Targeting eukaryotic Rab proteins: a smart strategy for chlamydial survival and replication

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Chlamydia co-opt Rab-controlled vesicular transport

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Abstract

Chlamydia, an obligate intracellular bacterium which passes its entire lifecycle within a membrane-bound vacuole called the inclusion, has evolved a variety of unique strategies to establish an advantageous intracellular niche for survival. This review highlights the mechanisms by which *Chlamydia* subverts vesicular transport in host cells, particularly by hijacking the master controllers of eukaryotic trafficking, the Rab proteins. A subset of Rabs and Rab interacting proteins that control the recycling pathway or the biosynthetic route are selectively recruited to the chlamydial inclusion membrane. By interfering with Rab-controlled transport steps, this intracellular pathogen not only prevents its own degradation in the phagocytic pathway, but also creates a favorable intracellular environment for growth and replication. *Chlamydia*, a highly adapted and successful intracellular pathogen, has several redundant strategies to re-direct vesicles emerging from biosynthetic compartments that carry host molecules essential for bacterial development. Although current knowledge is limited, the latest findings have shed light on the role of Rab proteins in the course of chlamydial infections and could open novel opportunities for anti-chlamydial therapy.

Chlamydia trachomatis and *Chlamydia pneumoniae* are clinically important intracellular pathogens, causative agents of highly prevalent diseases in humans. *C. trachomatis* is the most common sexually-transmitted bacterium in western countries. It also constitutes the leading cause of infectious blindness worldwide. In the urogenital tract, a subset of serovars (D to K) causes urethritis, cervicitis and proctitis. Recurring infections can result in pelvic inflammatory disease and permanent sequelae such as tubal obstruction, ectopic pregnancy and female infertility. In the ocular epithelium, chronic infections elicited by serovars A to C result in trachoma. The serovars L1 to L3 pass the epithelium and invade lymphatic tissues, causing lymphogranuloma venereum. *C. trachomatis* has lately been associated with inflammatory pathologies such as ulcerous colitis, Crohn´s disease and arthritis (Choroszy-Król *et al.*, 2012). On the other hand, *C. pneumoniae* causes acute respiratory infections such as bronchitis, pharyngitis, sinusitis and pneumonia. There is increasing research interest in this pathogen for its role in the development of atherosclerosis and asthma (Roulis *et al.*, 2013). Additionally, *C. psittaci* infects birds but can be transmitted to humans causing psittacosis, a severe and difficult to treat pulmonary infection. Therefore, it is considered a potential candidate for use in biological warfare (Harkinezhad *et al.*, 2009).

Developmental lifecycle in Chlamydia

Chlamydia is a Gram negative obligate intracellular bacterium that undergoes a unique biphasic developmental cycle, comprising two functionally and morphologically distinct bacterial forms. The first form is the metabolically inert small elementary body (EB), which is environmentally-resistant and constitutes the infectious organism. The second form is the large pleiomorphic reticulate body (RB), unstable outside the host and

non-infectious, that displays metabolic and multiplicative activities (Choroszy-Król *et al.*, 2012). Under stressful conditions, such as those imposed by γ-interferon, antibiotics or deprivation of nutrients, *Chlamydia* enters a reversible persistent state characterized by an incomplete developmental cycle and the formation of aberrant bodies (ABs). These giant bacterial forms may remain within infected cells for long periods of time. Upon removal of the persistence inducer, the ABs re-enter the normal chlamydial developmental cycle (Mpiga and Ravaoarinoro, 2006).

Chlamydia completes its entire lifecycle within eukaryotic cells; hence, it has evolved various strategies to enter the host cell and create an environment conducive to replication. First, highly infectious EBs attach to the epithelial cell and promote their own uptake into a modified phagosome called an inclusion (Bastidas *et al.*, 2013). *Chlamydia* then inject TARP - a bacterial actin recruiting protein - through a type III secretion system, promoting the remodeling of the actin network at the invasion site (Clifton *et al.*, 2004). Once within the host cells, the EBs rapidly differentiate into RBs that asynchronously replicate by binary fission within the protected confines of the inclusion. After 48 to 72 hours -depending on the species and strains- the majority of the RBs re-differentiate into EBs. Finally, they are released into the extracellular environment either by lysis of the infected cell or by extrusion, an exocytic process without host cell death (Hybiske and Stephens, 2007). Thereafter, a multitude of infectious EBs disseminate, eliciting new rounds of infection (Figure 1).

