Published in final edited form as:
Pathog Dis. 2013 November ; 69(2): . doi:10.1111/2049-632X.12057.

Host Organelle Hijackers: A similar *modus operandi* for *Toxoplasma gondii* and *Chlamydia trachomatis* - Co-infection model as a tool to investigate pathogenesis -

Julia D. Romano and **Isabelle Coppens***
Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA

Abstract

The bacterium *Chlamydia trachomatis* and the protozoan parasite *Toxoplasma gondii* are the causative agents of chlamydiosis and toxoplasmosis in humans, respectively. Both micro-organisms are obligate intracellular pathogens and notorious for extensively modifying the cytoskeletal architecture and the endomembrane system of their host cells to establish productive infections. This review highlights the similar tactics developed by these two pathogens to manipulate their host cell despite their genetic unrelatedness. By using an *in vitro* cell culture model whereby single fibroblasts are infected by *C. trachomatis* and *T. gondii* simultaneously, thus setting up an intracellular competition, we demonstrate that the solutions to the problem of intracellular survival deployed by the parasite and the bacterium may represent an example of convergent evolution, driven by the necessity to acquire nutrients in a hostile environment.

Introduction

Obligate intracellular pathogens represent a subset of consummate microbes typified by an overarching requirement for growth within an eukaryotic cell (reviewed in Casadevall, 2008; Kumar & Valdivia, 2009). Inherently to their intracellular residence and strategies to remain inside their host cells, these microbes induce damage to the host, either inadvertently or as a necessary condition for replication and transmission to a new host. As such, many obligate intracellular parasites, bacteria and viruses are indeed responsible for a significant negative impact on worldwide human health by causing debilitating or fatal disease. Among them, the apicomplexan parasite *Toxoplasma gondii* is one of the most successful parasites on earth; it is globally spread, affecting up to one-third of the human population. In healthy individuals, toxoplasmosis is usually asymptomatic and self-limiting, with the parasite remaining encysted during the whole lifetime of the host. However, *Toxoplasma* can cause severe and life-threatening disease (e.g., encephalitis, necrotizing retinochoroiditis, myocarditis) in immunocompromised individuals (Luft & Remington, 1992). In addition, *Toxoplasma* infection in pregnant women causes serious brain defects of the fetus (e.g., hydrocephalus, blindness). In the prokaryotic domain, *Chlamydia trachomatis* is widely distributed, affects ten percent of the human population and represents a major infectious cause of human genital (e.g., cervitis, urethritis) and eye disease (e.g., conjunctivitis, blindness) worldwide (Schachter, 1999). Table 1 summarizes commonalities and differences between *T. gondii* and *C. trachomatis*.

The luxury of an obligate intracellular life with its bountiful access to host resources and protection from immune confrontations comes, however, with a price. Survival within a host

*To whom all correspondence should be addressed, Tel. (443) 287-1589; Fax (410) 955-0105, icoppens@jhsph.edu.

cell requires physiological adaptations of the microbe to the host. In fact, the strategies of intracellular pathogens are constantly co-evolving with their respective eukaryotic hosts over hundreds of millions of years. While adaptation and specialization increase the microbe's fitness for interacting with the host, it can limit the ability of the microbe to thrive outside of a cell (Pallen & Wren, 2007). For instance, during evolution, the genomes of obligate intracellular pathogens have undergone dynamic remodeling, including the massive loss of genes that encode for entire metabolic pathways (Fraser-Liggett, 2005). As a part of their obligatory intracellular lifestyle, both *T. gondii* and *C. trachomatis* have undergone a massive condensation of their genome, compared to their non-parasitic relatives. The genome of *T. gondii* contains ~8,000 predicted genes while to the genome of Colpodellids, the free-living ancestors of *Toxoplasma* (Kuvardina *et al.*, 2002), harbors at least 40,000 genes. Likewise, the genome of *C. trachomatis* totals only ~900 genes compared to ~2,000 present in the genome of environmental *chlamydiae* (Horn *et al.*, 2004). For the two pathogens, loss of genes results in reduced central biosynthetic pathways. *T. gondii* and *C. trachomatis* possess only ~800 and ~200 genes coding for predicted metabolic enzymes respectively, compared to ~1,900 genes in humans (www.ToxoDB.org; Stephens *et al.*, 1998; Dean *et al.*, 2006). In return, genes promoting nutrient scavenging have expanded, and these genetic replacements have become indispensable for the pathogen to meet its basic metabolic requirements. Patho-adaptation to an obligatory lifestyle carries the danger that the loss of self, in the form of irreversible genome decay, may lead to extinction if the micro-organism narrows the cell types it invades and its subcellular replication niches; as it increases its reliance on host cell resources, the microbe ultimately links its own survival to the continued existence of its host (Andersson & Kurland, 1998). Thus, both *T. gondii* and *C. trachomatis* have become auxotrophic with maximal nutritional needs. As such, they have acquired redundant compensatory mechanisms to import nucleotides, sugars, amino acids, lipids, and several co-factors from the host cell. Proportionally to the number of genes, *T. gondii* contains three times more genes with similarity to known transporters or proteins with transporter-like properties than humans (820 transporter genes/8,000 total protein-coding genes in *T. gondii* vs. 1,000 transporter genes/30,000 total protein-coding genes in *H. sapiens*). Similarly, *C. trachomatis* harbors an extensive collection of transporters (summarized in Saka & Valdivia, 2010).

To access pools of host-derived nutrients, obligate intracellular pathogens must sculpt the intracellular environment of the host to their advantage. This situation is particularly challenging for pathogens that reside within a membrane-bound compartment in their host cell, which separates them from pools of soluble molecules present in the host cytosol and organelles. Despite this restriction, intravacuolar pathogens, such as *T. gondii* and *C. trachomatis*, covertly co-opt host resources to gain access to the needed nutrients by attracting host organelles to their vacuole or redirecting host transport vesicles (reviewed in Laliberté & Carruthers, 2008; Saka & Valdivia, 2010). Remarkably, *T. gondii* and *C. trachomatis* not only share a patho-adapted genome, but also operate in a similar way to manipulate the host cell and hijack organelles for their own benefit. Despite differences in their phylogenetic origin and their physico-chemical properties of their vacuolar compartments, both pathogens subvert host cytoskeleton elements, hijack the microtubule-organizing center (MTOC), attract endocytic and exocytic organelles, re-route Rab GTPases vesicle to their vacuole and engulf cytoplasmic organelles into their vacuoles (Table 1). In the first part of this review, we will summarize the extensive interactions that occur between host cell structures and the parasitophorous vacuole (PV) of *Toxoplasma* or the vacuole of *C. trachomatis*, termed the inclusion, and highlight the similar subversive tactics used by the two pathogens. In Figures 1 and 2, we illustrate the host cell interactions mediated by *T. gondii* and *C. trachomatis*, respectively. To evaluate the important contribution of host organelles to the intracellular development of *T. gondii* and *C. trachomatis*, and therefore the necessity for these pathogens to recruit host organelles to their vacuoles, we have

established an *in vitro* cell culture model whereby single fibroblasts are infected by the two pathogens simultaneously (Romano *et al.*, 2012; 2013a). In a co-infection system, the success and failure of an infection established by a pathogen depends on the skills of that pathogen to adhere to its normal developmental program. Since the parasite and the bacterium usurp the same host organelles, their presence in the same cell would lead to a severe competition for nutrients. In the second part of the review, we will collate our studies using the co-infection model and emphasize the unique opportunities provided by the dual infection system to evaluate the (in)compatibility of *T. gondii* and *C. trachomatis* and to gain fundamental knowledge on each of these pathogens. New aspects on the intracellular parasitism of *C. trachomatis* and *T. gondii* revealed by our co-infection assays will be discussed.

