Published in final edited form as:

Cell Microbiol. 2012 July ; 14(7): 1010–1018. doi:10.1111/j.1462-5822.2012.01794.x.

Lipid Acquisition by Intracellular *Chlamydiae*

Cherilyn A. Elwell^{1,3,*} and Joanne N. Engel^{1,2,3}

¹Department of Medicine

²Department of Microbiology and Immunology

³Department of Microbial Pathogenesis and Host Defense Program

Abstract

Chlamydia species are obligate intracellular pathogens that are important causes of human genital tract, ocular, and respiratory infections. The bacteria replicate within a specialized membrane-bound compartment termed the inclusion and require host-derived lipids for intracellular growth and development. Emerging evidence indicates that *Chlamydia* has evolved clever strategies to fulfill its lipid needs by interacting with multiple host cell compartments and redirecting trafficking pathways to its intracellular niche. In this review, we highlight recent findings that have significantly expanded our understanding of how *Chlamydia* exploit lipid trafficking pathways to ensure the survival of this important human pathogen.

Introduction

Chlamydiae are obligate intracellular, Gram-negative pathogens that have a significant impact on public health. Two human-adapted species, *C. trachomatis* and *C. pneumoniae*, are responsible for the majority of *Chlamydia* infections. *C. trachomatis* is the leading cause of sexually transmitted disease and non-congenital blindness (Mandell *et al.*, 2010). *C. pneumoniae* is responsible for ~10% of upper and lower respiratory tract infections and has been implicated as a risk factor in chronic diseases, such as atherosclerosis (Blasi *et al.*, 2009). One key aspect of *Chlamydia* pathogenesis is its ability to acquire host cell lipids and other nutrients while at the same time avoid immune detection. Understanding the molecular mechanisms of host-*Chlamydia* interactions may lead to new approaches for the development of novel therapeutics, diagnostics, and preventative strategies.

All *Chlamydia* species share a dimorphic developmental cycle in which they alternate between an extracellular, spore-like infectious elementary body (EB) and an intracellular, metabolically active but non-infectious reticulate body (RB) (Scidmore, 2011). Following binding and entry, the EB is sequestered within a unique membrane bound compartment (inclusion) where the EB differentiates into an RB and replicates by binary fission. The inclusion rapidly segregates from the endocytic pathway where it avoids fusion with lysosomes and is transported via microtubules to the peri-Golgi region. During this time, the inclusion interacts with multiple host cell compartments and trafficking pathways to acquire host-derived nutrients. Interactions between the inclusion and the host cell are likely mediated by a set of *Chlamydia* inclusion membrane proteins (Incs) that are inserted into the inclusion membrane by type III secretion or by *Chlamydia* proteins that are directly secreted into the host cytosol (Valdivia, 2008). A large number of Inc proteins are encoded in the *Chlamydia* genome and are thought to act as scaffolds for recruiting host cell proteins to the

*Corresponding author. Division of Infectious Disease, Box 0654, University of California, San Francisco, CA 94143-0654. Phone: (415)-476-3433. Fax: (415)-476-9364. Cherilyn.elwell@ucsf.edu.

inclusion, where they can modulate host cell trafficking pathways, signal transduction, or the host immune response. In some strains of *Chlamydia*, infection of cells by more than one EB leads to the formation of multiple individual inclusions that undergo homotypic fusion to form one larger inclusion in a process that requires at least one bacterial membrane protein, IncA (Hackstadt *et al.*, 1999, Delevoye *et al.*, 2004) and likely other host and/or bacterial factors (Paumet *et al.*, 2009). After 24–72 hours (hrs) of replication, RBs redifferentiate back to EBs and are released from the host cell by cell lysis or active extrusion (Hybiske *et al.*, 2007). Under some circumstances, such as exposure to interferon-gamma, penicillin, or tryptophan starvation, *Chlamydiae* can enter into a reversible, altered growth state called persistence which is characterized by the presence of non-replicating, aberrant RBs and which may contribute to chronic infection (Wyrick, 2010).

Chlamydiae rely on host-derived lipids for survival and have evolved efficient methods to acquire glycerophospholipids, sphingolipids, and cholesterol from the host cell (see below). These lipids are not uniformly distributed throughout the host cell and help to define the identity and function of each unique membrane-bound organelle (Lev, 2010). The Golgi complex plays a central role in vesicular transport of lipids along the endocytic and exocytic pathways and between organelles. Non-vesicular transport also plays an important role in lipid trafficking between organelles and is thought to be facilitated by cytosolic lipid transfer proteins (LTPs) (Lev, 2010). LTPs likely act at membrane contact sites (MCS), which are sub-regions (10–20 nm) where two organelle membranes are arranged in close proximity. Recent studies have begun to shed some light on how *Chlamydia* actively modulates its lipid composition within bacteria and at the inclusion membrane, what lipids are present, and what function they perform. Here we review the mechanisms of lipid acquisition by *Chlamydiae* and highlight recent studies that reveal novel strategies by which this important pathogen exploits both vesicular and non-vesicular pathways to acquire host cell lipids.

Lipid Composition of *Chlamydia* and the Inclusion

Chlamydiae are capable of synthesizing various lipids commonly found in bacteria, such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylserine (PS) (Hatch *et al.*, 1998, Wylie *et al.*, 1997). However, analysis of the lipid composition of *C. trachomatis* membranes reveals the presence of lipids typically found in eukaryotic cells, including phosphatidylcholine (PC), phosphatidylinositol (PI), sphingomyelin (SM), and cholesterol (Hackstadt *et al.*, 1995, Hackstadt *et al.*, 1996, Wylie *et al.*, 1997, Hatch *et al.*, 1998, Carabeo *et al.*, 2003). Approximately 3.69% and 6.47% of total lipids from *C. trachomatis* EBs is composed of SM and cholesterol, respectively (Carabeo *et al.*, 2003, Hatch *et al.*, 1998, Wylie *et al.*, 1997). Since the *Chlamydia* genome does not encode the enzymes required for the synthesis of these lipids (Stephens *et al.*, 1998), the bacteria must obtain them from the host.

