–148.
Ohlson, M.B., Fluhr, K., Birmingham, C.L., Brumell, J.H. & Miller, S.I. (2005) SseJ deacylase activity by *Salmonella enterica* serovar Typhimurium promotes virulence in mice. *Infection and Immunity*, *73*, 6249–6259.
Ohsaki, Y., Cheng, J., Fujita, A., Tokumoto, T. & Fujimoto, T. (2006) Cytoplasmic lipid droplets are sites of convergence of proteasomal and autophagic degradation of apolipoprotein B. *Molecular Biology of the Cell*, *17*, 2674–2683.
Olzmann, J.A. & Carvalho, P. (2019) Dynamics and functions of lipid droplets. *Nature Reviews. Molecular Cell Biology*, *20*, 137–155.
Orlicky, D.J., Monks, J., Stefanski, A.L. & McManaman, J.L. (2013) Dynamics and molecular determinants of cytoplasmic lipid droplet clustering and dispersion. *PLoS ONE*, *8*, e66837.
Paludan, S.R., Pradeu, T., Masters, S.L. & Mogensen, T.H. (2021) Constitutive immune mechanisms: mediators of host defence and immune regulation. *Nature Reviews. Immunology*, *21*, 137–150.
Pandey, A.K. & Sassetti, C.M. (2008) Mycobacterial persistence requires the utilization of host cholesterol. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 4376–4380.
Papackova, Z. & Cahova, M. (2015) Fatty acid signaling: the new function of intracellular lipases. *International Journal of Molecular Sciences*, *16*, 3831–3855.
Park, H.D., Guinn, K.M., Harrell, M.I., Liao, R., Voskuil, M.I., Tompa, M. et al. (2003) Rv3133c/dosR is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. *Molecular Microbiology*, *48*, 833–843.
Personnic, N., Bärlocher, K., Finsel, I. & Hilbi, H. (2016) Subversion of retrograde trafficking by translocated pathogen effectors. *Trends in Microbiology*, *24*, 450–462.
Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F. et al. (2008) Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. *PLoS Pathogens*, *4*, e1000204.
Pfisterer, S.G., Gateva, G., Horvath, P., Pirhonen, J., Salo, V.T., Karhinen, L. et al. (2017) Role for formin-like 1-dependent acto-myosin assembly in lipid droplet dynamics and lipid storage. *Nature Communications*, *8*, 14858.
Pidoux, G., Witczak, O., Jarnaess, E., Myrvold, L., Urlaub, H., Stokka, A.J. et al. (2011) Optic atrophy 1 is an A-kinase anchoring protein on lipid droplets that mediates adrenergic control of lipolysis. *The EMBO Journal*, *30*, 4371–4386.
Qiu, J. & Luo, Z.Q. (2017) *Legionella* and *Coxiella* effectors: strength in diversity and activity. *Nature Reviews Microbiology*, *15*, 591–605.
Rabhi, I., Rabhi, S., Ben-Othman, R., Rasche, A., Daskalaki, A., Trentin, B. et al. (2012) Transcriptomic signature of *Leishmania* infected mice macrophages: a metabolic point of view. *PLoS Neglected Tropical Diseases*, *6*, e1763.
Rabhi, S., Rabhi, I., Trentin, B., Piquemal, D., Regnault, B., Goyard, S. et al. (2016) Lipid droplet formation, their localization and dynamics during *Leishmania major* macrophage infection. *PLoS ONE*, *11*, e0148640.
Rai, P., Kumar, M., Sharma, G., Barak, P., Das, S., Kamat, S.S. et al. (2017) Kinesin-dependent mechanism for controlling triglyceride secretion from the liver. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 12958–12963.
Rakotonirina-Ricquebourg, R., Costa, V. & Teixeira, V. (2022) Hello from the other side: membrane contact of lipid droplets with other organelles and subsequent functional implications. *Progress in Lipid Research*, *85*, 101141.
Rambold, A.S., Cohen, S. & Lippincott-Schwartz, J. (2015) Fatty acid trafficking in starved cells: regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. *Developmental Cell*, *32*, 678–692.
Renne, M.F., Klug, Y.A. & Carvalho, P. (2020) Lipid droplet biogenesis: a mystery "unmixing"? *Seminars in Cell & Developmental Biology*, *108*, 14–23.
Renvoise, B., Malone, B., Falgairolle, M., Munasinghe, J., Stadler, J., Sibilla, C. et al. (2016) Reep1 null mice reveal a converging role for hereditary spastic paraplegia proteins in lipid droplet regulation. *Human Molecular Genetics*, *25*, 5111–5125.
Rivera-Serrano, E.E., Gizzi, A.S., Arnold, J.J., Grove, T.L., Almo, S.C. & Cameron, C.E. (2020) Viperin reveals its true function. *Annual Review of Virology*, *7*, 421–446.
Rodriguez, J.G., Hernandez, A.C., Helguera-Repetto, C., Aguilar Ayala, D., Guadarrama-Medina, R., Anzola, J.M. et al. (2014) Global adaptation to a lipid environment triggers the dormancy-related phenotype of *Mycobacterium tuberculosis*. *mBio*, *5*, e01125-14.
Roingeard, P. & Melo, R.C. (2017) Lipid droplet hijacking by intracellular pathogens. *Cellular Microbiology*, *19*, e12688. Available from: https://doi.org/10.1111/cmi.12688
Romanauska, A. & Kohler, A. (2018) The inner nuclear membrane is a metabolically active territory that generates nuclear lipid droplets. *Cell*, *174*, 700–715.
Roque, N.R., Lage, S.L., Navarro, R., Fazolini, N., Maya-Monteiro, C.M., Rietdorf, J. et al. (2020) Rab7 controls lipid droplet-phagosome association during mycobacterial infection. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1865*, 158703.
Rowe, E.R., Mimmack, M.L., Barbosa, A.D., Haider, A., Isaac, I., Ouberai, M.M. et al. (2016) Conserved amphipathic helices mediate lipid droplet targeting of perilipins 1-3. *The Journal of Biological Chemistry*, *291*, 6664–6678.
Russell, D.G., Cardona, P.J., Kim, M.J., Allain, S. & Altare, F. (2009) Foamy macrophages and the progression of the human tuberculosis granuloma. *Nature Immunology*, *10*, 943–948.
Saitoh, T., Satoh, T., Yamamoto, N., Uematsu, S., Takeuchi, O., Kawai, T. et al. (2011) Antiviral protein viperin promotes toll-like receptor 7- and toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. *Immunity*, *34*, 352–363.

