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Review

Membrane contact sites between pathogen-containing compartments and host organelles☆

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ABSTRACT

Intracellular pathogens survive and replicate within specialised membrane-bound compartments that can be considered as pseudo-organelles. Using the obligate intracellular bacterium *Chlamydia* as an illustrative example, we consider the modes of lipid transport between pathogen-containing compartments and host organelles, including the formation of static membrane contact sites. We discuss how lipid scavenging can be mediated via the reprogramming of cellular transporters at these interfaces and describe recent data suggesting that pathogen effectors modulate the formation of specific membrane contacts. Further study of these emerging mechanisms is likely to yield new insights into the cell biology of lipid transport and organelle communication, which highlights potential new targets and strategies for future therapeutics.

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1. Introduction

Many medically important bacterial pathogens engage in complex interactions with cells of their eukaryotic hosts. This interplay is mediated by bacterial virulence effector proteins, which are delivered directly into the host cell via sophisticated molecular nanomachines that span the bacterial envelope [1]. These translocated effectors often mimic eukaryotic functions to subvert key cellular processes, thereby promoting bacterial survival and replication [2]. Understanding this pathogen–host interplay not only reveals new insights into the molecular basis of disease, but often illuminates new aspects of cell biology. In turn, effector activities can be exploited to probe physiological cellular events including signal transduction, cytoskeletal dynamics, lipid transport and organelle positioning (*e.g.* pathogenic *Escherichia coli*) [3].

After effector-driven entry into the host cell, intracellular bacterial pathogens replicate either in the host cytosol directly or reside within modified membrane-bound compartments. Whereas remaining in the cytosol affords easy access to nutrients, the bacteria are rendered vulnerable to rapid detection and consequent immune responses. Conversely, intra-vacuolar replication helps to avoid immune recognition but restricts access to essential nutrients [4]. Amongst these nutrients, lipids are central both to provide the biochemical precursors for bacterial metabolism and also to enable the physical expansion of the boundary membrane of pathogen-containing vacuoles.

Membrane contact is an essential requirement for the transfer of host lipids into pathogen-containing vacuoles. In cells, such contact can be transient, and is followed by the fusion of the two compartments enabling lipid mixing and content transfer [5]. Alternatively, static zones of close apposition termed membrane contact sites (MCSs) allow non-vesicular trafficking of small molecules via transfer proteins [*e.g.* 6]. Pathogens harness both these mechanisms to commandeer host lipid transport, as pathogen effectors can specifically target host membranes to control fusion or to initiate the formation of stable MCSs, which act as critical host–pathogen interfaces. In this review, we will consider the intracellular bacterial pathogen *Chlamydia* as an illustrative example.

The *Chlamydiae* are a genus of obligate intracellular bacteria that infect humans (*Chlamydia trachomatis* and *Chlamydia pneumoniae*), animals with the possibility of zoonoses (*Chlamydia psittaci*, *Chlamydia abortus*, *Chlamydia felis*), and can also exclusively cause veterinary infections (*Chlamydia pecorum*, *Chlamydia caviae*, *Chlamydia suis*, *Chlamydia muridarum*) [7]. In humans, *C. trachomatis* remains the leading bacterial agent of sexually transmitted disease worldwide [8], and ocular strains cause blinding trachoma, which is designated as a neglected tropical disease by the World Health Organisation [9]. The *Chlamydiae* adopt an unusual biphasic lifecycle. Extracellular metabolically inert yet highly infectious elementary bodies (EBs) trigger their own actin-dependent entry into a target cell and internalise into tight vacuoles that are rapidly segregated from the canonical endocytic pathway. These vacuoles then traffic to the microtubule organising centre in the perinuclear region of

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the cell, where they coalesce to form a single membrane-bound compartment termed an 'inclusion'. Subsequently, EBs differentiate to form larger reticulate bodies (RBs) that divide by binary fission within the inclusion until they re-differentiate into EBs and exit the cell by inclusion extrusion or induced lysis [10]. Multiple bacterial effectors are delivered into the inclusion membrane via a type III secretion system (T3SS) that promote inclusion biogenesis and pathogen replication [11]. Historically, the inclusion was considered to be an inert compartment, but more recent evidence suggests that it actively engages in complex crosstalk with the host secretory pathway and organelles, interactions essential for bacterial survival and replication. In the following sections we describe how the chlamydial inclusion scavenges host lipids via transient interaction and engulfment of lipid droplets and multivesicular bodies, through the hijack of secretory vesicular traffic by fragmenting the Golgi apparatus, and via the assembly of stable MCSs with the endoplasmic reticulum and potentially mitochondria. We also consider how this relates to the activities of other intracellular bacterial pathogens and protozoan parasites.