The inclusion is a dynamic lipid-containing compartment. Initially, the inclusion membrane is small and contains only a single bacterium, however the inclusion must expand during infection to accommodate the replicating bacteria. Defining the lipid composition of the inclusion membrane has been challenging, as it is difficult to isolate this fragile compartment *in vitro*. Immunofluorescence studies, however, have demonstrated the presence of some host lipids, including SM, ceramide (SM precursor), cholesterol, phosphatidic acid (PA), PI, PE, and PS in the inclusion membrane (Beatty, 2008, Hackstadt *et al.*, 1996, Hackstadt *et al.*, 1995, Carabeo *et al.*, 2003, Elwell *et al.*, 2011). Emerging evidence reveals that a number of host proteins that regulate lipid trafficking and biosynthesis also localize to the inclusion, possibly by binding to one or more Inc proteins. While the potential functions of a few Inc proteins have been revealed and are discussed

throughout this review, the majority of Incs have no known function and are attractive candidates for bacterial factors that may facilitate lipid acquisition.

Acquisition of SM and Cholesterol from the Golgi by Vesicular Trafficking

Seminal studies by Hackstadt and colleagues over 15 years ago paved the way to understanding one of the mechanisms by which *Chlamydiae* acquire SM (Hackstadt *et al.*, 1996). These investigators found a close association between the inclusion and the Golgi complex where SM is synthesized prior to its transport to the plasma membrane. Using a fluorescently labeled ceramide analogue (C6-NBD-ceramide), they showed that this lipid is converted to SM in the Golgi and subsequently accumulates within the inclusion membrane as early as 2 hrs post infection, with up to ~50% of SM synthesized from C6-NBD-ceramide ultimately accumulating within the bacteria (Hackstadt *et al.*, 1995, Hackstadt *et al.*, 1996). Acquisition of SM by *C. trachomatis* is partially blocked when cells are treated with Brefeldin A (BFA), an inhibitor of vesicular trafficking, and this block correlates with a decrease in inclusion size (Hackstadt *et al.*, 1996). These observations led to the hypothesis that *C. trachomatis* intercepts an exocytic pathway by fusing with SM-containing vesicles exiting the *trans*-Golgi in transit to the plasma membrane, and that SM is important for growth of the inclusion membrane. Subsequent studies demonstrate that interception of SM by this pathway is a common feature of several *Chlamydia* species (Wolf *et al.*, 2001, Rockey *et al.*, 1996). Interestingly, glucosylceramide, a lipid closely related to SM that is also synthesized in the Golgi, is not delivered to the inclusion, suggesting that the inclusion specifically interacts with a subset of exocytic vesicles (Moore *et al.*, 2008). Further specificity for this interaction is demonstrated by the observation that SM is intercepted from a basolaterally directed pathway in polarized cells (Moore *et al.*, 2008). In addition to SM, cholesterol appears to be co-transported to the inclusion in vesicles in a partially BFA-dependent manner where it is incorporated into the inclusion membrane as well as replicating bacteria (Carabeo *et al.*, 2003).

It is now evident that the ability of the host cell to synthesize SM is absolutely essential for multiple steps of *Chlamydia* development (van Ooij *et al.*, 2000, Robertson *et al.*, 2009). *C. trachomatis* serovar L2 replication is severely impaired in cells that carry a temperature sensitive mutation in Serine Palmitoyltransferase (SPT), the first enzyme in the biosynthetic pathway for SM biosynthesis (van Ooij *et al.*, 2000). Pharmacological inhibition of SM biosynthesis in *C. trachomatis* serovar E-infected cells results in loss of inclusion membrane stability, premature differentiation of RBs to EBs, early release of EBs from host cells, and disruption of homotypic fusion of multiple inclusions (Robertson *et al.*, 2009). Inhibition of SPT also severely impairs the ability of *C. trachomatis* serovar B RBs to recover from a persistent state induced by interferon-gamma (Robertson *et al.*, 2009). Taken together, these studies reveal that SM is essential not only for bacterial replication but also for both biogenesis of the inclusion membrane and reactivation from persistence.

Recent studies have yielded further mechanistic insight into BFA-sensitive vesicular trafficking of SM from the Golgi to the inclusion and reveal that only a subset of Golgi trafficking proteins are co-opted by *C. trachomatis*, including Arf1 GTPase (Moorhead *et al.*, 2010, Mehlitz *et al.*, 2010) and its *cis*-Golgi localized guanine exchange factor (GEF), GBF1 (Robertson *et al.*, 2009, Elwell *et al.*, 2011). Arf GTPases are key players in vesicular trafficking as they mediate the formation of vesicle carriers by recruiting COPI coat proteins (Bui *et al.*, 2009). Arf1 activation in the Golgi is regulated through differential localization of specific GEFs, GBF1 and BIG1/BIG2, which are the direct targets of BFA (Manolea *et al.*, 2008). GBF1 is restricted to the *cis*-Golgi and is required for the assembly and maintenance of the Golgi stack whereas BIG1/2 are found in the *trans*-Golgi and are required for maintenance of the *trans*-Golgi network (Manolea *et al.*, 2008). Arf1 localizes

to the inclusion membrane of *C. trachomatis*, although conflicting roles in infection have been reported (Moorhead *et al.*, 2010, Mehltitz *et al.*, 2010, Elwell *et al.*, 2011). Depletion of GBF1, but not BIG1/2, reduces SM transport to the inclusion with a concomitant loss of inclusion membrane integrity and decrease in inclusion size (Elwell *et al.*, 2011). In addition, inhibition of GBF1 results in a slight disruption of homotypic fusion (Robertson *et al.*, 2009). These results indicate that *Chlamydia* acquires SM at least in part through GBF1-dependent vesicular trafficking and that this source of SM contributes to inclusion membrane stability and growth as well as fusion. Despite the reduction in SM transport following loss of GBF1 activity, there is no effect on production of infectious progeny (Elwell *et al.*, 2011). These observations are consistent with the original report that BFA treatment does not block replication (Hackstadt *et al.*, 1996), and unexpectedly, indicate that *Chlamydia* requires only one of the BFA-sensitive targets (GBF1) for SM acquisition. Since host SM biosynthesis is necessary for replication and inhibiting GBF1-dependent trafficking is not sufficient to impair replication, SM must also be acquired through GBF1-independent pathways.