Saka, H.A. & Valdivia, R. (2012) Emerging roles for lipid droplets in immunity and host-pathogen interactions. *Annual Review of Cell and Developmental Biology*, *28*, 411–437.

Salo, V.T., Belevich, I., Li, S., Karhinen, L., Vihinen, H., Vigouroux, C. et al. (2016) Seipin regulates ER-lipid droplet contacts and cargo delivery. *The EMBO Journal*, *35*, 2699–2716.

Sandoz, K.M., Valiant, W.G., Eriksen, S.G., Hruby, D.E., Allen, R.D., 3rd & Rockey, D.D. (2014) The broad-spectrum antiviral compound ST-669 restricts chlamydial inclusion development and bacterial growth and localizes to host cell lipid droplets within treated cells. *Antimicrobial Agents and Chemotherapy*, *58*, 3860–3866.

Schulze, R.J., Krueger, E.W., Weller, S.G., Johnson, K.M., Casey, C.A., Schott, M.B. et al. (2020) Direct lysosome-based autophagy of lipid droplets in hepatocytes. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 32443–32452.

Segal, G., Feldman, M. & Zusman, T. (2005) The Icm/Dot type-IV secretion systems of *Legionella pneumophila* and *Coxiella burnetii*. *FEMS Microbiology Reviews*, *29*, 65–81.

Sharma, M., Recuero-Checa, M.A., Fan, F.Y. & Dean, D. (2018) *Chlamydia trachomatis* regulates growth and development in response to host cell fatty acid availability in the absence of lipid droplets. *Cellular Microbiology*, *20*, e12801. Available from: https://doi.org/10.1111/cmi.12801

Shen, W.J., Azhar, S. & Kraemer, F.B. (2016) Lipid droplets and steroidogenic cells. *Experimental Cell Research*, *340*, 209–214.

Shibata, Y., Shemesh, T., Prinz, W.A., Palazzo, A.F., Kozlov, M.M. & Rapoport, T.A. (2010) Mechanisms determining the morphology of the peripheral ER. *Cell*, *143*, 774–788.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M. et al. (2009) Autophagy regulates lipid metabolism. *Nature*, *458*, 1131–1135.

Singh, V., Jamwal, S., Jain, R., Verma, P., Gokhale, R. & Rao, K.V. (2012) *Mycobacterium tuberculosis*-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. *Cell Host & Microbe*, *12*, 669–681.

Skinner, J.R., Shew, T.M., Schwartz, D.M., Tzekov, A., Lepus, C.M., Abumrad, N.A. et al. (2009) Diacylglycerol enrichment of endoplasmic reticulum or lipid droplets recruits perilipin 3/TIP47 during lipid storage and mobilization. *The Journal of Biological Chemistry*, *284*, 30941–30948.

Smirnova, E., Goldberg, E.B., Makarova, K.S., Lin, L., Brown, W.J. & Jackson, C.L. (2006) ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. *EMBO Reports*, *7*, 106–113.