Part I: Commonalities between *Toxoplasma gondii* and *Chlamydia trachomatis* in Host Cell Interactions and Nutrient Acquisition

Host cytoskeleton reorganization and MTOC capture

The initial phases of invasion by *T. gondii* and *C. trachomatis* differ completely from one another but both result in the formation of a specialized pathogen-containing vacuole whose the limiting membrane is derived from the host plasma membrane. *Toxoplasma* can invade any nucleated mammalian cell, irrespective of the cell type or host origin (Sibley, 2003). The parasite penetrates into mammalian cells in a rapid process called active invasion lasting about one minute (Carruthers & Boothroyd, 2007). Upon contact with the host cell surface, the parasite secretes proteins that induce the formation of a host cell-parasite junction (Aikawa *et al.*, 1978; Besteiro *et al.*, 2011) that mediates the contact between the parasite and the host cell plasma membranes. This structure is pulled toward the posterior end of the parasite as the latter invades the cell and forms the PV. As it forms its vacuole, the parasite excludes most host plasma membrane transmembrane proteins from the PV membrane and incorporates its own proteins into this membrane, creating a compartment unrecognizable by host endo-lysosomal organelles (Mordue, 1999; Charron and Sibley, 2004; Cesbron-Delauw *et al* 2008; Nam, 2009). In contrast, *C. trachomatis* invades predominantly epithelial cells and macrophages. The invasive form of *C. trachomatis* (or the elementary body, EB) is internalized into a phagosomal compartment in mammalian cells within ~10 min, in a process driven by both the host cell and the bacterium (Dautry-Varsat *et al* 2005). Comparatively, the bacterium disengages its inclusion from host degradative pathways by eliminating host proteins from the inclusion membrane and adding chlamydial proteins (reviewed in Fields and Hackstadt, 2002).

Regardless of the mechanism of microbial entry, the cortical host cell cytoskeleton, especially actin filaments, represents a physical and mechanical barrier for the entry of any intruder. At their site of entry, *T. gondii* and *C. trachomatis* actively induces a local re-organization of the cortical actin network to facilitate their penetration though using distinct mechanisms and specific effectors (Gonzalez *et al.*, 2009; Carabeo *et al.*, 2002). Upon invasion, *Toxoplasma* secretes a protein, toxofilin, from anterior organelles termed rhoptries, into the host cytoplasm at the entry site. Toxofilin binds and severs host actin filaments, thus aiding the parasite's penetration into the host cell (Delorme-Walker *et al.*, 2012). Afterwards, the parasite induces the formation of a stable ring-shaped F-actin structure at the parasite-cell junction, and recruits the Arp2/3 complex, an actin-nucleating factor essential for the formation of many F-actin-based structures, to the ring structure to provide a solid anchor for pulling the parasite inside the cell (Gonzalez *et al.*, 2009). As opposed to the *T. gondii* situation, *de novo* polymerization of host actin is important for the entry of *C. trachomatis*. Within minutes upon attachment to the host cell, the bacterium transiently recruits actin to the site of invasion leading to the formation of an actin-rich

pedestal underneath the attachment site. Chlamydial and host cell actin nucleators cooperate to increase the rate of actin filament formation for *C. trachomatis* internalization. Using a type III secretion system, *C. trachomatis* injects into the host cytoplasm a translocated actin recruiting phosphoprotein (Tarp) that, upon phosphorylation by the Abelson kinase (Abl kinase), associates with globular actin to nucleate the formation of linear actin filaments (Jewett *et al.*, 2006; Elwell *et al.*, 2008; Jiwani *et al.*, 2012; 2013). Phosphorylated Tarp is implicated in the GTPase mediated activation of the host cell Arp2/3 complex (Lane *et al.*, 2008). Binding of *C. trachomatis* to the host platelet Derived Growth Factor Receptor β (PDGFRβ) at the cell surface results in the activation of both PDGFRβ and Abl kinase signaling pathways, leading to the phosphorylation of VAv2, a Rac guanine nucleotide exchange factor, and the actin nucleators WAVE2 and cortactin, which are two activators of the Arp2/3 complex (Elwell *et al.*, 2008).

The replicative cycle of *C. trachomatis* is initiated by the conversion of the EBs to reticulate bodies (RBs) that undergo successive binary fissions. During replication, the bacterium continues to remodel the host actin cytoskeleton and recruits intermediate filaments (Kumar and Valdivia, 2008a; 2008b). It encases its inclusion in a network of F-actin and intermediate filaments that act cooperatively to stabilize the pathogen-containing vacuole. Recruitment of F-actin at the inclusion is dependent on host RhoA, a regulator of the actin cytoskeleton that forms stress fibers. Disruption of RhoA leads to the loss of inclusion integrity, resulting in the leakage of the inclusion's content into the host cytosol, and consequently the activation of cytoplasmic innate immune surveillance pathways (Buchholz and Stephens, 2008). *Chlamydia* also specifically modifies vimentin filaments around the inclusion by secreting the chlamydial proteasome like activity factor (CPAF). This protease cleaves the head domain of vimentin, which is essential for filament assembly, thus allowing for the expansion of the inclusion through the removal of the physical constraints imposed by these static cytoskeletal structures on the growing inclusion. Unlike Chlamydia, there is no evidence to date that *Toxoplasma* remodels the host actin cytoskeleton post-invasion but the parasite does rearrange vimentin filaments around its PV (Halonen and Weidner, 1994). As with the chlamydial inclusion, the intermediate filament network may serve to dock the PV to the host cell nuclear surface as its disruption results in displacement of the vacuole away from the host perinuclear region.

In addition to actin and intermediate filaments, host microtubules are largely exploited by *Toxoplasma* and *C. trachomatis* to penetrate into their host cell but the pathogens operate differently in accordance to their distinctive modes of invasion. Pharmacological disruption of the host microtubular network significantly increases the time before the parasite initiates invasion (Sweenet *et al.*, 2010). Selectively concentrated on one side of the moving junction, host microtubules may help in stabilizing the site of parasite invasion, providing support to help withstand the compressive force provoked by *Toxoplasma* contacting the host cell. Once internalized, the parasite migrates to the host nucleus by an unknown mechanism. Concerning *C. trachomatis*, host microtubules are required for the phagocytosis of the bacterium into cells, the coalescence of vesicles containing chlamydiae EBs and the movement of the inclusion to the perinuclear region (Ward and Murray, 1984; Clausen *et al.*, 1997; Grieshaber *et al.*, 2003). The inclusion travels toward the host nucleus along microtubules by recruiting the microtubule-based motor dynein and kinesin II, and some components of the dynactin complex, which are required for the activity of dynein. Inhibitors of microtubules and dynein interfere with both chlamydial invasion and intracellular locomotion (Clausen *et al.*, 1997). At the perinuclear region, the PV and the chlamydial inclusion are positioned at the center of the microtubular network and remain surrounded by host microtubules throughout infection (Coppens *et al.*, 2006; Walker *et al.*, 2008; Clausen *et al.*, 1997). In addition, the parasite nucleates host microtubule growth via γ-tubulin-associated sites, which suggests a physical interaction between the PV membrane

and host microtubules (Walker *et al.*, 2008). In conclusion, due the unique mode of invasion of *T. gondii and C. trachomatis*, the host cytoskeletal components recruited by these pathogens and the microbial effectors used for the reorganization of cellular architecture are different for the parasite and the bacterium. However, what remains intriguing and unique to these two pathogens is the encasement of the PV and inclusion by both host microtubules and vimentin throughout replication.