Acquisition of Ceramide and SM by the Cytosolic Lipid Transporter, CERT

SM synthesis is a multistep process (Hanada *et al.*, 2003, Huitema *et al.*, 2004, Hanada *et al.*, 2009). The SM precursor, ceramide, is synthesized on the cytosolic surface of the Endoplasmic Reticulum (ER). It is subsequently transported to the *trans*-Golgi, where it is converted to SM by SM synthases, SMS1 or SMS2 (Huitema *et al.*, 2004). It was previously thought that transport of ceramide from the ER to the *trans*-Golgi for *de novo* SM biosynthesis followed canonical vesicular trafficking through the Golgi stacks. However, Hanada and colleagues discovered that the major pathway for *de novo* SM biosynthesis requires a novel ceramide transfer protein, called CERT (Hanada *et al.*, 2003, Hanada *et al.*, 2009). CERT binds to the ER membrane proteins VAP-A and VAP-B by a short motif containing two phenylalanines in an acidic tract (FFAT), where it extracts newly synthesized ceramide using its steroidogenic acute regulatory protein-related lipid transfer (START) domain (Hanada *et al.*, 2009). CERT then transfers ceramide by non-vesicular trafficking to the *trans*-Golgi where it binds phosphatidylinositol 4-phosphate (PI4P) through its pleckstrin homology (PH) domain and releases ceramide (Hanada *et al.*, 2009). CERT-mediated transfer of ceramide is thought to occur at ER-Golgi MCS.

Two new studies now suggest that *Chlamydia* hijacks CERT to facilitate the transfer of ceramide from the ER to the inclusion membrane, possibly for subsequent conversion to SM (Derre *et al.*, 2011, Elwell *et al.*, 2011). As early as 2 hrs post infection, CERT, as well as VAP-A and VAP-B, localize to the inclusion membrane in discrete domains. Ultrastructural studies confirm direct recruitment of CERT and further reveal ER tubules containing VAP-B in close apposition to the inclusion, suggesting the creation of ER-inclusion MCS (Derre *et al.*, 2011). Indeed, an intimate association of the ER with the chlamydial inclusion was previously reported (Peterson *et al.*, 1988, Majeed *et al.*, 1999, Giles *et al.*, 2008).

While the PH domain of CERT is necessary and sufficient for binding to the inclusion (Derre *et al.*, 2011), surprisingly, the regions that target CERT to the ER (FFAT) and to the Golgi (PI4P binding) are not required for CERT localization to the inclusion (Elwell *et al.*, 2011). Previous studies demonstrated that SM acquisition by the inclusion required bacterial protein synthesis (Hackstadt *et al.*, 1996), suggesting involvement of a chlamydial protein. Immunoprecipitation and mass spectrometry analysis of *C. trachomatis*-infected cells identified a bacterial inclusion membrane protein, IncD, as a binding partner for CERT (Derre *et al.*, 2011). IncD was found to interact with the PH domain of CERT and to partially colocalize with CERT at the inclusion membrane (Derre *et al.*, 2011). A strain that infects guinea pigs, *C. caviae*, lacks IncD and does not recruit CERT to the inclusion (Derre

et al., 2011), thus making IncD an exciting candidate mediating CERT recruitment. Despite the lack of IncD and CERT recruitment, *C. caviae* acquires SM from host cells (Rockey *et al.*, 1996), suggesting that *C. caviae* relies on other pathways for SM acquisition.

Immunofluorescence analysis reveal an enrichment of ceramide at the inclusion and to the adjacent region, suggesting that CERT may function to mobilize this lipid not only to the Golgi but also to the inclusion (Elwell *et al.*, 2011). While SMS1 and SMS2 are found in close proximity to the inclusion, only SMS2 localizes at the inclusion membrane (Elwell *et al.*, 2011). Interestingly, inclusion-localized SMS2 partially overlaps with CERT (Elwell *et al.*, 2011). Depletion of CERT, VAPs, SMS1, and SMS2 significantly reduces the production of infectious progeny (Derre *et al.*, 2011, Elwell *et al.*, 2011). Furthermore, inhibition of CERT activity results in a significant defect in SM acquisition by the inclusion (Elwell *et al.*, 2011). Based on these observations, it is possible that CERT mediates the transfer of ceramide (a) from the ER to the inclusion where it may be converted to SM by SMS2, and/or (b) from the ER to the Golgi where it is converted to SM by SMS1/SMS2 and subsequently transferred to the nearby inclusion. Consistent with either of these possibilities, diacylglycerol (DAG), a product generated during SM synthesis, accumulates at the inclusion (Tse *et al.*, 2005). It is also possible that CERT-mediated ceramide transfer may function at ER-inclusion MCS to generate specialized metabolic and/or signaling platforms necessary for replication (Derre *et al.*, 2011). It will be interesting to determine whether these specialized lipid platforms are similar to the recently described microdomains located at the inclusion membrane enriched in cholesterol, Src family kinases, and a subset of Incs (Mital *et al.*, 2010).

The ability of *Chlamydia* to exploit both vesicular and non-vesicular trafficking pathways for SM acquisition has provided a possible explanation for a longtime paradox as to why inhibitors of vesicular trafficking have no effect on replication despite an absolute requirement for host SM biosynthesis in *Chlamydia* development (Elwell *et al.*, 2011). This finding raises the possibility that vesicular and non-vesicular pathways contribute to different aspects of *Chlamydia* development, with GBF-dependent vesicular trafficking being essential for inclusion membrane stability and CERT-dependent transport being necessary for replication.

The ability of *Chlamydia* to redirect specific components of host SM biosynthetic machinery, such as CERT, VAPs, and SMS2, directly to the inclusion provides a novel strategy in which a pathogen creates its own nutrient factory. Future work will likely focus on whether *Chlamydia* co-opts additional LTPs to obtain other lipids, such as cholesterol and glycerophospholipids.