Stanhope, R., Flora, E., Bayne, C. & Derré, I. (2017) IncV, a FFAT motif-containing *Chlamydia* protein, tethers the endoplasmic reticulum to the pathogen-containing vacuole. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 12039–12044.

Steiner, B., Swart, A.L., Welin, A., Weber, S., Personnic, N., Kaech, A. et al. (2017) ER remodeling by the large GTPase atlastin promotes vacuolar growth of *Legionella pneumophila*. *EMBO Reports*, *18*, 1817–1836.

Steiner, B., Weber, S. & Hilbi, H. (2018) Formation of the *Legionella*-containing vacuole: phosphoinositide conversion, GTPase modulation and ER dynamics. *International Journal of Medical Microbiology*, *308*, 49–57.

Steiner, B., Weber, S., Kaech, A., Ziegler, U. & Hilbi, H. (2018) The large GTPase atlastin controls ER remodeling around a pathogen vacuole. *Communicative & Integrative Biology*, *11*, 1–5.

Steinert, M. & Heuner, K. (2005) *Dictyostelium* as host model for pathogenesis. *Cellular Microbiology*, *7*, 307–314.

Stone, S.J., Levin, M.C., Zhou, P., Han, J., Walther, T.C. & Farese, R.V., Jr. (2009) The endoplasmic reticulum enzyme DGAT2 is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. *The Journal of Biological Chemistry*, *284*, 5352–5361.

Sun, Z., Gong, J., Wu, H., Xu, W., Wu, L., Xu, D. et al. (2013) Perilipin1 promotes unilocular lipid droplet formation through the activation of Fsp27 in adipocytes. *Nature Communications*, *4*, 1594.

Swart, A.L., Gomez-Valero, L., Buchrieser, C. & Hilbi, H. (2020) Evolution and function of bacterial RCC1 repeat effectors. *Cellular Microbiology*, *22*, e13246.

Swart, A.L., Harrison, C.F., Eichinger, L., Steinert, M. & Hilbi, H. (2018) *Acanthamoeba* and *Dictyostelium* as cellular models for *Legionella* infection. *Frontiers in Cellular and Infection Microbiology*, *8*, 61.

Swart, A.L. & Hilbi, H. (2020) Phosphoinositides and the fate of *Legionella* in phagocytes. *Frontiers in Immunology*, *1*, 25.

Szymanski, K.M., Binns, D., Bartz, R., Grishin, N.V., Li, W.P., Agarwal, A.K. et al. (2007) The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 20890–20895.

Thiam, A.R., Antonny, B., Wang, J., Delacotte, J., Wilfling, F., Walther, T.C. et al. (2013) COPI buds 60-nm lipid droplets from reconstituted water-phospholipid-triacylglyceride interfaces, suggesting a tension clamp function. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 13244–13249.

Thiam, A.R. & Foret, L. (2016) The physics of lipid droplet nucleation, growth and budding. *Biochimica et Biophysica Acta*, *1861*, 715–722.

Traber, M.G. & Kayden, H.J. (1987) Tocopherol distribution and intracellular localization in human adipose tissue. *The American Journal of Clinical Nutrition*, *46*, 488–495.

Truong, N.T.T., Lydic, T.A., Bazil, J.N., Suryadevara, A. & Olson, L.K. (2020) Regulation of lipid metabolism in pancreatic beta cells by interferon gamma: a link to anti-viral function. *Cytokine*, *133*, 155147.

Valachovic, M., Garaiova, M., Holic, R. & Hapala, I. (2016) Squalene is lipotoxic to yeast cells defective in lipid droplet biogenesis. *Biochemical and Biophysical Research Communications*, *469*, 1123–1128.

Vallochi, A.L., Teixeira, L., Oliveira, K.D.S., Maya-Monteiro, C.M. & Bozza, P.T. (2018) Lipid droplet, a key player in host-parasite interactions. *Frontiers in Immunology*, *9*, 1022.

Valm, A.M., Cohen, S., Legant, W.R., Melunis, J., Hershberg, U., Wait, E. et al. (2017) Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature*, *546*, 162–167.

van Dierendonck, X., Vrieling, F., Smeehuijzen, L., Deng, L., Boogaard, J.P., Croes, C.A. et al. (2022) Triglyceride breakdown from lipid droplets regulates the inflammatory response in macrophages. *Proceedings of the National Academy of Sciences of the United States of America*, *119*, e2114739119.

Varghese, M., Kimler, V.A., Ghazi, F.R., Rathore, G.K., Perkins, G.A., Ellisman, M.H. et al. (2019) Adipocyte lipolysis affects perilipin 5 and cristae organization at the cardiac lipid droplet-mitochondrial interface. *Scientific Reports*, *9*, 4734.