Inclusion biogenesis

Normally, phagosomes fuse sequentially with early endosomes, late endosomes and lysosomes, a process that leads to the degradation of the internalized microorganisms (Vieira *et al.*, 2002). In contrast, chlamydial inclusions are non-fusogenic with components of the endocytic pathway as evinced in the lack of acquisition of endocytic tracers (e.g. dextran) or typical molecules of endo/lysosomal structures (e.g. early endosomal antigen 1, mannose 6-phosphate receptors and cathepsin D) (Fields and Hackstadt, 2002). Furthermore, neither the recruitment of the proton-ATPase pump nor intra-inclusion acidification occur (Heinzen *et al.*, 1996; Al-Younes *et al.*, 1999). Recent data show that *Chlamydia* require transferrin and functional lysosomes in close apposition to the inclusion for optimal bacterial growth (Ouellette and Carabeo, 2010; Ouellette *et al.*, 2011). Nevertheless, a consensus exists that once the inclusion is formed it quickly deviates from the endo/phagocytic route, and instead intersects with structures of the biosynthetic/exocytic pathways. At the earliest stages, *Chlamydia* apparently use preformed effectors; later on bacterial protein synthesis is required for subverting intracellular transport of the host cell (Scidmore *et al.*, 2003).

Shortly following the entry, *Chlamydia* actively control the movement of the nascent inclusion by recruiting the host motor protein dynein, which drives transport along the microtubule network towards the microtubule-organizing center. The migration to the perinuclear region is src family kinase dependent, and independent of p50 dynamitin (Grieshaber *et al.*, 2003; Mital *et al.*, 2010).

Interplay of chlamydial inclusions and host Rabs

Rab proteins are master controllers of intracellular trafficking, membrane fusion and organelle identity in eukaryotic cells. They comprise the largest branch of the Ras superfamily of small GTPases, monomeric enzymes that bind and hydrolyze GTP. Thus, the GTP moiety serves as a biochemical switch, shifting the protein from a membrane-associated active state when GTP-bound to an inactive cytosolic GDP-bound state. Rab switching is modulated by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) that respectively, promote the GTP hydrolysis, or the replacement of GDP by GTP. For membrane anchoring, Rab proteins require the addition of C-20 isoprenyl moieties to one or two C-terminal cysteine residues by the action of a Rab geranygeranyltransferase in association with a chaperone-like Rab escort protein (REP). After performing their function, Rab-GDP proteins are extracted from membranes by GDI (GDP dissociation inhibitor). Cycling between the cytosol and membranes is an essential feature of Rab action. At present, more than 60 different Rabs have been described; each one localizes to a distinct intracellular structure and controls a specific transport step. Rab targeting to a determined membrane is dictated by a complex mechanism involving GEFs, GAPs, effectors, and the hypervariable C-terminal domain of the Rab protein. By recruiting a multitude of Rab-specific effectors, including tethering complexes and motor proteins, Rabs direct vesicular transport (Zerial and McBride, 2001; Ali and Seabra, 2005; Stenmark, 2009). Progression along the endo/phagocytic pathway is tightly regulated by around eighteen Rab proteins. The most significant are Rab5, which controls homo/heterotypic fusion between early endosomes and early phagosomes; and Rab7, which accumulates on mature phagosomes, controlling fusion with late endosomes and the formation of phagolysosomes (Vieira *et al.*, 2002; Vieira *et al.*, 2003).