Acquisition of SM following Golgi Fragmentation

During *C. trachomatis* infection, the Golgi is fragmented into mini-stacks that surround the inclusion and enhance *C. trachomatis* replication (Heuer *et al.*, 2009). Golgi fragmentation correlates with cleavage of the Golgi matrix protein, Golgin-84, which is mediated by host proteases and the *Chlamydia* protease, CPAF (Heuer *et al.*, 2009, Christian *et al.*, 2011). Inhibiting fragmentation of the Golgi by blocking Golgin-84 cleavage reduces progeny production and decreases SM uptake. Conversely, inducing Golgi fragmentation enhances progeny formation (Heuer *et al.*, 2009). It is speculated that Golgi fragmentation enhances SM transport to the inclusion during infection. Whether the enhanced SM uptake observed following Golgi fragmentation occurs via vesicular trafficking or whether altering the Golgi structure somehow promotes non-vesicular trafficking to the inclusion is not known. One attractive hypothesis is that fragmentation of the Golgi may increase the number of ER-Golgi-inclusion MCS for efficient lipid transfer. Although SM acquisition is observed as

early as two hours post infection, Golgi fragmentation occurs during later stages of infection (Hackstadt *et al.*, 1996, Heuer *et al.*, 2009). This finding suggests that *Chlamydia* may utilize different mechanisms of lipid transport at early and late times of development.

Acquisition of Lipids by Rab GTPases and Phosphoinositides

Rab GTPases are critical regulators of membrane trafficking between specific intracellular compartments (Stenmark, 2009). A subset of Rabs are recruited to the inclusion in a species-dependent and independent manner, including Rab1, Rab6, and Rab10, which are involved in ER-Golgi trafficking, and Rab4, Rab11, and Rab14, which are associated with endosomes (Rzomp *et al.*, 2003, Capmany *et al.*, 2010). Recruitment of Rabs to the inclusion may be mediated by specific Inc proteins. Rab4 interacts with *C. trachomatis* CT229 (Rzomp *et al.*, 2003) while *C. pneumonia* Cpn0585 interacts with Rab1, Rab10, and Rab11 (Cortes *et al.*, 2007). Interaction of specific Rabs with the inclusion likely allows for selective fusion with host vesicles and access to essential lipids. Thus far, Rab6, 11, and 14 have been shown to play a role specifically in SM acquisition (Rejman Lipinski *et al.*, 2009, Capmany *et al.*, 2010). Rab6 and Rab11 regulate *C. trachomatis*-induced Golgi fragmentation following Golgin-84 cleavage (Rejman Lipinski *et al.*, 2009). Depletion of Rab6 or Rab11 impairs progeny formation, SM uptake, and Golgi fragmentation (Rejman Lipinski *et al.*, 2009). Remarkably, progeny formation and SM uptake are rescued when Golgi fragmentation is induced by depletion of p115, another Golgi matrix protein (Rejman Lipinski *et al.*, 2009). These results suggest that Rab6 and Rab11 may increase the efficiency of SM acquisition by mobilizing the Golgi fragments. Rab14 has also been implicated in SM acquisition by *C. trachomatis* (Capmany *et al.*, 2010). Interestingly, Rab14-positive vesicles are present within the lumen of the inclusion, suggesting that the inclusion may directly fuse with Rab14 vesicles containing SM. In addition to the recruitment of Rabs, *Chlamydia* may regulate fusion with host vesicles for delivery of essential lipids by recruiting Golgi-associated host soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) (Moore *et al.*, 2011, Pokrovskaya *et al.*, 2012), which are key components of intracellular fusion machinery, and/or expression of Inc proteins that contain SNARE-like motifs (Delevoye *et al.*, 2008).

Phosphoinositides are short-lived phosphorylated derivatives of PI that regulate the localization of PI-binding proteins and thus contribute to host cell lipid transport and signaling (D'Angelo *et al.*, 2008). Rabs control the levels of phosphoinositides on membranes through recruitment of lipid phosphatases, such as oculocerebrorenal syndrome of Lowe protein (OCRL1), which interacts with Rab1, 6, 8, and 14 (Behnia *et al.*, 2005). OCRL hydrolyzes PI(4,5)P₂ to produce PI4P and is recruited to the inclusion along with another PI4P producing enzyme, PI4KII α (Moorhead *et al.*, 2010). Pools of PI4P, which are normally highly enriched at the Golgi, are present at the inclusion (Moorhead *et al.*, 2010). Depletion of OCRL or PI4KII α reduces production of infectious progeny and inclusion formation, suggesting that PI4P generation at the inclusion is important for infectivity (Moorhead *et al.*, 2010). The exact role of PI4P at the inclusion is unclear. Although PI4P is not required for CERT recruitment to the inclusion (Derre *et al.*, 2011, Elwell *et al.*, 2011), this lipid may recruit other PI4P-binding host proteins that help define a specialized compartment that is necessary for survival and/or regulate lipid trafficking to the inclusion. Indeed, the PH domain of OSBP, a sterol transporter, which binds to PI4P at the Golgi, is recruited to the inclusion in a PI4P-dependent manner (Moorhead *et al.*, 2010), however it is not clear whether this is necessary for *Chlamydia* infection. Interestingly, SM and sterol metabolism are co-regulated at the Golgi through CERT and OSBP (Lev, 2010), therefore *Chlamydia* may co-opt CERT and OSBP to coordinately regulate the local lipid composition at the inclusion.

PI4P production at the Golgi membrane can be modulated by Arf1 GTPases, through their ability to recruit enzymes involved in the biosynthesis of PI4P, such as PI4KIII β (Shin *et al.*, 2004). Although Arf1 is not required for recruitment of PI4KII α to the inclusion, simultaneous depletion of OCRL, PI4KII α , and Arf1 results in a greater reduction in infectivity than single or double depletions (Moorhead *et al.*, 2010). These results suggest that PI4P production and Arf1 play non-redundant roles in infection.