Both pathogens actively hijack the host MTOC (or centrosome) by detaching it from the nuclear envelope and relocating the MTOC to their vacuole. Evidence for MTOC-PV association in *Toxoplasma*-infected cells is supported by the detection of centrosomal material at the PV surface, including pericentriolar matrix proteins and components of the γ-tubulin ring complex, which are critical for microtubule nucleation (Coppens *et al.*, 2006; Walker *et al.*, 2008; Wang *et al.*, 2010; Romano *et al.*, 2013b). Similarly, the host centrosomal foci associated with the chlamydial inclusion contain both centriolar and pericentriolar matrix proteins (Johnson *et al.*, 2009). Host centrosome positioning at the *Toxoplasma* PV requires the function of the host mammalian target of rapamycin complex 2 (mTORC2), which activates the Akt signaling pathway (Wang *et al.*, 2010). In mTORC2-deficient cells infected with the parasite, the host centrosome-PV association is abolished and the microtubules display an altered distribution. In addition, treatment of infected cells with an Akt inhibitor interferes with the interaction between the PV and host centrosomes. The Akt signaling pathway plays a pivotal role in growth factor regulation of microtubule stability, resulting in the phosphorylation of the glycogen synthase kinase 3β (GSK3β), which is a master regulator of the microtubule cytoskeleton (Kumar *et al.*, 2008). Moreover, inhibition of GSK3β restores the host centrosome-PV association in infected cells treated with an Akt inhibitor and in mTORC2 deficient cells. Inhibition of GSK3β in untreated cells increases the association of the host centrosomes and the PV. The chlamydial inclusion also maintains a tight association with host centrosomes. While this association is dependent on host dynein (Grieshaber *et al.*, 2006), chlamydial *de novo* transcription and translation is required to promote host MTOC-inclusion association, suggesting that chlamydial proteins at the inclusion membrane mediate this interaction. *In silico* predictions reveal that *C. trachomatis* may express up to ~50 inclusion membrane proteins (Incs). Furthermore, subdomains on the inclusion membrane that are enriched in cholesterol, active host Src-family kinases and four Inc proteins have been shown to associate with host centrosomes (Mital *et al.*, 2010). One *C. trachomatis* Inc protein, Inc850, even co-localizes with host centrosomes when ectopically expressed in uninfected cells (Mital *et al.*, 2010).

At the cellular level, controlling MTOC functions may allow intravacuolar pathogens to perturb the host cell cycle by creating centrosomal defects and/or disorganizing mitosis. By stalling host cell division prior to cytokinesis, these pathogens ensure for themselves a stable and spacious environment offered by a multinucleated cell (Walker *et al.*, 2008;Brown *et al* 2012). To this point, infection of quiescent cells with *T. gondii* induces an increase in D and E cyclins, thus promoting progression through G1 and transition into S-phase, respectively (Molestina *et al.*, 2008). *C. trachomatis* also modifies the host cell cycle but by targeting different cellular pathways, which include the dysregulation of the G2/M transition by cleavage of mitotic cyclin B1 and mitotic arrest via the destruction of microtubular networks by the chlamydial type III secreted effector CopN (Johnson *et al.*, 2009; Knowlton et al., 2011; Archuleta *et al.*, 2011; Brown *et al.*, 2012). Therefore, in both *Toxoplasma*- and *Chlamydia*-infected cells, there is not only an induction of entry into S-phase triggered by the pathogens but also an arrest in cell cycle progression in the S/G2 transition, based on similar host cyclin expression levels and accumulation in the infected cells. More generally, this phenotype may be viewed as a vantage point of evolution for pathogens sequestered within a membrane-bound compartment as, contrarily, pathogens residing freely in the host

cell cytoplasm, e.g., *Theileria* parasites are more prone to trigger the proliferation of the infected cell by interacting with the host mitotic machinery (Dobbelaere and Küenzi, 2004).

During *Chlamydia* infection, centrosomal abnormalities such as chromosomal segregation defects and supernumerary chromosomes that result from the dysregulation of centrosome duplication, are frequently observed (Grieshaber *et al.*, 2006; Johnson *et al.*, 2009). These dysfunctional centrosomes may be a factor in the epidemiological links between chlamydial infection and certain cancers (Koskela *et al.*, 2000; Anttila *et al.*, 2001; Wallin *et al.*, 2002; Smith *et al.*, 2004). Several studies report that *Toxoplasma* is also a possible oncogenic pathogen in humans, especially in the brain where the parasite resides (Wrensch *et al.*, 1993; Ryan *et al.*, 1993; Thomas *et al.*, 2012). The underlying molecular mechanism of *Toxoplasma*- mediated brain carcinogenesis is not clearly understood but seems to be linked to a dysregulation in the host miRNA processing pathway by parasite effectors, which in turn promotes host cell survival (Thirugnanam *et al.*, 2013). However, no information exists about a connection between host MTOC defects in *Toxoplasma*-infected brain cells and brain tumors. Finally, controlling MTOC functions may also permit intravacuolar pathogens to regulate the movement of host organelles and attract them to their vacuoles. Located at the intersection of the exocytic and endocytic pathway, the MTOC-Golgi region of the cell is rich in both endosomes and lysosomes. Therefore, the positioning of the PV and inclusion in the peri-Golgi/MTOC region of the cell could facilitate the interception of host vesicular trafficking, which may satisfy the pathogens' requirements for essential nutrients.

Host exocytic pathway diversion

Two main organelles involved in the exocytic pathway comprised the ER and Golgi apparatus. The ER plays a central role in a number of cellular pathways: the synthesis of phospholipids, cholesterol and ceramides; the trafficking of proteins destined for secretion or transport to other organelles; the glycosylation of proteins via the addition of *N*-linked oligosaccharides or GPI anchors; and the processing and presentation of antigens via MHC class I. The Golgi is a major site of carbohydrate, glycolipid and sphingomyelin synthesis; the *O*-glycosylation and sorting of proteins; and the packaging of glycoproteins for delivery to other organelles. Both *Toxoplasma* and *C. trachomatis* have developed strategies to recruit ER and Golgi elements, and intercept the trafficking of host exocytic vesicles.

A- Endoplasmic reticulum—Rapidly after penetration into the cell, *Toxoplasma* induces a dramatic change in the distribution of the host rough ER. This organelle becomes concentrated around the PV, and by 4 h post-infection, about 50% of the PV membrane is covered by host ER structures (de Melo *et al.*, 1992; Sinai *et al.*, 1997). In fact, the PV is immobilized in the host perinuclear region of the cell by the anchorage of the PV membrane to the nuclear envelope, which is contiguous with the cytoplasmic ER network (Romano *et al.*, 2008). These peri-vacuolar ER elements are closely apposed to the PV membrane (distance within ~20 nm) and ribosomes are restricted to the opposite face of the ER away from the PV. The retention of the ER at the PV has been proposed to be mediated by two parasite proteins that are anchored to the PV membrane: ROP2, which contains ER-targeting domains exposed to the host cytosol and GRA3, which interacts with the host ER type II transmembrane protein calcium modulating ligand (CAMLG) (Sinai and Joiner, 2001; Kim *et al.*, 2008). By comparison, the interaction of the inclusion of *C. trachomatis* with the host ER is more intimate than for the *Toxoplasma* PV as the inclusion membrane forms direct Membrane Contact Sites (MCSs or zones of close apposition < 50 nm) with ER elements (Derre *et al.*, 2011; Dumoux *et al.*, 2012). These MCSs are numerous and persist for long distances along the inclusion membrane. The needle structures of the type III secretion system extend from the bacterial surface and connect to the inclusion membrane at these host ER-inclusion MCSs (Dumoux *et al.*, 2012). Select host ER proteins are enriched, in

patches, on the inclusion membrane. Located at ER-Golgi MCSs in mammalian cells, the ceramide transfer protein (CERT) is responsible for the transfer of ceramides from the ER to the Golgi (Lebiedzinska *et al.*, 2009; Levine and Loewen, 2006). Interestingly, CERT is present at the host ER-inclusion MCSs (Derre *et al.*, 2011). CERT bridges the host ER via association with the ER vesicle-associated membrane protein-associated proteins (VAP-A/VAP-B) and the inclusion via binding to IncD through its pleckstrin domain. Finally, these contact sites may represent portals for the selective influx of ER material into the inclusion. Indeed, host CERT-containing vesicles have been observed within the chlamydial inclusion, suggesting that *C. trachomatis* is able to engulf portions of the ER (Dumoux *et al.*, 2012).