Chlamydia are not passive microorganisms travelling along the phagocytic pathway towards the phagolysosomes as their final destination. On the contrary, the bacteria disrupt vesicular trafficking by promoting recruitment to the inclusion membrane of certain host Rabs and by avoiding the acquisition of others. A subset of Rabs that belong to the recycling or biosynthetic pathways selectively associates with chlamydial inclusions (Rzomp *et al.*, 2003). Despite the long interval since this was discovered, the actual roles of these Rabs on the course of infection and the mechanisms involved in their recruitment are far from fully understood. Fortunately, in the last few years, functional studies have increased exponentially and now constitute an active field of chlamydial research.

Initial work describing the localization of small GTPases at the inclusion membrane was performed in *Chlamydia*-infected cells over-expressing Rabs fused to the green fluorescent protein. Those studies showed that Rab proteins controlling transport along the phagocytic pathway, such as Rab5, Rab7 and Rab9, do not associate with inclusions of any chlamydial species. In contrast, Rab4 and Rab11, GTPases that regulate fast- and slow-recycling transport respectively, localize at the inclusion membrane. Interestingly, Rab1, which is involved in endoplasmic reticulum (ER)-to-Golgi and in intra-Golgi trafficking, decorates inclusions. In fact, Rab1, Rab4, and Rab11 are recruited to inclusions of all chlamydial species. Intriguingly, Rab6, which regulates Golgi-to-ER retrograde trafficking and endosome-to-trans-Golgi network (TGN) transport, only associates with *C. trachomatis*-inclusions; whereas Rab10, which is involved in ER dynamics and post-Golgi transport, exclusively surrounds *C. pneumoniae*-inclusions (Rzomp *et al.*, 2003; Brumell and Scidmore, 2007). A genome-wide RNA interference screen, performed in *Drosophila* SL2 cells infected with species of *Chlamydia* that are non-pathogenic for humans, revealed

the involvement of several GTPases in bacterial growth (Elwell and Engel, 2005; Derré *et al.*, 2007; Elwell *et al.*, 2008).

The first images of an endogenous Rab decorating a chlamydial inclusion were of Rab14, an endosomal GTPase involved in Golgi-to-endosomes and TGN-to-plasma membrane transport (Capmany and Damiani, 2010). Subsequently, endogenous Rab11 was also seen surrounding chlamydial inclusions as a rim-like staining pattern (Leiva *et al.*, 2013). On the other hand, Rab39a (localized at multivesicular bodies and lysosomes) and Rab39b (a Golgi-associated Rab) are recruited to chlamydial inclusions through a bacteria-driven process (Gambarte et al, unpublished results). These findings were shown in cells infected with *C. trachomatis* serovar L2. The biological meaning and the consequences for the host of the recruitment of Rab proteins in a species-specific manner remain elusive. It might be implicated in tissue tropism or in the particularities of the disease caused by each species or strains of *Chlamydia*. Further investigation is necessary to answer this open query.

A common feature is that only Rabs bound to GTP are recruited to chlamydial inclusions. Most of them (Rab4, Rab6, Rab11 and Rab14) do not require intact microtubule or actin networks to associate with chlamydial inclusions. Strikingly, Golgi disorganization caused by brefeldin A (BFA) does not alter the recruitment of Golgi-related Rabs. Conversely, treatment with chloramphenicol impairs the binding of all Rabs, demonstrating a requirement for bacterial protein synthesis in this process. The subset of Rabs that decorates the inclusions varies along the developmental cycle, in accordance to the differential gene expression of *Chlamydia*, Rab11 being one of the first to be recruited (Rzomp *et al.*, 2003; Cocchiaro and Valdivia, 2009).