Acquisition of Lipids from Multivesicular Bodies

Multivesicular bodies (MVBs) are late endocytic organelles which have been proposed as another source of SM and cholesterol, possibly acting as a post-Golgi trafficking intermediate (Beatty, 2006, Beatty, 2008, Robertson *et al.*, 2009). Several MVB constituents, including CD63, MLN64, and lysobisphosphatidic acid (LBPA) localize to the inclusion, and vesicles positive for CD63 have been observed within the inclusion (Beatty, 2006, Beatty, 2008), suggesting direct fusion of MVBs with the inclusion. Despite the presence of CD63 within the inclusion, CD63 itself is not required for the association of MVBs with the inclusion (Beatty, 2008). Treatment of infected cells with U18666A, a pharmacological inhibitor of MVB biogenesis, disrupts inclusion maturation, decreases SM and cholesterol acquisition, blocks homotypic inclusion fusion, and inhibits reactivation from a persistent infection (Beatty, 2006, Beatty, 2008, Robertson *et al.*, 2009), suggesting that MVBs may be an important source of SM and cholesterol lipids for multiple steps in *C. trachomatis* development (Robertson *et al.*, 2009). It should be noted that inhibitors of MVB biogenesis might have pleiotropic effects on other cellular functions (Cenedella, 2009). In addition, others have reported no effect of U18666A on SM uptake (Ouellette *et al.*, 2010). While there is a clear interaction of the *Chlamydia* inclusion with MVBs, the precise role of this organelle in *Chlamydia* infection and lipid transport remains an open question. MLN64 localization at the inclusion is interesting since it contains a StAR-related lipid transfer (START) domain that binds cholesterol acquired from endocytosed LDL and may play a role in cholesterol transport to the inclusion, as *Chlamydia* acquires cholesterol from an LDL pathway and from *de novo* synthesis (Carabeo *et al.*, 2003).

Interactions with Lipid Droplets

Lipid droplets (LD) have been proposed as a source of neutral lipids, such as long chain fatty acids (LCFAs), for *C. trachomatis* (Kumar *et al.*, 2006, Cocchiario *et al.*, 2008). LDs accumulate near the periphery of the inclusion and are translocated into the lumen at InCA-rich sites (Kumar *et al.*, 2006, Cocchiario *et al.*, 2008). At least 2 *Chlamydia* proteins, Lda1 and Lda3, localize with LDs adjacent to the inclusion (Kumar *et al.*, 2006), and Lda3 causes disassociation of adipocyte differentiation related protein (ADRP), a host protein that inhibits lipases that degrade LDs (Cocchiario *et al.*, 2008). This has led to the hypothesis that Lda3 docks LDs to the inclusion and removes ADRP so that lipases can degrade LD lipids following translocation into the lumen (Cocchiario *et al.*, 2008). Since LCFAs are important precursors in phospholipid synthesis, it has been postulated that *Chlamydia* utilizes the LCFA released from LDs for membrane biosynthesis (Cocchiario *et al.*, 2008). Inhibition of neutral lipid biosynthesis impairs *Chlamydia* development, suggesting an important function of LDs in *Chlamydia* pathogenesis (Cocchiario *et al.*, 2008). Recent studies have revealed that Arf1 and GBF1 also play important roles in lipid droplet biogenesis (Soni *et al.*, 2009, Guo *et al.*, 2008); thus it will be interesting to determine whether these protein play dual roles in SM acquisition and LD mobilization during *Chlamydia* infection.

Signaling and Lipid Transport

A number of host signaling molecules have been implicated in the transport of glycerophospholipids and SM to the inclusion. Host glycerophospholipids, such as PC and

PI, are trafficked to the inclusion in a vesicle-independent manner (Hatch *et al.*, 1998, Wylie *et al.*, 1997). Unlike SM and cholesterol, glycerophospholipids are selectively modified by *Chlamydia* which replace the straight chain fatty acid with a bacteria-derived branched chain fatty acid (Wylie *et al.*, 1997), in a process mediated by host Ca^{2+} -dependent cytosolic phospholipase A_2 (cPLA $_2$) that is activated by ERK1/2 (Su *et al.*, 2004).

A role for serine/threonine kinases in SM uptake by *Chlamydia* has been suggested by the observation that treatment with rottlerin, an inhibitor of several kinases, including protein kinase C delta (PKC δ), calmodulin-dependent protein kinase III, and p38-regulated/activated kinase, reduces SM transport to the inclusion (Shivshankar *et al.*, 2008). This reduction in SM transport correlates with inhibition of chlamydial growth (Shivshankar *et al.*, 2008). While it is unclear how rottlerin exerts its effect, one of the major targets of rottlerin, PKC δ is selectively recruited to the inclusion by binding to DAG produced at the inclusion and plays a role in protecting infected cells from apoptosis during infection (Tse *et al.*, 2005). PKC also plays a role in regulating vesicular trafficking at the Golgi (Diaz Anel *et al.*, 2005) and could direct SM transport to the inclusion.

A recent siRNA screen to identify host proteins required for SM acquisition by *C. trachomatis* has also implicated Src family kinases (SFK), specifically Fyn, in SM acquisition (Mital *et al.*, 2011b). Activated Fyn and Src kinases localize in discrete microdomains at the inclusion that are enriched in cholesterol and overlap with a subset of Incs, including IncB, Inc101, Inc222, and Inc850 (Mital *et al.*, 2010). Depletion of Fyn decreases C6-NBD-sphingolipid retention by both the inclusion and EBs, however, depletion of Fyn alone does not impair production of infectious progeny, indicating that SM trafficking mediated by Fyn signaling functions redundantly with other lipid trafficking pathways (Mital *et al.*, 2011b). SFK regulate microtubule- and dynein-dependent trafficking of the inclusion to the microtubule organizing center (MTOC) (Mital *et al.*, 2011a), therefore, it is speculated that Fyn may mediate linkage of the inclusion to the microtubule network and thereby intersect SM containing vesicles trafficking along microtubules. Whether Fyn regulates vesicle-mediated trafficking from the Golgi and/or MVBs and whether Fyn plays a role in cholesterol acquisition is not known.