The host rough ER is essential for the biogenesis and integrity of the inclusion of *C. trachomatis* since disruption of ER architecture impedes chlamydial infectivity (Dumoux et al., 2012). In particular, host-derived sphingolipids play a crucial role in the replication of *C. trachomatis* as the bacterium does not grow in cells unable to produce sphingolipids (van Ooij *et al.*, 2000). The close apposition of ER tubules to the inclusion membrane may represent a dynamic environment specialized in non-vesicular trafficking of lipids such as ceramides, leading to metabolism and signaling events that ensure proper bacterial development. Of interest, host CERT is required for bacterial replication and sphingolipid acquisition (Elwell *et al.*, 2011). Similarly to *Chlamydia* infection, a possible function for associated ER to the PV would be to provide essential constituents for the parasite. To this point, *Toxoplasma* shows growth defect in cells impaired in ceramide biogenesis, suggesting the importance with host sphingolipid metabolism in the ER for the parasite (Romano *et al.*, 2013b). Moreover, *Toxoplasma* makes *N*-glycans from a mixture of the 14-sugar precursor (Glc3Man9GlcNAc2) scavenged from the host ER and its own 10-sugar precursor (Glc3Man5GlcNAc2) (Bushkin *et al.*, 2010) and incorporate them onto newly synthesized proteins (Garenaux *et al.*, 2008).

The close association of *Toxoplasma* and *C. trachomatis* with the host ER may also be exploited by the pathogens to modify the host cell's presentation of their antigens. In the case of *Toxoplasma*, the ER association may facilitate the presentation of parasite antigens at the host plasma membrane (Goldsmith et al., 2009), thereby eliciting a CD8+ T cell response and the secretion of INF-γ, which plays a role in the persistence of the parasite in the host. A molecular exchange between the host ER and the PV has been documented (Goldsmith *et al.*, 2009), suggesting that host ER may be the export route of *Toxoplasma*-derived antigens into the cytosol via ERAD translocon subunit Sec61 before processing by the proteasome. Antigenic peptides produced by proteolysis in the cytoplasm are then transported back into the ER via TAP and further trimmed by the aminopeptidase ERAAP to the appropriate length for presentation by MHC class I molecules (Blanchard *et al.*, 2008). The host ER is also a destination for chlamydial antigens, particularly the Major Outer Membrane Protein (MOMP), lipopolysaccharide (LPS) and IncA (Giles and Wyrick, 2008). These antigens are presented via MHC class I molecules and stimulate a CD8+ T cell response and IFN-γ secretion (Gervassi et al., 2003). Three possible scenarios for the release of chlamydial antigens from the inclusion have been proposed: i) IncA-laden fibers that are derived from the inclusion membrane and extend into the host cytosol (Brown *et al.*, 2002); ii) the direct injection of LPS, MOMP and IncA into the host cytosol via the type III secretion machinery (Fields *et al.*, 2005); and iii) vesicles derived from the inclusion membrane that contain LPS, MOMP and IncA that either protrude into the host cell or are detached from the inclusion (Giles *et al.*, 2008). These latter vesicles may then fuse with the ER via the SNARE-like fusion properties of IncA, thereby delivering antigens directly into the ER for processing and presentation.

B- The Golgi apparatus—The morphology of the host Golgi apparatus is dramatically altered in cells infected either with *Toxoplasma* or *C. trachomatis*. Astonishingly, the Golgi

alterations look very similar in both infections as the organelle is fragmented and sliced into functional mini-stacks that encircle the PV or the inclusion (Heuer *et al.*, 2009; Romano *et al.*, 2013b). In the case of a *C. trachomatis* infection, host matrix protein golgin84 is cleaved and this cleavage is responsible for the destabilization of the Golgi structure (Heuer *et al.*, 2009). A secreted protease of bacterial origin has been proposed to be involved in the cleavage of host golgin84 but the nature of the protease is controversial (Christian *et al.*, 2011; Chen *et al.*, 2012). The process of Golgi fragmentation during a *Toxoplasma* infection, however, seems to involve a different process since no cleavage of host golgins is detected (Romano *et al.*, 2013b).

As observed with the recruitment of host ER, the closeness of the pathogens to host Golgi elements may facilitate the scavenging of nutrients, e.g., sphingolipids from this organelle. As a case in point, *C. trachomatis* recruits two host Golgi enzymes implicated in sphingomyelin synthesis, the sphingomyelin synthase 1 and 2 (SMS1 and SMS2), to distinct compartments of the inclusion to produce its own sphingomyelin using host enzymes. Although *Toxoplasma* is capable of *de novo* sphingolipid synthesis (Azzouz *et al.*, 2002; Sonda *et al.*, 2005; Bisanz *et al.*, 2006), its growth also relies on host sources of sphingolipids as exogenously added ceramides that are processed in the Golgi, enhance parasite replication (Romano *et al.*, 2013b). Additionally, the destabilization and dispersion of the Golgi structure in *Toxoplasma*- and *C. trachomatis*- infected cells may be related to the interception of host Golgi-derived vesicles by these pathogens. Indeed, *C. trachomatis* has developed a vesicular-mediated access of ceramides to its inclusion (Hackstadt *et al.*, 1995; 1996) involving the Golgi-specific brefeldin A resistance guanine nucleotide exchange factor 1 (GBF1), which is required for the assembly and maintenance of the Golgi stacks (Elwell *et al.*, 2011). The bacterium selectively co-opts GBF1 within the *cis*-Golgi compartment for vesicle-mediated sphingolipid acquisition. Moreover, treatment of infected cells with brefeldin A that inhibits GBF1, depletion of host GBF1 or silencing of host golgin84 to prevent Golgi fragmentation lead to a decrease in sphingolipid scavenging and the formation of smaller inclusions (Hackstadt *et al.*, 1995; Heuer *et al.*, 2009; Elwell *et al.*, 2011). Unlike chlamydial infection in epithelial cells where Golgi stacks are recruited by the pathogen, the efficiency of the inclusions to capture Golgi-derived vesicles is reduced significantly in macrophages, and parallels a reduction in the replication rate of the bacterium in macrophages, which underscores the importance of this process for the bacterium development (Sun *et al.*, 2012). It has been proposed that the two trafficking paths for sphingolipid acquisition, molecular and vesicular, by *C. trachomatis* may operate simultaneously and contribute to distinct aspects of the developmental cycle of *Chlamydia*, the CERT pathway being important for bacterial replication while the GBF1 pathway may contribute to inclusion membrane integrity. Both *Toxoplasma* and *C. trachomatis* divert several host Golgi-derived vesicles to their vacuoles (Hackstadt *et al.*, 1996; Capmany and Damiani, 2010; Pokrovskaya *et al.*, 2012; Romano *et al.*, 2013b). Rab14- associated Golgi vesicles, which deliver sphingomyelin to the plasma membrane, are intercepted by the two pathogens. In addition, *Toxoplasma* scavenges sphingolipids from Rab30-, or Rab43-associated Golgi vesicles that accumulate within the PV and the parasite incorporates these lipids into its plasma membrane and organelles.

Host endocytic network exploitation

Both the PV and chlamydial inclusion are impervious to the host endosomal/lysosomal degradative pathway. Yet, host endocytic organelles, by virtue of their catabolytic functions, represent a rich source of nutrients derived from the external medium, and both *Toxoplasma* and *C. trachomatis* have developed strategies to take advantage of the content of these organelles while avoiding destruction. Both pathogens localize near the MTOC in the perinuclear region of the cell where endo-lysosomes accumulate around their vacuoles. The

host mTORC2-Akt signaling is exploited by *Toxoplasma* to maintain endo-lysosomes around the PV (Wang *et al.*, 2010). The parasite manipulates the host microtubular network to create invaginations of the PV membrane, and through these microtubule-based invaginations, host endo-lysosomes are retained within the PV (Coppens *et al.*, 2006). Mirroring the uptake of host Golgi-derived vesicles by the parasite, the sequestration of intact host endo-lysosomes into the PV lumen allows the parasite to acquire the needed nutrients by circumventing the fusion with host organelles. In case of *C. trachomatis*, host structures containing the transferrin receptor, a component of early endosomes, the cation-independent mannose-6-phosphate receptor (CI-M6PR) or LAMP-1 from late endosomes, and CD-63 and lysobisphosphatidic acid, two constituents of multivesicular bodies (MVB) gather around the inclusion. The bacterium can also inserts some of these proteins into the inclusion membrane and induce the fusion of host recycling endosomes with the inclusion (van Ooij *et al.*, 1997; Carabeo et al., 2003; Ouellette *et al.*, Beatty, 2006; 2008; Ouellette et al., 2011).