2. Lipid scavenging by transient contact and engulfment of host organelles

2.1. Lipid droplets

The interaction between the chlamydial inclusion and cellular lipid droplets (LDs) is probably the best mechanistically characterised of all organelle-inclusion interactions (Fig. 1). Initially using a yeast two-hybrid approach, Kumar and colleagues [12] showed that *C. trachomatis* encodes at least three secreted LD-associated proteins (Ldas) that are translocated into the host cytosol and interact with LDs, promoting

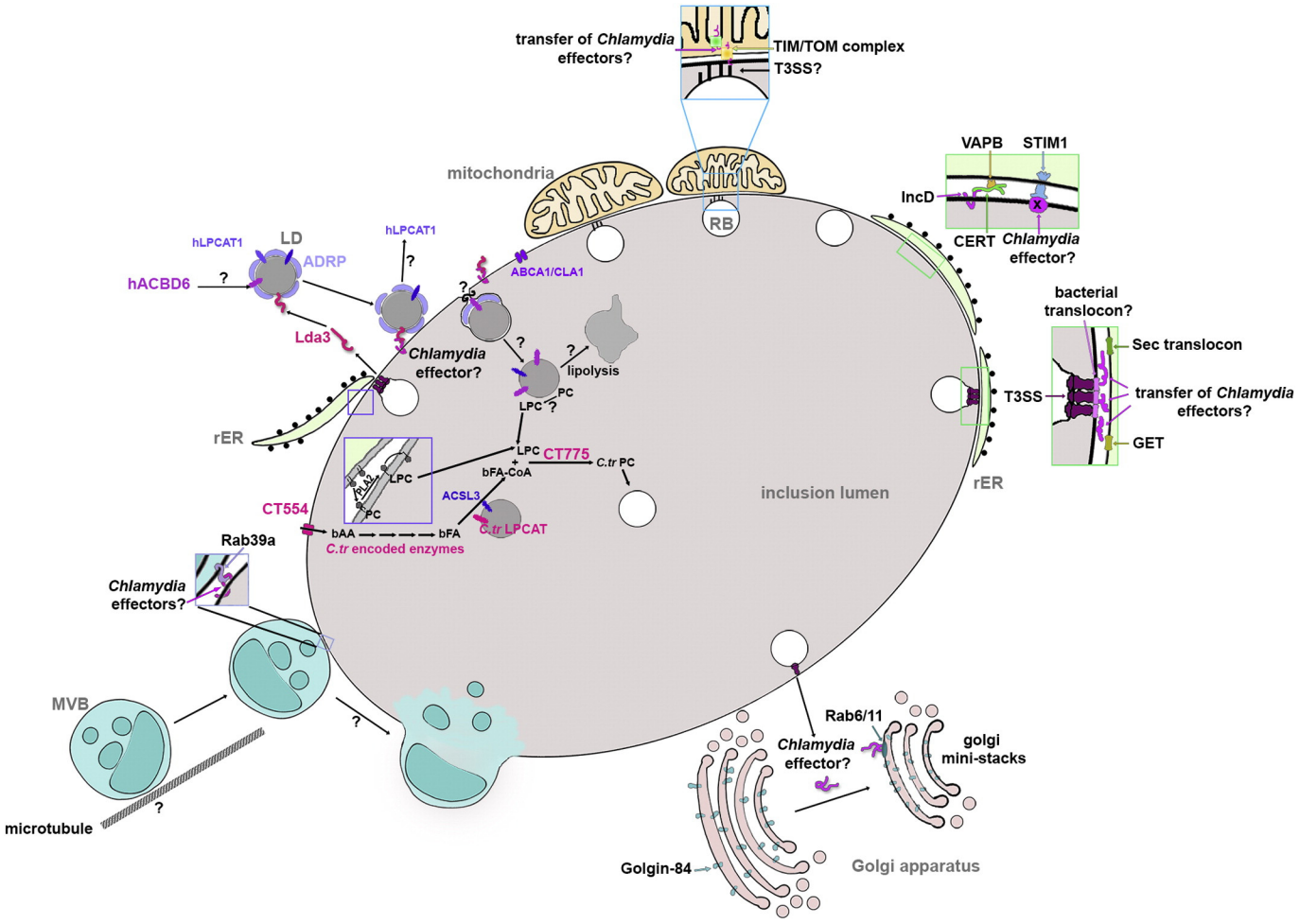

Fig. 1. Schematic of the *Chlamydia* inclusion in contact with host cell organelles. The chlamydial inclusion (grey) contains replicative reticulate bodies (RB) in contact with the inclusion membrane. Host organelles contact the cytosolic face of the inclusion membrane. *C. psitacci* or *C. caviae* inclusions form tight contacts with mitochondria (orange). Unidentified projections on the RBs face the inclusion membrane–mitochondria contact site. In *C .caviae*, the mitochondrial transporter inner/outer membrane (TIM/TOM) complex is important for the bacterial lifecycle. Interaction between the *Chlamydia* inclusion and lipid droplets (LDs, grey) is transient. The *Chlamydia* effector LD-associated protein 3 (Lda3) directs the transport of LDs to the inclusion. LDs then dock at the inclusion membrane, adipose differentiation-related protein (ADRP) is detached, and LDs translocated into the inclusion lumen by an unknown mechanism. *Chlamydia* mimics the Land's pathway using a combination of translocated host and secreted bacterial factors to synthesise a modified form of phosphatidylcholine (C.tr PC) in the inclusion lumen that can be incorporated into the RB membrane. For full detail, see reference 52. Multivesicular bodies (MVBs, blue) are recruited by *Chlamydia* using the cellular GTPase Rab39a, and fuse to allow the release of MVB contents into the inclusion lumen. An unknown *Chlamydia* effector triggers the cleavage of the golgin-84 (light blue), a protein resident in the Golgi apparatus (pink), in a Rab6/11 (dark blue ellipse) dependant manner. This leads to Golgi fragmentation and the contact of some mini-stacks with the inclusion. The rough endoplasmic reticulum (rER, green) contacts the inclusion