Concluding Remarks

Chlamydiae employ a surprising variety of strategies to acquire essential host cell lipids (Figure 1), including co-opting vesicular trafficking from the Golgi and MVBs (1), hijacking non-vesicular trafficking from Lipid droplets (2) and the ER (3), recruiting lipid modifying enzymes (4), and activating signaling transduction pathways (5). Subversion of multiple transport mechanisms helps to ensure the survival of this highly successful pathogen within the hostile intracellular environment. With the recent development of new genetic tools to study *Chlamydiae* (Binet *et al.*, 2009, Wang *et al.*, 2011, Nguyen *et al.*, 2012, Kari *et al.*, 2011), future studies will likely focus on identifying the bacterial factors that mediate lipid acquisition and understanding how these factors target specific pathways. Ideally, finding ways to disrupt lipid acquisition by *Chlamydiae* without disrupting overall host lipid synthesis may prove to be a useful strategy to combat *Chlamydia* infections. These studies will likely yield new insights into mechanistic details of host lipid trafficking.

Acknowledgments

We kindly thank Dr. Kathleen Mirrashidi for comments on the manuscript. We apologize to those investigators whose work was not cited due to space constraints. Work in the laboratory is supported by grants from NIH RO1-AI073770.

References

- Beatty WL. Trafficking from CD63-positive late endocytic multivesicular bodies is essential for intracellular development of *Chlamydia trachomatis*. *J Cell Sci*. 2006; 119:350–359. [PubMed: 16410552]
- Beatty WL. Late endocytic multivesicular bodies intersect the chlamydial inclusion in the absence of CD63. *Infect Immun*. 2008; 76:2872–2881. [PubMed: 18426873]
- Behnia R, Munro S. Organelle identity and the signposts for membrane traffic. *Nature*. 2005; 438:597–604. [PubMed: 16319879]
- Binet R, Maurelli AT. Transformation and isolation of allelic exchange mutants of *Chlamydia psittaci* using recombinant DNA introduced by electroporation. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:292–297. [PubMed: 19104068]
- Blasi F, Tarsia P, Aliberti S. *Chlamydophila pneumoniae*. *Clin Microbiol Infect*. 2009; 15:29–35. [PubMed: 19220337]
- Bui QT, Golinelli-Cohen MP, Jackson CL. Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. *Mol Genet Genomics*. 2009; 282:329–350. [PubMed: 19669794]
- Capmany A, Damiani MT. *Chlamydia trachomatis* intercepts Golgi-derived sphingolipids through a Rab14-mediated transport required for bacterial development and replication. *PLoS One*. 2010; 5:e14084. [PubMed: 21124879]
- Carabeo RA, Mead DJ, Hackstadt T. Golgi-dependent transport of cholesterol to the *Chlamydial* inclusion. *Proc Natl Acad Sci U S A*. 2003; 100:6771–6776. [PubMed: 12743366]
- Cenedella RJ. Cholesterol synthesis inhibitor U18666A and the role of sterol metabolism and trafficking in numerous pathophysiological processes. *Lipids*. 2009; 44:477–487. [PubMed: 19440746]
- Christian JG, Heymann J, Paschen SA, Vier J, Schauenburg L, Rupp J, et al. Targeting of a chlamydial protease impedes intracellular bacterial growth. *PLoS Pathog*. 2011; 7:e1002283. [PubMed: 21990969]
- Cocchiari JL, Kumar Y, Fischer ER, Hackstadt T, Valdivia RH. Cytoplasmic lipid droplets are translocated into the lumen of the *Chlamydia trachomatis* parasitophorous vacuole. *Proc Natl Acad Sci U S A*. 2008; 105:9379–9384. [PubMed: 18591669]
- Cortes C, Rzomp KA, Tvinnereim A, Scidmore MA, Wikel B. *Chlamydia pneumoniae* inclusion membrane protein Cpn0585 interacts with multiple Rab GTPases. *Infect Immun*. 2007
- D'Angelo G, Vicinanza M, Di Campli A, De Matteis MA. The multiple roles of PtdIns(4)P -- not just the precursor of PtdIns(4,5)P₂. *J Cell Sci*. 2008; 121:1955–1963. [PubMed: 18525025]
- Delevoye C, Nilges M, Dautry-Varsat A, Subtil A. Conservation of the biochemical properties of IncA from *Chlamydia trachomatis* and *Chlamydia caviae*: oligomerization of IncA mediates interaction between facing membranes. *J Biol Chem*. 2004; 279:46896–46906. [PubMed: 15316015]
- Delevoye C, Nilges M, Dehoux P, Paumet F, Perrinet S, Dautry-Varsat A, Subtil A. SNARE protein mimicry by an intracellular bacterium. *PLoS Pathog*. 2008; 4:e1000022. [PubMed: 18369472]
- Derre I, Swiss R, Agaisse H. The lipid transfer protein CERT interacts with the *Chlamydia* inclusion protein IncD and participates to ER-*Chlamydia* inclusion membrane contact sites. *PLoS Pathog*. 2011; 7:e1002092. [PubMed: 21731489]
- Diaz Anel AM, Malhotra V. PKC ϵ is required for beta1gamma2/beta3gamma2- and PKD-mediated transport to the cell surface and the organization of the Golgi apparatus. *The Journal of cell biology*. 2005; 169:83–91. [PubMed: 15824133]
- Elwell CA, Jiang S, Kim JH, Lee A, Wittmann T, Hanada K, et al. *Chlamydia trachomatis* Co-opts GBF1 and CERT to Acquire Host Sphingomyelin for Distinct Roles during Intracellular Development. *PLoS Pathog*. 2011; 7:e1002198. [PubMed: 21909260]
- Giles DK, Wyrick PB. Trafficking of chlamydial antigens to the endoplasmic reticulum of infected epithelial cells. *Microbes Infect*. 2008; 10:1494–1503. [PubMed: 18832043]
- Guo Y, Walther TC, Rao M, Stuurman N, Goshima G, Terayama K, et al. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature*. 2008; 453:657–661. [PubMed: 18408709]