Rab-interacting proteins associate with chlamydial inclusions

Rab proteins in their active state interact with specific effectors, usually assembled in multi-molecular complexes, leading ultimately to membrane targeting and fusion. Despite the importance of these effectors in vesicular transport, little is known about Rab-interacting proteins that directly or indirectly associate with chlamydial inclusions. At present, the scarce available data proceed from epithelial cells infected with *C. trachomatis* serovar L2. Rab6-interacting protein Bicaudal D1 (BICD1) was the first to be identified at the inclusion membrane. The binding of BICD1 is Rab6-independent, suggesting a direct interaction with a bacterial protein (Moorhead *et al.*, 2007). Additionally, the oculocerebrorenal syndrome of Lowe protein 1 (OCRL1), a Golgi-localized phosphatidylinositol 5-phosphatase that binds to multiple Rabs, is also recruited to the inclusions (Moorhead *et al.*, 2010). The finding of two Rab6-interacting proteins, BICD1 and OCRL1, at the chlamydial inclusion membrane suggests that multi-molecular complexes could be involved in intercepting certain Rab-controlled trafficking pathways in infected host cells. Recently, the Family of Interacting Protein-2 (FIP2), a dual effector of Rab11 and Rab14, was described as surrounding the inclusions. The recruitment of FIP2 is specific since other members of the family (such as the Rab Coupling Protein or FIP3) do not associate with chlamydial inclusions (Leiva *et al.*, 2013).

Host Rabs interact with bacterial inclusion (Inc) proteins

One of the major tasks of *Chlamydia* is the modification of the inclusion membrane to promote the recruitment of the most convenient Rabs for the generation of a suitable niche for its survival and replication. Hence, the identification of the bacterial proteins involved in these processes constitutes one of the main challenges of current research.

Incs constitute a group of bacterial proteins enriched in segments predicted to form coiled coils. Owing to their strategic position at the inclusion membrane, facing the cytoplasm, they likely mediate the interactions with the host cell. Surprisingly, putative Incs are poorly conserved among chlamydial species, considering that they exert key functions. At present, limited numbers of interactions between Incs and eukaryotic proteins have been demonstrated (Li *et al.*, 2008; Dehoux *et al.*, 2011). CT229 is the only Inc protein of *C. trachomatis* proven to be a Rab binding partner. It has been demonstrated that CT229 directly interacts with Rab4-GTP, using the two-hybrid system, pull-down experiments and confocal microscopy (Rzomp *et al.*, 2006). In the case of *C. pneumoniae* Cpn0585 is the only Inc described as a Rab-interacting protein, and is able to bind Rab1, Rab10, and Rab11 (Cortes *et al.*, 2007). Unfortunately, neither structural nor functional homologs of CT229 and Cpn0585 have been identified among different chlamydial species.

The recruitment of eukaryotic Rabs and their Rab-binding proteins facilitate the transport of chlamydial inclusions and their selective interactions with intracellular structures of infected host cells. Table 1 summarizes our current knowledge of the eukaryotic Rabs and the bacterial Incs that participate in the interaction between *Chlamydia* and host cells.

Inclusion growth and acquisition of nutrients

Capture of nutrients through vesicular pathways

During migration towards the perinucleus, individual *Chlamydia*-containing vacuoles (except the ones with *C. pneumoniae*) fuse to form a single inclusion that grows rapidly, harboring an increasing number of replicating RBs (Delevoye *et al.*, 2008). The

restricted metabolic and biosynthetic activities of these bacteria are compensated for a wide variety of strategies that parasitize host cells, among them the hacking of Rab-controlled trafficking pathways to acquire nutrients and structural molecules essential for bacterial survival and growth (Saka and Valdivia, 2010).

Rab4 and Rab11 control transferrin recycling pathways intercepted by the inclusion. In spite of a strong dependence on host iron, the silencing of Rab4 failed to reveal any role in bacterial growth. However, the simultaneous depletion of Rab4 and Rab11 causes the retention of transferrin near to the inclusion and impairs chlamydial growth (Al-Younes *et al.*, 2001; Ouellette and Carabeo, 2010).