Interactions with the early and late endosomal compartments may provide a source of membrane or nutrients for the maintenance of a productive infection. *Toxoplasma* and *C. trachomatis* both acquire nutrients from the endo-lysosomal pathway. The parasite scavenges cholesterol from the host LDL receptor-mediated endocytic pathway, a process that is specifically increased in infected cells (Coppens *et al.*, 2000). Interference with LDL endocytosis, lysosomal degradation of LDL or cholesterol translocation across lysosomal membranes results in blockade of cholesterol delivery to the PV and significantly reduces parasite replication. More generally, the sequestration of host endo-lysosomal organelles within the PV membrane invaginations allows the acquisition of a diverse range of molecules supplied by the endocytic circuit to the parasite. Plasma transferrin, LDL-derived cholesterol and albumin are detected within the inclusion, indicating that exogenously added ligands can be transported to the lumen of the vacuole (van Ooij et al., 1997; Carabeo et al., 2003; Ouellette et al., 2011). Blockade of lysosomal acidification and functions impairs the growth of *C. trachomatis*. The inclusion also interacts with host MVBs, which are temporary storage compartments enriched in sphingolipids and cholesterol. Inhibition of MVB biogenesis leads to disruption of sphingolipid and cholesterol trafficking to the chlamydial inclusion, and therefore delays inclusion maturation (Beatty, 2006; 2008). Of interest, *Toxoplasma* and *Chlamydia* are not only taking advantage of the heterophagic properties of its host cell to gain access to components present in the environment, but they also exploit the nutritive function of host autophagic compartments (Wang *et al.*, 2008; Pachikara *et al.*, 2009; Ouellette *et al.*, 2011; Sun *et al.*, 2012). The parasite induces autophagy in the host cell by a mechanism dependent on calcium but independent of mTOR, and attracts autophagosomes to its PV to enhance the flow of autophagic degradative products to the vacuole. Disabling of the host cell autophagy process by deleting autophagy-like proteins such asAtg5 abrogates the ability of the parasite to maintain optimal proliferation under nutrient limitation conditions. These observations illustrate the parasite's ability to shift the balance from one source of nutrients (from heterophagy) to another one (from autophagy) in function of the anabolic resources of the host cell. *C. trachomatis* induces autophagy in the middle of its developmental cycle, in response to the declines in the nutrient pools of host cells. The bacterium attracts host autophagosomes to its inclusion but autophagosomes rarely fuse with the chlamydial inclusion. However, neither the augmentation nor blockade of host cell autophagic functions has a detectable effect on chlamydial infection, suggesting that unlike for *T. gondii*, host autophagic activities are dispensable for chlamydial growth.

Part II: Co-infection *Toxoplasma* and *Chlamydia trachomatis*

Relevance of co-infection models in vitro

For many decades, cells in culture have been infected with two different viruses, or with a viral and a nonviral pathogen, and these co-infection models *in vitro* have subserved fundamental discoveries such as the phenomena of recombination and phenotypic mixing, and the role of interferons in virus eradication. Cells dually infected with different prokaryotic and/or eukaryotic pathogens, however, have more rarely been reported (for examples, see Meirelles and De Souza, 1983; Black *et al.*, 1990; Heinzen *et al.*, 1996; Rabinovitch and Veras, 1996; de Chastellier *et al.*, 1999; Sinai *et al.*, 2000; Vanover *et al.*, 2008; Borel *et al.*, 2010; Real *et al.*, 2010; Romano *et al.*, 2012; 2013a). Although these instances represent artificially constructed *in vitro* model systems, dually infected cells with different nonviral pathogens may provide unique opportunities to evaluate the compatibility of two different pathogens during co-infection and to gain fundamental knowledge on each of the pathogens (Box 1). For example, a study on fibroblasts co-infected with *Toxoplasma* and the bacterium *Coxiella burnetti*, which flourishes within a mature phago-lysosomal compartment, highlights how refractory the parasite is to any interaction with the bacterium, despite the hyperactive fusion machinery associated with the *C. burnetti* vacuole (Sinai *et al.*, 2000). A parallel study where cells are co-infected with *C. trachomatis and C. burnetti* demonstrates that both bacteria remain segregated from one another (Heinzen *et al.*, 1996). In the *C. burnetti*-infected cells, *Toxoplasma* or *Chlamydia* replicate normally, suggesting that their intracellular survival depends on following their respective programs of infection, which are very different from that of *Coxiella*, to establish a permissive environment.

Cohabitation of Toxoplasma and C. trachomatis - an evolving battle to catch host organelles

For a *Toxoplasma* and *C. trachomatis* co-infection, a completely different scenario would be expected since both pathogens have similar requirements for their host cells to support their intracellular growth. The shared ability of the parasite and the bacterium to usurp some host organelles would cause these pathogens to ferociously compete for the same pool of nutrients. To verify the hypothesis of rivalry between these two pathogens and address the importance of the manipulation of host cell pathways for them, we have established a novel co-infection model in which human cells are simultaneously infected with *Toxoplasma* and *C. trachomatis*. Using this system, we have examined each pathogen's ability to exploit host cell resources and replicate (Romano *et al.*, 2012; 2013a).

Figure 3 illustrates the morphology of an inclusion developing either alone or in the presence of a PV (panel A) and presents our model regarding the association of host structures with the inclusion and PV during 24-h of co-infection (panel B). A summary of the findings is as follows. A single mammalian cell can harbor both *Chlamydia* and *Toxoplasma*. The parasite and the bacterium never share the same vacuolar compartment in the host cell, which is consistent with their distinct modes of cell entry and their dissimilar vacuolar membrane composition that precludes mutual recognition and fusion. Following invasion, the two pathogen-containing vacuoles migrate independently to the host perinuclear region. *Toxoplasma*, which invades mammalian cells more rapidly than *C. trachomatis*, arrives the first at the host nucleus, hijacks the MTOC and distributes centrosomal microtubules around its vacuole. The parasite associates with host ER elements and the nuclear envelope. When the bacterium arrives in the perinuclear region, it is still able to recruit host microtubules, which enfold the inclusion, even though it is distant from the MTOC. This highlights that the control of the MTOC is not essential for the bacterium's ability to recruit host microtubules. When *C. trachomatis* invades a cell before *Toxoplasma*, the host MTOC is systematically associated with the inclusion, suggesting that the targeting

of the MTOC is a programmed post-invasion event for both pathogens. In co-infected cells, the host Golgi is severely fragmented into small mini-stacks by the two pathogens. The parasite and the bacterium attract and share the Golgi mini-stacks. *C. trachomatis* but not *Toxoplasma*, recruits CERT, and both pathogens efficiently diverts host sphingolipids. The redundancy in sphingolipid salvage pathways of both pathogens averts the need for severe competition for these lipids. *Toxoplasma*, though, is able to more effectively scavenge other nutrients from the host cell and outcompetes *Chlamydia* for cholesterol, essential amino acids, and to a lesser extent iron.