and the ribosomes (black complexes) are excluded to the inclusion distal face. Chlamydial inclusion protein D (IncD) interacts with ceramide transfer protein (CERT), which binds vesicle-associated membrane protein-associated protein (VAPB) in the ER. The IncD/CERT/VAPB complex allows the selective transfer of host lipids at the inclusion membrane. Stromal interaction molecule (STIM1) is also recruited to the inclusion membrane. Pathogen synapses also form at distinct inclusion-rER contact sites. These synapses connect T3SSs in the RB envelope to the host rER via the inclusion membrane and could provide an interface for the delivery of hydrophobic chlamydial effectors into the host cell via the host Sec and GET machinery.

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their interaction with the inclusion membrane and subsequent retrograde translocation into the lumen [12]. Lda1 is involved in LD biogenesis, and inhibiting the interaction between the Ldas and LDs impaired chlamydial replication. Lda3 facilitates the translocation of LDs into the inclusion at sites enriched for the effector IncA, a bacterial mimic of eukaryotic SNARE proteins that integrates into the inclusion membrane [13]. Associated Lda3 displaces adipose differentiation-related protein (ADRP) that normally coats the LDs rendering them translocation-permissive [14]. However, how requisitioned LD components are accessed and metabolised by chlamydial RBs in the inclusion lumen still remains unknown. More indirect functions are also possible, as the interaction of LD with pathogen-containing phagosomes has been recognised as a broader response to infection via the stimulation of toll like receptors (TLRs) [15,16]. In this context, LD may be harnessed to retain inflammatory mediators thus prolonging the survival of the pathogen within host cells. Such interactions were also detected in neuronal biopsies and monocytes from patients infected with *Mycobacterium leprae*, as well as in macrophages from infected mice, confirming a similar interaction between LD and the mycobacterial phagosome *in vivo* [17]. *M.leprae* induces TLR2/TLR6-dependent LD biogenesis, and the resultant membrane contacts require phosphatidylinositol-3-kinase-dependent reorganisation of the actin cytoskeleton [18].