Inclusion development is accompanied by a considerable requirement for lipids coming from different host cell organelles, the major sources being multivesicular bodies (MVBs) and the Golgi apparatus (Hackstadt *et al.*, 1996). Pioneering studies established that inclusions preferentially intercept basolaterally-directed Golgi-derived vesicles for the acquisition of sphingomyelin and cholesterol (Hackstadt et al., 1996; Wolf and Hackstadt, 2001; Carabeo et al., 2003). In fact, *C. trachomatis* hijacks Golgi-associated Rabs (Rab6, Rab11 and Rab14) to capture exocytic vesicles enriched in endogenously synthesized host lipids (Rejman Lipinski *et al.*, 2009; Capmany *et al.*, 2011). Recent data show that this bacterium usurps Golgi vesicular trafficking via Arf1 (ADP-ribosylation factor 1) and GBF1 (Golgi-specific Brefeldin A resistance Factor 1) (Elwell *et al.*, 2011). Interestingly, *C. trachomatis* causes fragmentation of the Golgi and the formation of mini-stacks that surround the inclusion. Rab6 and Rab11 are probably involved in this process which enhances the delivery of sphingolipids to the growing inclusion (Heuer *et al.*, 2009; Rejman Lipinski *et al.*, 2009). Furthermore, Rab14 promotes the transport of newly biosynthesized

sphingomyelin towards the *C. trachomatis*-inclusion and the incorporation of these lipids by the bacteria (Capmany and Damiani, 2010; Capmany *et al.*, 2011). Depleting Rab6, Rab11 or Rab14 decreases the transport of endogenously synthesized sphingolipids from the Golgi to the inclusion, though lipid acquisition is far from blocked. In double silenced cells, the impairment of lipid transport is higher, demonstrating that *C. trachomatis* usurps redundant pathways to ensure the capture of essential molecules from host cells (Rejman Lipinski *et al.*, 2009; Capmany and Damiani, 2010). In addition, the depletion of FIP2, a dual Rab11- and Rab14-effector, reduces the delivery of sphingolipids from the Golgi to *C. trachomatis*-inclusions and consequently generates inclusions of smaller size (Leiva *et al.*, 2013). Sphingolipid deprivation not only has a detrimental impact on inclusion growth, but also simultaneously provokes the appearance of atypical bacteria. In fact, ABs are mainly observed in cells treated with inhibitors of sphingomyelin synthesis and in cells depleted of Rab6, Rab11 and Rab14 (Rejman Lipinski *et al.*, 2009; Robertson *et al.*, 2009; Capmany and Damiani, 2010).

MVBs are another important source of cholesterol and sphingolipids for the developing inclusions. These complex organelles occupy a crucial position at the intersection of endo/lysosomal and exocytic pathways (Woodman and Futter, 2008). The internal membranes of MVBs are rich in lyso-bis-phosphatidic acid (LBPA). The pharmacological interruption of transport from MVBs decreases both the arrival of lipids to chlamydial inclusions and bacterial growth (Beatty, 2006; Beatty, 2008; Robertson *et al.*, 2009). The participation of Rabs in the regulation of lipid transport from MVBs remains elusive. Interestingly, Rab11 is involved in the biogenesis of MVBs and in the release of

exosomes (Savina *et al.*, 2005), thus constituting a good candidate for controlling MVB-inclusion interactions.

The supply of neutral lipids is provided by lipid droplets (LD), ER-derived organelles, that are translocated into the inclusion through a mechanism that involves the chlamydial proteins Lda1 and Lda3 (Kumar *et al.*, 2006; Cocchiaro *et al.*, 2008). Proteomic screens reveal the presence of eighteen different Rabs in LDs; however, their role in lipid transport and processing is barely understood. Rab1, Rab6, Rab10, Rab11, Rab14 and Rab39, are present in both LDs and chlamydial inclusions of certain species. This finding suggests a possible participation of some of these Rabs in the fusion between LDs and inclusions (Murphy *et al.*, 2009).