Throughout co-infection, the parasite replicates normally, displaying a superior competitive fitness over the bacterium. Due to its poor ability to scavenge many host molecules in the presence of *Toxoplasma*, *C. trachomatis* shifts to a stress-induced persistent growth as a direct result from being barred from its normal nutrient supplies. Parasite killing engenders an ordered return to normal chlamydial development. Reintroduction of excess nutrients into the medium also results in a substantial recovery of chlamydial infectivity. Furthermore, co-infection of *C. trachomatis* and either slow-growing strains of *Toxoplasma* or a mutant impaired in nutrient acquisition, allows the bacterium to develop unhampered. Likewise, pre-infection of a cell with *Chlamydia* prior to *Toxoplasma* results in slower parasite growth than in a cell infected concurrently.

T. gondii and C. trachomatis: A case for convergent evolution?

Host-associated microbes, whether endosymbionts, commensals or pathogens, all require some adaptive capacity for intracellular life. There is no fossil record to estimate when some organisms have acquired the ability to survive inside other cells. Based on the endosymbiotic origin of some eukaryotic organelles (Margulis, 1971), the emergence of an intracellular lifestyle is likely ancient. Each attempt of microbes to adjust to an intracellular residence must have been subjected to many selection pressures that have contributed to various types of host-microbe relationships and to the development of diverse intracellular lifestyle strategies that are still constantly evolving to handle the stresses.

Similarities in intracellular pathogenic strategies between phylogenetically distant microbes suggest convergent evolution. Obligate intracellular pathogens, such as *T. gondii* and *C. trachomatis*, must evolve strategies to scavenge nutrients within a host cell and avoid host cytosolic assaults. Comparisons of these two pathogens uncover that both the parasite and the bacterium manifest similar traits in adapting to the interior of a mammalian cell, although their molecular and cellular mechanisms differ. Both microbes develop within vacuoles created from the host cell's plasma membrane, which is subsequently modified by the pathogens. Despite different invasion strategies, which influences the development of their vacuolar membranes, both *Toxoplasma* and *Chlamydia* gather host vimentin and microtubules around their vacuoles and retain these associations throughout infection. The two microbes have evolved the ability to hijack the host MTOC, possibly to control host vesicular trafficking and organelle placement and/or to interfere with the host cell cycle. Host organelles, such as the ER, Golgi and endocytic vesicles, associate with the PV and inclusion presumably enabling the scavening of nutrients like lipids from these organelles, or in the case of the ER, controling antigen presentation. In addition, the host Golgi is fragmented into mini-stacks during an infection with either pathogen though the molecular mechanism used is different. In sum, both have evolved similar (thought not identical) strategies to exploit same host cell organelles and scavenge nutrients thereof (Laliberté and Carruthers, 2008; Saka and Valdivia, 2010; Cocchiaro and Valdivia, 2009; Romano *et al.*, 2013b). Of note, these similarities are restricted to the subcellular level as *Chlamydia* and *Toxoplasma* infect different cell types in their mammalian hosts, which leads to different disease caused these pathogens.

Since the divergence of prokaryotes and protozoa is ancient (1–2 billion years ago) and antedated the emergence of mammals (400–500 million years ago), the selective pressure responsible for the similar adaptations to a mammalian cell of these phylogenetically unrelated microorganisms could not have been linked their pathogenicity in humans. As an alternative, it is tempting to hypothesize that a process of adaptive convergent evolution - possibly in response to interactions with hosts such as protozoan predators in the environment - may be at the origin of the similar survival strategies of nutrient scavenging developed by *T. gondii* and *C. trachomatis*.

Another intriguing case of convergent evolution between genetically unrelated pathogens is illustrated by the bacterium *Yersinia pestis* (human plague), the fungus *Cryptococcus neoformans* (cryptococcosis) and protozoan parasites from the *Leishmania mexicana* complex (cutaneous leishmaniasis) (Bliska and Casadevall, 2009; Antoine *et al.*, 1998). These microorganisms are facultative intracellular pathogens. Inside their human hosts, *Y. pestis*, *C. neoformans* and *L. mexicana* parasites are taken up by macrophages wherein they can survive and replicate in a very large phagolysosome. To circumvent the cytocidal response of macrophages, these three different pathogens alter the intravacuolar environment of their phagolysosomes using analogous mechanisms: they interfere with phagosomal microbicidal activities by diluting lysosomal contents. The mechanism of spacious phagosome formation by *Y. pestis* has not been identified, but unidirectional fusion of endocytic compartments with the phagosome seems to occur (Grabenstein *et al.*, 2006). *C. neoformans* secretes in the host cytoplasm capsular polysaccharide-containing vesicles that subsequently modify the membrane properties of phagosomes and promote their homotypic fusion, resulting into giant phagosomes sheltering the fungus (Alvarez and Casadevall, 2006). *L. mexicana* parasites expand their compartments by secreting proteophosphoglycans in the phagolysosomal lumen. Due to their polyanionic properties, these specific glycans induce the vacuolization of the phagosomes (Peters *et al.*, 1997). Additionally, these three pathogens are protected by unique surface structures: *Y. pestis* has a modified coat of lipopolysaccharides, *C. neoformans*, a polysaccharide capsule, and *L. mexicana* parasites, high levels of glycosphingolipids (Bliska and Casadevall, 2009; McConville & Ralton, 1997). It could be interesting to analyze the outcome of the co-infection of a macrophage with the bacterium, the fungus and the protozoan. It is tempting to hypothesize that during a co-infection, the growth of *Y. pestis*, *C. neoformans* and *L. mexicana* parasites may be enhanced by some sort of cooperativity between themselves in coping with the defenses of their host cells. Altogether, these examples emphasize common adaptive strategies, such as the neutralization of lysosomal acidic contents by dilution, developed by intracellular pathogens that arise in distant evolutionary branches.

Concluding remarks

By comparing the program of infectivity of *T. gondii* and *C. trachomatis*, this review explores the strategies of these two intracellular pathogens for manipulating the host at a cellular level. First, the key role of cellular remodeling and host organelle interception for *Toxoplasma* and *Chlamydia* to gain access to host intracellular resources was highlighted. Second, a pathogen's strict dependence on its ability to successfully adhere to a finely tuned developmental program to achieve productive infection was demonstrated. For instance, *Toxoplasma* and *Chlamydia* tend to conform to their respective intracellular developmental program regardless of the presence of the other organism within the cell. The normal growth of each pathogen (i.e., the production of infectious progeny) is highly dependent on the pathogen's ability to maintain a threshold level of interaction between its vacuole and host cell organelles. Third, despite the uniqueness of each host-microbe interaction, there are relatively few solutions to the problem of intracellular entry, survival, and escape. For phylogenetically distant microbial species, similarities in intracellular pathogenic strategies

are probably most easily explained by convergent evolution. For *Toxoplasma* and *Chlamydia*, the most fascinating example is certainly the hijacking of the host Golgi, which is further reorganized into mini-stacks that these pathogens similarly and conveniently align just outside their vacuole in the cell.

Acknowledgments

The authors are grateful to the members of the Coppens lab who provided comments during the course of these studies. We would like to thank Patrik P. Bavoil at the University of Maryland, our close collaborator who introduced us to the field of *Chlamydia* and intravacuolar bacteria. We thank Jose Carrasco at the University of Maryland for providing the seeds of *Chlamydia*. The work was supported by the grant from the NIH AI060767 to I.C.