2.2. Multivesicular bodies

Although historically considered to be segregated from the endosomal pathway immediately after cell entry, Beatty (2006) demonstrated that multivesicular bodies (MVBs) are recruited to the chlamydial inclusion and that endosome-derived material is translocated into the inclusion lumen [19]. CD63, a general marker of late endosomes, is involved in the transport of MVBs to the inclusion but not for membrane contact between the inclusion and MVBs [20]. These longstanding enigmatic observations were recently reinforced by live imaging and immunogold electron microscopy, demonstrating that the small cellular GTPase Rab39a labelled a subset of late endocytic compartments, mainly MVBs, which migrated along microtubules to the surface of the inclusion [21]. Subsequent content mixing occurs, allowing the delivery of sphingolipids into the inclusion lumen. Although this process requires chlamydial viability, the effector(s) involved still remains unknown. This pathway would appear distinct from the mechanism of endo-lysosomal transport to the parasitophorous vacuole (PV) in *Toxoplasma gondii*-infected cells. The parasite recruits host microtubules, which allows the unidirectional transport of a subset of host endo-lysosomes to the PV. Invaginations of the PV membrane then act as conduits for the delivery of endo-lysosomal content into the PV lumen. These conduits are decorated by parasite proteins, including GRA7, which is sufficient to tubulate and constrict model membranes *in vitro* [22].

3. Sphingomyelin acquisition upon Golgi fragmentation

Chlamydiae induce the fragmentation of the host Golgi apparatus in infected cells [23]. To date, exactly how fragmentation enhances lipid acquisition is unclear in any system. The small GTPases Rab6 and Rab11 regulate Golgi fragmentation [24], and sphingolipids derived from the Golgi apparatus are intercepted by the inclusion in a Rab14-dependent manner [25]. Indeed, these Rab GTPases are enriched at specific sites on the cytoplasmic face of the inclusion membrane, coincident with the association of Golgi-derived vesicles. Interaction with the Golgi apparatus is not limited to bacterial pathogens, as *T. gondii* and *Neospora caninum* also recruit the Golgi apparatus to their respective PVs [26,27]. Although both PVs contact the host Golgi apparatus, only *Toxoplasma* induces efficient fragmentation, demonstrating that fragmentation is not a pre-requisite for the formation of membrane contacts between the Golgi and pathogen-containing vacuoles. There is also apparent mechanistic overlap with bacteria, as both *T. gondii* and *N. caninum* Golgi-PV interactions are mediated by Rab14, Rab30 and Rab43, with Rab30- and Rab43-positive vesicles present in the PV lumen [26,27].

Rather than fragmenting the Golgi, the emerging human pathogen *Anaplasma phagocytophilum* recruits Rab10 to its intracellular vacuole to redirect exocytic traffic from the trans-Golgi network (TGN). Rab10-positive TGN-derived vesicles not only associate with the *A. phagocytophilum* vacuole but are also translocated into the lumen, events critical for the generation of infectious progeny. This is consistent with bacterial incorporation of host sphingolipids, which are even retained when the bacteria subsequently exit the host cell [28].

4. Membrane contact sites

4.1. Static membrane contact sites

Early electron microscopy studies of mature *C. psittaci* inclusions revealed extensive contacts between the inclusion membrane and mitochondria that were apparently absent from *C. trachomatis* and *C. pneumoniae* inclusions [29]. Indeed, the isolation of *C. psittaci* inclusions allowed the specific co-fractionation of the inclusion with mitochondria and demonstrated that these were ordered contacts with a defined spacing [29]. The RBs facing the junctions exhibited cylindrical projections 10–13 nm in diameter that were apparently perforate the inclusion membrane at the interface with mitochondria [29]. The host and *C. psittaci* proteins that mediate these contacts between mitochondria and the inclusion membrane still remain unknown. Nevertheless, these *C. psittaci* platforms are reminiscent of those formed between the *Toxoplasma* parasitophorous vacuole (PV) and mitochondria [30]. Naturally occurring wild-type *T. gondii* type I and III strains are able to recruit mitochondria whereas type II is not. This enabled a subtractive approach to identify and characterise a mitochondrial-associated factor (MAF1) expressed by *Toxoplasma* responsible for mediating the PV-mitochondrial contacts [31]. The existence of species and strains of *Toxoplasma* and *Chlamydia* that are differentially able to associate with host mitochondria suggests this activity is not essential. However, these contacts may confer advantages in the colonisation of particular niches where access to specific metabolic or other mitochondrial functions is beneficial.