In addition, other organelles from host cells are important for chlamydial growth, including the mitochondria that localize in the vicinity of the inclusions and act as essential energy providers (Matsumoto *et al.*, 1991; Derré *et al.*, 2007). Recently, it has been shown that Rab11 facilitates the redistribution of mitochondria near energy-requiring actin-rich structures. Consequently, this Rab might be implicated in the localization of mitochondria to the surrounding of inclusions (Frederick and Shaw, 2007). On the other hand, lysosomes are required to supply amino acids derived from host protein hydrolysis (Ouellette *et al.*, 2011). However, Rab7, the most iconic lysosome-associated Rab, is excluded from *C. trachomatis*- and *C. pneumoniae*-inclusions in epithelial cells (Rzomp *et al.*, 2003). Further research is necessary to unravel the underlying mechanisms involved in the interplay between certain subcellular organelles and the vacuole harboring *Chlamydia*.

Acquisition of nutrients through non-vesicular pathways

The inclusion membrane is a barrier that isolates the bacteria from the nutrient-rich environment of host cell cytoplasm. It is unlikely that metabolites such as sugars, amino acids, fatty acids, and nucleotides are delivered to inclusions by fusion with vesicles coming from the endo/lysosomal pathway (Heinzen and Hackstadt, 1997; Grieshaber *et al.*, 2002). Present knowledge strongly suggests the active participation of bacterial transporters, porins and translocases for the acquisition of nutrients from host cells (Vandahl *et al.*, 2005; Trentmann *et al.*, 2007; Braun *et al.*, 2008; Saka and Valdivia, 2010).

Recent data from cells infected with *C. trachomatis* serovar L2 indicate that several eukaryotic enzymes involved in biosynthetic pathways are recruited to the inclusion membrane and supply essential lipids for chlamydial development, including the machinery for high density lipoproteins (HDL) biogenesis (Cox *et al.*, 2012). Certain phospholipids (e.g. phosphatidylinositol and phosphatidylcholine) are provided through the activation of phospholipase A_2 and ERK at the chlamydial inclusion membrane (Su *et al.*, 2004). An on-site sphingomyelin biosynthetic factory is established at the inclusion membrane through the recruitment of host sphingomyelin synthase 2 (Elwell *et al.*, 2011). Furthermore, the host lipid carrier CERamide Transfer protein (CERT) - involved in non-vesicular transfer of ceramide at ER-Golgi membrane contact sites - also associates with inclusion membranes, most likely through binding to the bacterial protein IncD (Derré *et al.*, 2011). Moreover, it has been shown that *C. trachomatis* makes contact with the ER to acquire lipids from host cells through direct interaction (Dumoux *et al.*, 2012).

Summarizing, *Chlamydia* hijacks vesicular and non-vesicular mechanisms to facilitate the acquisition of essential molecules from the host cell to secure inclusion growth and bacterial development (Figure 2).

Bacterial replication and infectivity

Evidence obtained from cells infected with *C. trachomatis* reveals that interference with the function of eukaryotic Rabs hinders not only inclusion development but also the generation of chlamydial progeny. Rab6, Rab11 and Rab14 are important regulators of *C. trachomatis* replication and depletion of these Rabs reduces the yield of infectious particles (Rejman Lipinski *et al.*, 2009; Capmany and Damiani, 2010). In line with these findings, silencing the Rab-binding effector OCRL1 and the dual Rab-interacting protein FIP2 also decreases bacterial progeny (Moorhead *et al.*, 2010; Leiva *et al.*, 2013). Interestingly, the simultaneous depletion of more than one of these eukaryotic proteins results in a much more dramatic decrease in chlamydial infectivity. This finding strongly suggest that these Rab and Rab-binding proteins exert partially overlapping functions that are crucial for *C. trachomatis* survival and multiplication (Rejman Lipinski *et al.*, 2009; Capmany and Damiani, 2010). Presently, it is desirable to extend this research to other species of *Chlamydia*, mainly to *C. pneumoniae*, to understand the precise role of Rabs and Rab-binding proteins at the molecular level in the replication and infectivity of these pathogens.