Vevea, J.D., Garcia, E.J., Chan, R.B., Zhou, B., Schultz, M., Di Paolo, G. et al. (2015) Role for lipid droplet biogenesis and microlipophagy in adaptation to lipid imbalance in yeast. *Developmental Cell*, *35*, 584–599.

Voeltz, G.K., Prinz, W.A., Shibata, Y., Rist, J.M. & Rapoport, T.A. (2006) A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell*, *124*, 573–586.

Volmer, R. & Ron, D. (2015) Lipid-dependent regulation of the unfolded protein response. *Current Opinion in Cell Biology*, *33*, 67–73.

Volmer, R., van der Ploeg, K. & Ron, D. (2013) Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 4628–4633.

Vormittag, S., Hüsler, D., Haneburger, I., Kroniger, T., Anand, A., Prantl, M. et al. (2023) *Legionella*- and host-driven lipid flux at LCV-ER membrane contact sites promotes vacuole remodeling. *EMBO Reports*, *24*, e56007.

Voskuil, M.I., Schnappinger, D., Visconti, K.C., Harrell, M.I., Dolganov, G.M., Sherman, D.R. et al. (2003) Inhibition of respiration by nitric

oxide induces a *Mycobacterium tuberculosis* dormancy program. *The Journal of Experimental Medicine*, *198*, 705–713.
Voth, D.E. & Heinzen, R.A. (2007) Lounging in a lysosome: the intracellular lifestyle of *Coxiella burnetii*. *Cellular Microbiology*, *9*, 829–840.
Walch, P., Selkrig, J., Knodler, L.A., Rettel, M., Stein, F., Fernandez, K. et al. (2021) Global mapping of *Salmonella enterica*-host protein-protein interactions during infection. *Cell Host & Microbe*, *29*, 1316–1332.
Walther, T.C. & Farese, R.V., Jr. (2012) Lipid droplets and cellular lipid metabolism. *Annual Review of Biochemistry*, *81*, 687–714.
Walther, T.C. & Farese, R.V., Jr. (2019) Un-phased: lipid droplets modulate the bioavailability of antibiotics. *Developmental Cell*, *50*, 530–532.
Wang, H., Becuwe, M., Housden, B.E., Chitraju, C., Porras, A.J., Graham, M.M. et al. (2016) Seipin is required for converting nascent to mature lipid droplets. *eLife*, *5*, e16582.
Weber, S., Wagner, M. & Hilbi, H. (2014) Live-cell imaging of phosphoinositide dynamics and membrane architecture during *Legionella* infection. *mBio*, *5*, e00839-13.
Weber, S.S., Ragaz, C., Reus, K., Nyfeler, Y. & Hilbi, H. (2006) *Legionella pneumophila* exploits PI(4)*P* to anchor secreted effector proteins to the replicative vacuole. *PLoS Pathogens*, *2*, e46.
Welte, M.A. (2009) Fat on the move: intracellular motion of lipid droplets. *Biochemical Society Transactions*, *37*, 991–996.
Welte, M.A. (2015) Expanding roles for lipid droplets. *Current Biology*, *25*, R470–R481.
Welte, M.A. & Gould, A.P. (2017) Lipid droplet functions beyond energy storage. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1862*, 1260–1272.
Wilfling, F., Thiam, A.R., Olarte, M.J., Wang, J., Beck, R., Gould, T.J. et al. (2014) Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. *eLife*, *3*, e01607.
Wilfling, F., Wang, H., Haas, J.T., Krahmer, N., Gould, T.J., Uchida, A. et al. (2013) Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Developmental Cell*, *24*, 384–399.
Witkos, T.M. & Lowe, M. (2015) The golgin family of coiled-coil tethering proteins. *Frontiers in Cell and Development Biology*, *3*, 86.
World Health Organization. (2022) *Global tuberculosis report 2022*. Geneva: World Health Organization.
Wright, H.R., Turner, A. & Taylor, H.R. (2008) Trachoma. *Lancet*, *371*, 1945–1954.
Zechner, R., Madeo, F. & Kratky, D. (2017) Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nature Reviews. Molecular Cell Biology*, *18*, 671–684.
Zehmer, J.K., Huang, Y., Peng, G., Pu, J., Anderson, R.G. & Liu, P. (2009) A role for lipid droplets in inter-membrane lipid traffic. *Proteomics*, *9*, 914–921.
Zhou, L., Yu, M., Arshad, M., Wang, W., Lu, Y., Gong, J. et al. (2018) Coordination among lipid droplets, peroxisomes, and mitochondria regulates energy expenditure through the CIDE-ATGL-PPARα pathway in adipocytes. *Diabetes*, *67*, 1935–1948.

How to cite this article: Hüsler, D., Stauffer, P. & Hilbi, H. (2023). Tapping lipid droplets: A rich fat diet of intracellular bacterial pathogens. *Molecular Microbiology*, *120*, 194–209. https://doi.org/10.1111/mmi.15120