- Hackstadt T, Rockey D, Heinzen R, Scidmore M. *Chlamydia trachomatis* interrupts an exocytic pathway to acquire endogenously synthesized sphingomyelin in transit from the Golgi apparatus to the plasma membrane. *EMBO J.* 1996; 15:964–977. [PubMed: 8605892]
- Hackstadt T, Scidmore M, Rockey D. Lipid metabolism in *Chlamydia trachomatis*-infected cells: directed trafficking of Golgi-derived sphingolipids to the chlamydial inclusion. *Proc Natl Acad Sci USA.* 1995; 92:4877–4881. [PubMed: 7761416]
- Hackstadt T, Scidmore-Carlson MA, Shaw EI, Fischer ER. The *Chlamydia trachomatis* IncA protein is required for homotypic vesicle fusion. *Cell Microbiol.* 1999; 1:119–130. [PubMed: 11207546]
- Hanada K, Kumagai K, Tomishige N, Yamaji T. CERT-mediated trafficking of ceramide. *Biochim Biophys Acta.* 2009; 1791:684–691. [PubMed: 19416656]
- Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, Nishijima M. Molecular machinery for non-vesicular trafficking of ceramide. *Nature.* 2003; 426:803–809. [PubMed: 14685229]
- Hatch GM, McClarty G. Phospholipid composition of purified *Chlamydia trachomatis* mimics that of the eukaryotic cell. *Inf Immun.* 1998; 66:3727–3735.
- Heuer D, Rejman Lipinski A, Machuy N, Karlas A, Wehrens A, Siedler F, et al. *Chlamydia* causes fragmentation of the Golgi compartment to ensure reproduction. *Nature.* 2009; 457:731–735. [PubMed: 19060882]
- Huitema K, van den Dikkenberg J, Brouwers JF, Holthuis JC. Identification of a family of animal sphingomyelin synthases. *Embo J.* 2004; 23:33–44. [PubMed: 14685263]
- Hybiske K, Stephens RS. Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. *Proc Natl Acad Sci U S A.* 2007; 104:11430–11435. [PubMed: 17592133]
- Kari L, Goheen MM, Randall LB, Taylor LD, Carlson JH, Whitmire WM, et al. Generation of targeted *Chlamydia trachomatis* null mutants. *Proc Natl Acad Sci U S A.* 2011; 108:7189–7193. [PubMed: 21482792]
- Kumar Y, Cocchiari J, Valdivia RH. The obligate intracellular pathogen *Chlamydia trachomatis* targets host lipid droplets. *Curr Biol.* 2006; 16:1646–1651. [PubMed: 16920627]
- Lev S. Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat Rev Mol Cell Biol.* 2010; 11:739–750. [PubMed: 20823909]
- Majeed M, Krause KH, Clark RA, Kihlstrom E, Stendahl O. Localization of intracellular Ca²⁺ stores in HeLa cells during infection with *Chlamydia trachomatis*. *J Cell Sci.* 1999; 112 (Pt 1):35–44. [PubMed: 9841902]
- Mandell, GL.; Bennett, JE.; Dolin, R. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 7. Philadelphia, PA: Churchill Livingstone/Elsevier; 2010. 1 online resource (2 v. (cl, 4028, xcvi p.))
- Manolea F, Claude A, Chun J, Rosas J, Melancon P. Distinct functions for Arf guanine nucleotide exchange factors at the Golgi complex: GBF1 and BIGs are required for assembly and maintenance of the Golgi stack and trans-Golgi network, respectively. *Mol Biol Cell.* 2008; 19:523–535. [PubMed: 18003980]
- Mehlitz A, Banhart S, Maurer AP, Kaushansky A, Gordus AG, Zielecki J, et al. Tarp regulates early *Chlamydia*-induced host cell survival through interactions with the human adaptor protein SHC1. *J Cell Biol.* 2010; 190:143–157. [PubMed: 20624904]
- Mital J, Hackstadt T. Diverse requirements for SRC-family tyrosine kinases distinguish chlamydial species. *MBio.* 2011a; 2
- Mital J, Hackstadt T. Role for the SRC family kinase fyn in sphingolipid acquisition by chlamydiae. *Infect Immun.* 2011b; 79:4559–4568. [PubMed: 21896774]
- Mital J, Miller NJ, Fischer ER, Hackstadt T. Specific chlamydial inclusion membrane proteins associate with active Src family kinases in microdomains that interact with the host microtubule network. *Cell Microbiol.* 2010
- Moore ER, Fischer ER, Mead DJ, Hackstadt T. The chlamydial inclusion preferentially intercepts basolaterally directed sphingomyelin-containing exocytic vacuoles. *Traffic.* 2008; 9:2130–2140. [PubMed: 18778406]