Studies of related *C. caviae* also revealed contacts between the mitochondria and the inclusion, but provided more functional insight, showing that the mitochondrial TIM–TOM complex (transporter inner/outer membrane) is involved in inclusion biogenesis and the generation of infectious bacterial progeny. It was proposed that these contacts might allow the transfer of bacterial effectors directly into mitochondria [32]. Intriguingly, this notion is supported by a recent screen of the *C. trachomatis* effector interactome that revealed multiple effectors potentially targeting the TIM–TOM complex [33]. However, these interactions and any extensive membrane contacts between the *C. trachomatis* inclusion and mitochondria are yet to be verified.

Although apparently non-essential and species specific, similar contacts might also enable certain intracellular pathogens to harness the energy resources of the host. The single cell protozoan parasite *Encephalitozoon cuniculi* that predominantly infects rabbits clusters the mitochondrial porin (VDAC) at points of contact between its replicative compartment and mitochondria to scavenge ATP directly from the host [34]. However, this is less likely in *Chlamydia*, which import ATP directly into the inclusion using dedicated mimics of ATP transporters [35].

Stable membrane contacts are not restricted to host mitochondria. Longstanding observations show that the intracellular vacuoles formed by the bacterial pathogens *Brucella* and *Legionella*, the causative agents of abortion in animals and Legionnaire's disease in humans respectively, transiently interact with endoplasmic reticulum (ER)-derived vesicles and ultimately alter the composition of their particular replicative phagosomes to closely resemble the ER of the host cell [36,37]. After entry *Brucella*-containing vacuoles (BCVs) undergo endocytic maturation to partially acquire phagolysosomal-like properties [38,39].

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Nascent BCVs interact with specialised ER exit sites (ERES) to establish sustained BCV-ERES MCSs where progressive interchange of ER and BCV membranes occurs [40]. *Legionella* employ a subtly different strategy whereby *Legionella*-containing vesicles (LCVs) fuse with ER-derived vesicles, ultimately transforming the LCVs into early secretory vesicles, which subsequently fuse with the ER [41–43].

Recent findings have revealed that the chlamydial inclusion maintains stable membrane contacts with the host rough ER (rER) [44]. rER tubules become tightly apposed to the cytoplasmic face of the inclusion membrane, patches of ER-derived membrane integrate into the inclusion membrane itself, and ER-derived material is translocated into the inclusion lumen, where calreticulin also associates with bacteria [45]. Indeed, the inclusion adopts sufficient rER-like character to render it susceptible to the action of aerolysin, a bacterial toxin from *Aeromonas hydrophila* that triggers specific vacuolation of the ER without disruption of the Golgi or other endocytic compartments. Treatment of infected cells with aerolysin early during infection stalls inclusion biogenesis, whereas later treatment disrupts inclusion integrity and impedes bacterial infectivity, demonstrating a critical role for rER interaction in inclusion development [45].

4.2. Lipid transfer at membrane contact sites

The inclusion establishes different classes of membrane contacts with the rER that allow the transfer of host lipids into the inclusion and bacterial effectors into the host cell via the T3SS [45]. Derré and colleagues [46] demonstrated that IncD, a chlamydial effector located in the inclusion membrane, interacts with the plextrin homology domain of the host ceramide transfer protein (CERT) [46]. In turn, the diphenylalanine FFAT motif of CERT binds ER-located VAPA/B, allowing the generation of an inclusion-ER membrane contact site that mediates direct transfer of lipids between the ER and the inclusion [46,47]. Stromal interaction molecule 1 (STIM1), an ER calcium (Ca^{2+}) sensor that endogenously relocates to ER-plasma membrane (PM) contact sites upon Ca^{2+} depletion co-localises with CERT and VAPB at the inclusion membrane throughout the chlamydial developmental cycle, whereas the PM Ca^{2+} channel Orai1 that usually interacts with STIM1 at ER-PM contact sites was not similarly recruited to the inclusion [48]. However, STIM1 depletion does not affect chlamydial growth [48]. The recruitment of additional factors like STIM1 to ER-inclusion contacts may indicate additional roles unrelated to lipid acquisition or suggests that STIM1 might be a bystander recruited to the inclusion with the functional CERT-VAPA/B complex [44].