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Box 1 - Some reasons to study cells co-infected with nonviral pathogens

- Capability of invasion, survival, multiplication, and/or differentiation
- Compositional and functional features of the intracellular vacuole of the pathogens
- Relative localization of one pathogen to the other one within the host cell
- Direct interactions between intracellular pathogens
- Indirect interactions between intracellular pathogens mediated by host cell factors
- Competition for host organelles and cell-derived nutrients
- Cooperation via exchange of substrates or growth factors
- Antagonism via toxins or antibiotics secreted by one of the pathogens
- Transfer or exchange of genetic elements between co-infection pathogens
- Changes in differential gene expression of pathogens in co-infected versus singly infected cells
- Induction or repression of host cell transduction cascades
- Induction or repression of host protective/inhibitory cytokines
- Induction or repression of host microbicidal/protective mechanisms
- Modulation of pathogen antigen expression

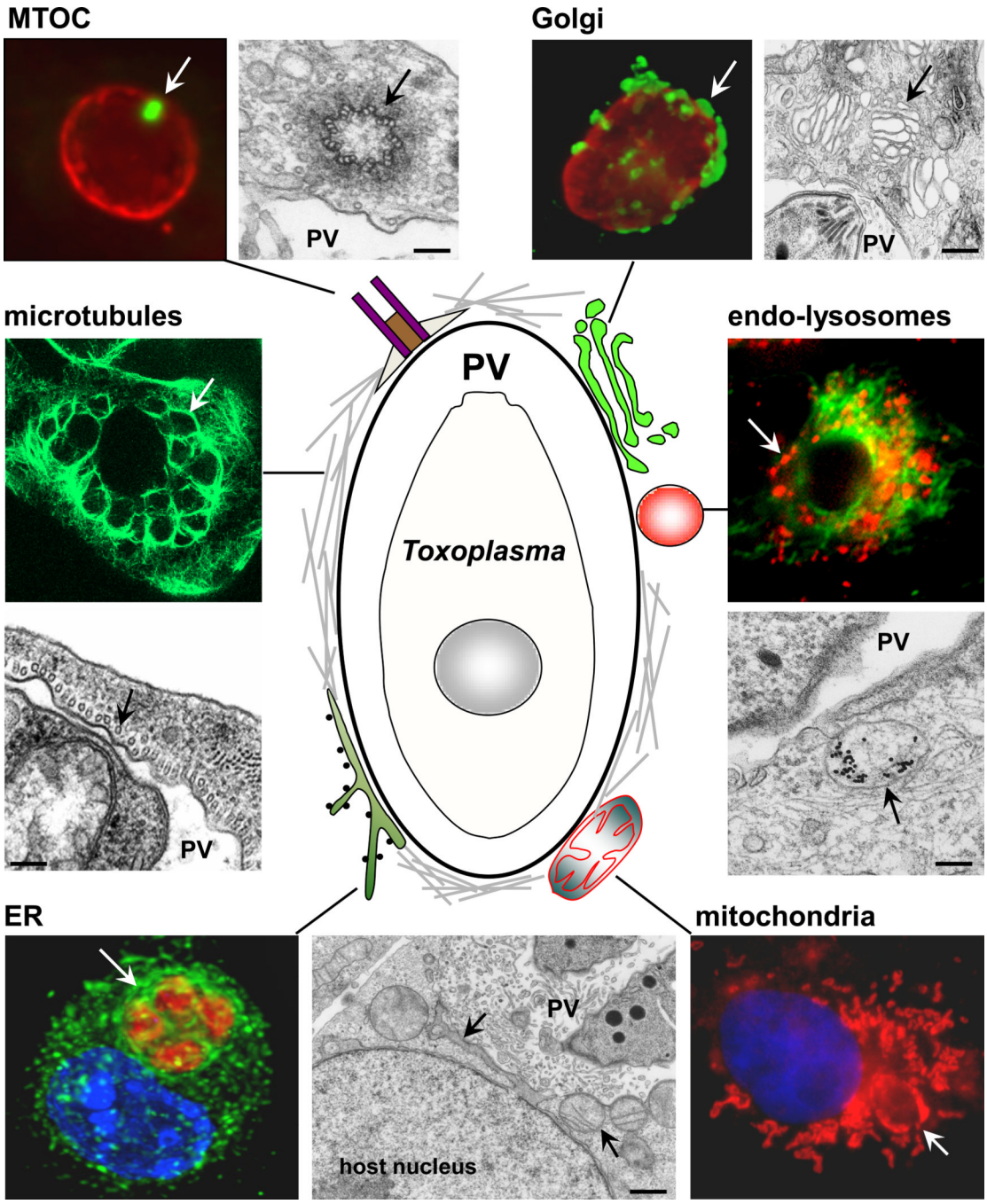

Figure 1. Model for the interaction of *Toxoplasma gondii* with host cells

A schematic representation of the PV and the host cell structures that are recruited by the parasite is shown. These host cell-PV interactions are illustrated by immunofluorescence and electron microscopy images. Host structures are indicated by an arrow. The following antibodies and dyes used for staining are: anti-γ-tubulin for MTOC (green) and anti-GRA7 for the PV membrane (red); anti-giantin for Golgi (green) and anti-GRA7 (red); anti-α-tubulin for microtubules in a multi-infected cell; anti-calnexin for ER, anti-SAG1 for the parasite plasma membrane and DAPI for nuclei; mito-Tracker for mitochondria and DAPI; Texas-red EGF (red) for endo-lysosomes and anti-α-tubulin (green). EM picture shows an endocytic organelle containing LDL-gold particles. Scale bars are 150 nm.