- Moore ER, Mead DJ, Dooley CA, Sager J, Hackstadt T. The trans-Golgi SNARE syntaxin 6 is recruited to the chlamydial inclusion membrane. *Microbiology*. 2011; 157:830–838. [PubMed: 21109560]
- Moorhead AM, Jung JY, Smirnov A, Kaufer S, Scidmore MA. Multiple host proteins that function in phosphatidylinositol-4-phosphate metabolism are recruited to the chlamydial inclusion. *Infect Immun*. 2010; 78:1990–2007. [PubMed: 20231409]
- Nguyen BD, Valdivia RH. Virulence determinants in the obligate intracellular pathogen *Chlamydia trachomatis* revealed by forward genetic approaches. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:1263–1268. [PubMed: 22232666]
- Ouellette SP, Carabeo RA. A Functional Slow Recycling Pathway of Transferrin is Required for Growth of *Chlamydia*. *Front Microbiol*. 2010; 1:112. [PubMed: 21607082]
- Paumet F, Wesolowski J, Garcia-Diaz A, Delevoye C, Aulner N, Shuman HA, et al. Intracellular bacteria encode inhibitory SNARE-like proteins. *PLoS One*. 2009; 4:e7375. [PubMed: 19823575]
- Peterson EM, de la Maza LM. *Chlamydia* parasitism: ultrastructural characterization of the interaction between the chlamydial cell envelope and the host cell. *J Bacteriol*. 1988; 170:1389–1392. [PubMed: 3343223]
- Pokrovskaya ID, Szewdo JW, Goodwin A, Lupashina TV, Nagarajan UM, Lupashin VV. *Chlamydia trachomatis* hijacks intra-Golgi COG complex-dependent vesicle trafficking pathway. *Cell Microbiol*. 2012
- Rejman Lipinski A, Heymann J, Meissner C, Karlas A, Brinkmann V, Meyer TF, Heuer D. Rab6 and Rab11 regulate *Chlamydia trachomatis* development and golgin-84-dependent Golgi fragmentation. *PLoS Pathog*. 2009; 5:e1000615. [PubMed: 19816566]
- Robertson DK, Gu L, Rowe RK, Beatty WL. Inclusion biogenesis and reactivation of persistent *Chlamydia trachomatis* requires host cell sphingolipid biosynthesis. *PLoS Pathog*. 2009; 5:e1000664. [PubMed: 19936056]
- Rockey DD, Fischer ER, Hackstadt T. Temporal analysis of the developing *Chlamydia psittaci* inclusion by use of fluorescence and electron microscopy. *Infect Immun*. 1996; 64:4269–4278. [PubMed: 8926099]
- Rzomp KA, Scholtes LD, Briggs BJ, Whittaker GR, Scidmore MA. Rab GTPases are recruited to chlamydial inclusions in both a species-dependent and species-independent manner. *Infect Immun*. 2003; 71:5855–5870. [PubMed: 14500507]
- Scidmore MA. Recent advances in *Chlamydia* subversion of host cytoskeletal and membrane trafficking pathways. *Microbes Infect*. 2011; 13:527–535. [PubMed: 21334451]
- Shin HW, Nakayama K. Dual control of membrane targeting by PtdIns(4)P and ARF. *Trends Biochem Sci*. 2004; 29:513–515. [PubMed: 15450602]
- Shivshankar P, Lei L, Wang J, Zhong G. Rottlerin inhibits chlamydial intracellular growth and blocks chlamydial acquisition of sphingolipids from host cells. *Appl Environ Microbiol*. 2008; 74:1243–1249. [PubMed: 18083882]
- Soni KG, Mardones GA, Sougrat R, Smirnova E, Jackson CL, Bonifacino JS. Coatamer-dependent protein delivery to lipid droplets. *J Cell Sci*. 2009; 122:1834–1841. [PubMed: 19461073]
- Stenmark H. Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol*. 2009; 10:513–525. [PubMed: 19603039]
- Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science*. 1998; 282:754–759. [PubMed: 9784136]
- Su H, McClarty G, Dong F, Hatch GM, Pan ZK, Zhong G. Activation of Raf/MEK/ERK/cPLA2 Signaling Pathway Is Essential for Chlamydial Acquisition of Host Glycerophospholipids. *J Biol Chem*. 2004; 279:9409–9416. [PubMed: 14676189]
- Tse SM, Mason D, Botelho RJ, Chiu B, Reyland M, Hanada K, et al. Accumulation of diacylglycerol in the *Chlamydia* inclusion vacuole: possible role in the inhibition of host cell apoptosis. *The Journal of biological chemistry*. 2005; 280:25210–25215. [PubMed: 15863503]
- Valdivia RH. *Chlamydia* effector proteins and new insights into chlamydial cellular microbiology. *Curr Opin Microbiol*. 2008; 11:53–59. [PubMed: 18299248]

- van Ooij C, van Ijzendoorn S, Nishijima M, Hanada K, Mostov K, Engel J. Host derived sphingolipids are required for the intracellular growth of *Chlamydia trachomatis*. *Cell Microbiol.* 2000; 2:627–638. [PubMed: 11207614]
- Wang Y, Kahane S, Cutcliffe LT, Skilton RJ, Lambden PR, Clarke IN. Development of a transformation system for *Chlamydia trachomatis*: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. *PLoS Pathog.* 2011; 7:e1002258. [PubMed: 21966270]
- Wolf K, Hackstadt T. Sphingomyelin trafficking in *Chlamydia pneumoniae*-infected cells. *Cell Microbiol.* 2001; 3:145–152. [PubMed: 11260137]
- Wylie JL, Hatch GM, McClarty G. Host cell phospholipids are trafficked to and then modified by *Chlamydia trachomatis*. *J Bacteriol.* 1997; 179:7233–7242. [PubMed: 9393685]
- Wyrick PB. *Chlamydia trachomatis* persistence in vitro: an overview. *J Infect Dis.* 2010; 201(Suppl 2):S88–95. [PubMed: 20470046]

Figure 1.

Chlamydiae Acquire Host-derived Lipids by Non-vesicular and Vesicular Trafficking from the ER, Golgi, MVBs, and Lipid Droplets. (1) *Chlamydia* induces Golgi fragmentation into ministacks that surround the inclusion in a process mediated by Rab6 and Rab11. *Chlamydiae* acquire SM and cholesterol by intercepting MVB and/or Golgi-derived exocytic vesicles where both lipids are incorporated into the inclusion and bacterial membranes. Trafficking of SM-containing vesicles from the Golgi is regulated by GBF1-dependent activation of Arf1, Rab14, and/or Fyn signaling from Inc microdomains. (2) Lipid droplets are targeted by Lda and translocated into the inclusion at IncA permissive sites. (3) CERT localizes to MCS formed between (a) the ER (via VAPs) and the Golgi (via PI4P), and (b) between the ER (via VAPs) and the inclusion (via IncD). Ceramide is transferred to the Golgi where it is converted to SM by SMS1/2 and SM is transported to the inclusion while ceramide transferred to the inclusion may serve as a signaling platform and/or may be converted to SM by SMS2. (4) Pools of PI4P are generated at the inclusion by OCRL and PI4KII α , which may recruit PI4P-binding proteins. (5) Activation of ERK and cPLA2 lead to modifications of host glycerophospholipids (PI and PC) that are taken up by the inclusion, modified by *Chlamydiae* enzymes, and incorporated into bacterial membrane.