Inclusion-rER contacts are also formed independently of CERT, which are enriched for host rER proteins including calreticulin, protein disulphide isomerase, and membrane-sculpting reticulons, a subset of which are additionally translocated into the inclusion lumen [45]. Electron tomography of RBs at the inclusion periphery revealed a polar array of T3SSs in contact with the luminal face of the inclusion membrane specifically formed at sites coincident with rER recruitment on the cytoplasmic face of the inclusion. These remarkable MCSs linking the rER in the host cytosol to the RB envelope through the inclusion membrane are termed 'pathogen synapses' [45]. Although the functions of pathogen synapses remain to be characterised in detail, the rER contains both the Sec translocon and the GET complex, responsible for the insertion of eukaryotic transmembrane proteins, which may be co-opted to facilitate T3SS effector delivery into the inclusion membrane [49]. Specialised inclusion-rER MCSs may therefore act as bidirectional portals facilitating the delivery of host lipids into the inclusion and the translocation of bacterial effectors into the inclusion membrane and beyond into the host cell [44,49].

4.3. Lipid transfer/acquisition without membrane contact

rER-located lipid transporters are recruited to the inclusion. The ER-localised acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT1) is recruited to the inclusion membrane and the functionally related Acyl-CoA synthetase (ACSL3) and Acyl-CoA binding protein (ACBD6) are translocated into the lumen [50]. Both the ER and LDs are a source of phosphatidylcholine in cells infected by *C. trachomatis*. *Chlamydia* mimics the Land's pathway to synthesise a modified phosphatidylcholine that is subsequently incorporated into the RB membrane. This results from the combined action of eukaryotic factors that are selectively translocated into the lumen and secreted bacterial enzymes [51]. Intriguingly, multiple components of the high-density lipoprotein biosynthesis machinery including the bi-directional phosphatidylcholine/cholesterol transporter (CLA1) and lipid efflux protein (ABCA1) are recruited to the inclusion, and their associated lipid acceptor ApoA-1 accumulates within the inclusion where it co-localises with pools of phosphatidylcholine [52,53]. These results indicate that *C. trachomatis* co-opts host phospholipid transporters normally used to assemble lipoproteins to acquire host phosphatidylcholine essential for growth. Whether LPCAT1 partitions with CERT or calreticulin at inclusion-rER MCSs remains to be determined, but the combined data suggest that multiple lipid transport platforms involving the ER exist at the inclusion membrane.

Stable MCSs between the rER and pathogen compartments are not restricted to *Chlamydia* or even intracellular bacteria. For example, the PV of *T. gondii* also forms tight junctions with the rER [54]. Although the parasite and host components of these PV-rER MCSs remain to be determined, these structures allow antigen presentation in dendritic cells, implying that active communication occurs between the parasite and host compartments [55].

5. Conclusion and outlook

The key roles of membrane contact sites between cellular organelles and membranes are now emerging. Many pathogens have evolved to occupy intracellular niches within modified membrane-bound compartments, which in some cases can be considered as pseudo-organelles. Recent observations suggest that pathogens can induce the *de novo* formation of novel classes of membrane contacts between pathogen-containing compartments and host organelles. In most cases, the mechanistic details of how this is achieved by pathogen effectors remain unknown. Studies of pathogenic mechanisms have revealed many insights into physiological cellular processes such as signal transduction, cytoskeletal reorganisation and cytokinesis, and so it is likely that further analysis of pathogen–host membrane contacts might yield similar advances as the detailed mechanisms emerge. Aside from identifying the pathogen effectors involved, many additional intriguing questions remain that will enhance our understanding of both host cell and pathogen biology. For example, how lipid droplets, multivesicular bodies, and organelle fragments are specifically translocated into the lumen of pathogen compartments. Although there are many reports of such translocation, care is still warranted as some of these apparent localisations might be due to fluorescence microscopy fixation artefacts [56]. However, live cell imaging, electron microscopy and alternative fixation techniques that better preserve cellular structures have reinforced the notion of luminal translocation in multiple recent studies [21,28,45]. It will also be important to probe the potential impact of pathogens on the function of pre-existing cellular membrane contacts, and whether the formation of pathogen–host contacts competes with or enhances membrane contact functions at sites distal from the pathogen-containing compartment itself. Understanding contacts between pathogen and host membranes could also reveal new avenues to the design of directed therapeutics and treatments.

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