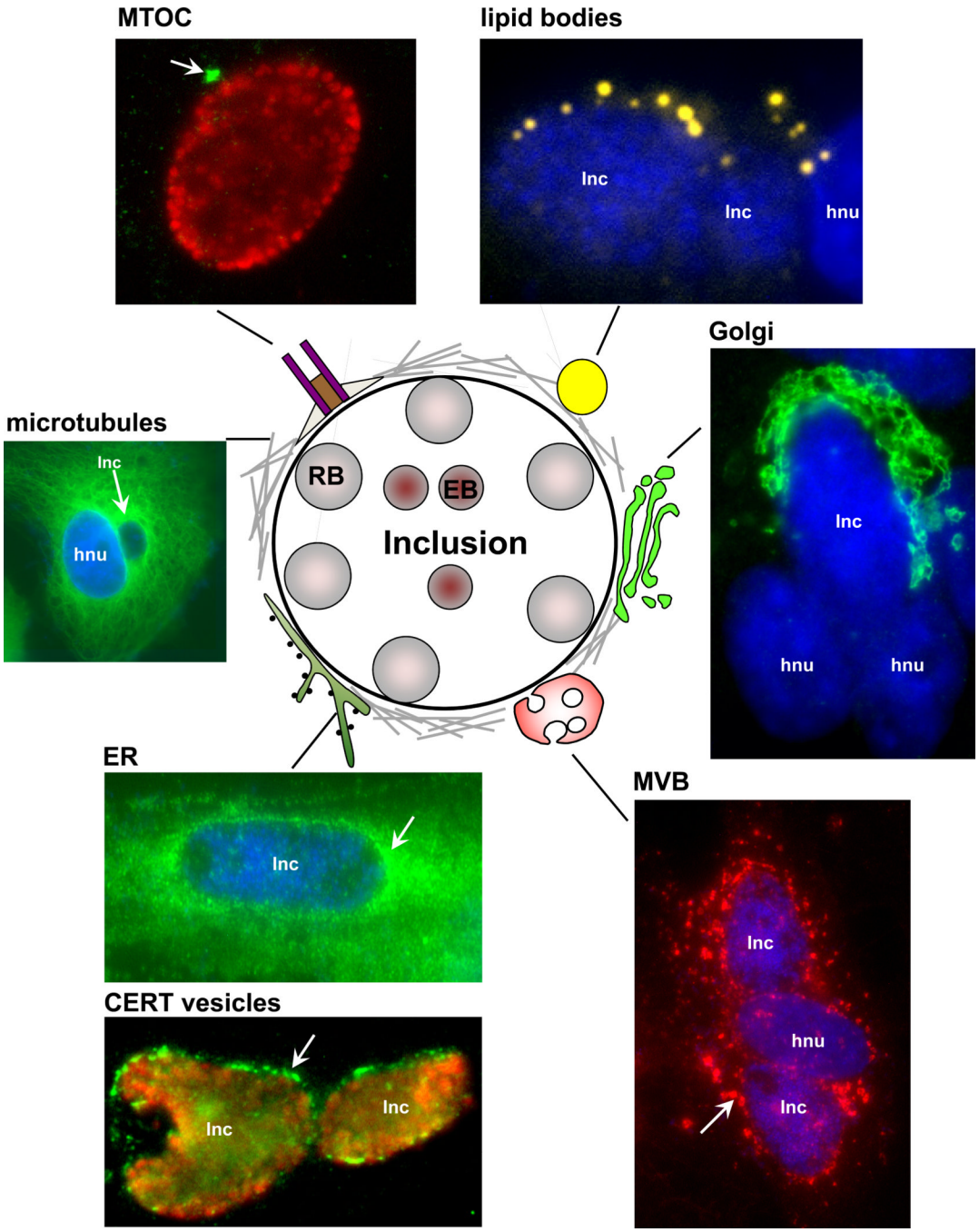

Figure 2. Model for the interaction of *Chlamydia trachomatis* with host cells

A schematic representation of an inclusion and host cell structures that are recruited by the bacterium is shown. These host cell-inclusion interactions are illustrated by immunofluorescence images. Host structures are indicated by an arrow. The following antibodies and dyes used for staining are: anti-γ-tubulin for MTOC (green) and anti-EF-Tu for *Chlamydia* (red); Nile Red for lipid bodies (yellow) and DAPI; anti-giantin for Golgi (green) and DAPI; anti-α-tubulin for microtubules and DAPI; anti-calnexin for ER and DAPI; anti-CERT for ER-derived vesicles (green) and anti-EF-Tu (red); anti-CD-63 for multivesicular bodies (red) and DAPI; Nile Red for lipid bodies (yellow) and DAPI. Inc, inclusion; hnu, host nucleus.

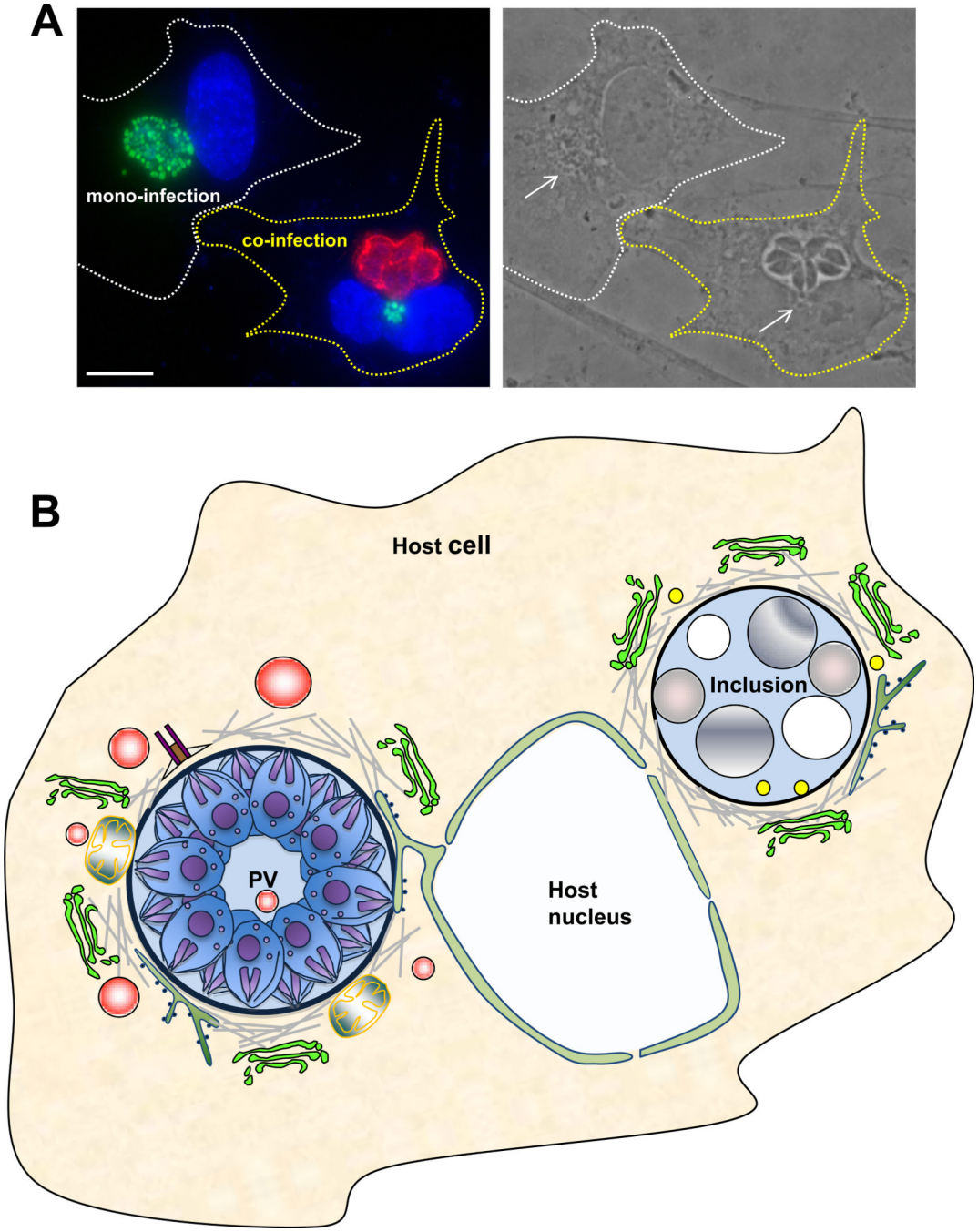

Figure 3. Co-infection of mammalian cells with *Toxoplasma gondii* and *Chlamydia trachomatis*, and host cell interactions with the PV and the inclusion during co-infection

A. A phase and immunofluorescence image of a mono- and a co-infected epithelial cellsshowing the poor development of the inclusion (stained for EF-Tu in green; arrow) during a 24-h co-infection while the parasites (stained for GRA7 in red) develop normally. Both the inclusion and the PV are located in the perinuclear region of the host cell. Scale bar is 10 μm. **B**. A schematic representation of a PV and an inclusion occupying the same cell for 24-h summarizing their interaction with host cell structures. Similarly as in a mono-infection, the PV is anchored to the host nuclear envelope, associates with the host MTOC, microtubules, ER, mitochondria and attracts host Golgi fragments and endocytic organelles

(in red) that are further delivered into the PV. In contrast, inclusions growing in a PV-containing cell shift to a stress-induced persistence state with large aberrant bodies despite its association with host microtubules (in the absence of the MTOC), Golgi fragments, ER and CERT vesicles, and the presence of lipid bodies in the vacuole.

Table 1

Comparative features of *Toxoplasma gondii* and *Chlamydia trachomatis*

	T. gondii	***C. trachomatis***
Domain	Eukaryota	Bacteria
Potential hosts	All blood-warmed animals	Humans
Disease	Encephalitis, retinochoroiditis, blindness, abortion, hydrocephalus	Trachoma, blindness, cervicitis, reactive arthritis
Infection	Ingestion of tissue cysts (bradyzoites) or oocysts (sporozoites)	Sexual or eye contacts with reticulate bodies
Pathogen size	2 × 7μm	1 × 1μm
Parasitism	Obligate intracellular	Obligate intracellular
Mode of entry	Active invasion	Phagocytic-like event (elementary bodies)
Intracellular compartment	Parasitophorous vacuole	Inclusion
Residence in the body	Brain and muscle cells	Epithelial cells
Multiplication	Endodyogeny of tachyzoites (intestine) and bradyzoites (brain)	Binary fission of reticulate bodies
Cycle	Lytic	Lytic
Stress response	Shift to tissue cyst	Persistence to elementary bodies
Lipids scavenged	Cholesterol, sphingolipids	Cholesterol, sphingolipids
Host structures recruited	Cytoskeleton, MTOC, ER, Golgi, mitochondria, endo-lysosomes, autophagosomes	Cytoskeleton, MTOC, Golgi, endo-lysosomes, autophagosomes lipid bodies