Conclusions and future directions

We have described a complex scenario with the participation of multiple bacterial and host players. *Chlamydia* intercepts numerous and redundant Rab-controlled transport pathways and non-vesicular mechanisms to ensure the delivery of essential nutrients and structural components from host cells to the developing inclusion. Conveniently, *Chlamydia* manipulates Rab proteins to prevent their degradation in the phagocytic

pathway and to create a favorable niche for replication. Although current knowledge is limited, recent studies have revealed valuable findings about the participation of Rabs in the course of chlamydial infections. This smart intracellular pathogen exploits specific trafficking pathways of infected host cells by means of hijacking Rabs that belong to recycling or biosynthetic compartments. This fruitful strategy allows the bacterium to gain access to nutrients and compounds indispensable for bacterial growth. At the same time, the camouflage of the inclusion with these Rabs hides it from the main intracellular innate immune defense mechanism: bacterial killing by fusion with lysosomes. This successful strategy might be referred as "two goals with one shot". The impossibility of knocking out or mutating chlamydial genes conspires against the identification of the Incs proteins responsible for the recruitment of host Rabs. Fortunately, novel approaches to manipulate the chlamydial genome are just beginning to appear. These new tools will shed light on the bacterial effectors involved in the generation of the friendly niche where *Chlamydia* hides within host cells.

Intriguing challenges for future research are the identification of bacterial recruiting factors for Rab proteins; a deeper understanding of the role played by eukaryotic Rabs along the bacterial developmental cycle; an overall analysis of the consequences for the infected cells of the interference in intracellular transport due to these bacteria; and finally, to expand the scope of the *in vitro* results to animal models and humans.

A detailed knowledge of the mechanisms involved in *Chlamydia*-host cell interaction will give us a better understanding of infections caused by these pathogens. More importantly, this insight might guide the development of vaccines and new therapeutic strategies for the effective control of acute and chronic chlamydial infections.

Figure Legends

Figure 1. **Chlamydial developmental cycle.** The cycle begins with the attachment of the infectious elementary body (EB) and its entry into the epithelial cell. Subsequently, the EB differentiates into the reticulate body (RB) within the confines of a modified phagosome called the inclusion. The nascent inclusion avoids fusion with endo/phagocytic compartments and migrates towards the perinucleus. Bacterial proteins (Incs) exposed at the inclusion membrane mediate the interaction with different host organelles and facilitate nutrient acquisition. RBs replicate by binary fission and finally re-differentiate into EBs that are released to the extracellular medium by host cell lysis or by extrusion of the inclusion. Under stressful conditions, *Chlamydia* transit to a quiescent state characterized by the presence of non-infectious persistent abnormal bacteria, the aberrant bodies (ABs).

Figure 2. **Interactions between *Chlamydia* and the host cell.** Multiple host cell organelles, including fragmented Golgi mini-stacks, MVBs (multivesicular bodies), the ER (endoplasmic reticulum), LD (lipid droplets), RE (recycling endosomes), L (lysosomes), and mitochondria serve as a source of sphingomyelin (SM), cholesterol, lysobisphosphatidic acid (LBPA), neutral lipids, amino acids, nucleotides, transferrin (Tf) and iron, among other nutrients. Rab-controlled vesicular transport and non-vesicular mechanisms are co-opted by *Chlamydia*, likely via bacterial Inc proteins, to acquire essential nutrients from the host cells. Conveniently, *Chlamydia* does not fuse with early endosomes (EE) and late endosomes (LE), thus escaping the degradative phago-lysosomal pathway.

Table 1. **Rab GTPases recruited to *C. trachomatis*- and *C. pneumoniae*- inclusions**. Brief summary of the subcellular localization and the transport step regulate by certain Rabs in eukaryotic cells. In addition, Rab recruitment to chlamydial inclusions, Rab-interacting Incs and Rab function in chlamydial-infected cells. ER: endoplasmic reticulum; EE: early endosomes; SE: sorting endosomes; PM: plasma membrane; TGN: trans-Golgi network; SM: sphingomyelin; RE: recycling endosomes; *Ct*: *Chlamydia trachomatis*; *Cpn*: *Chlamydia pneumoniae*; nd: not determined.

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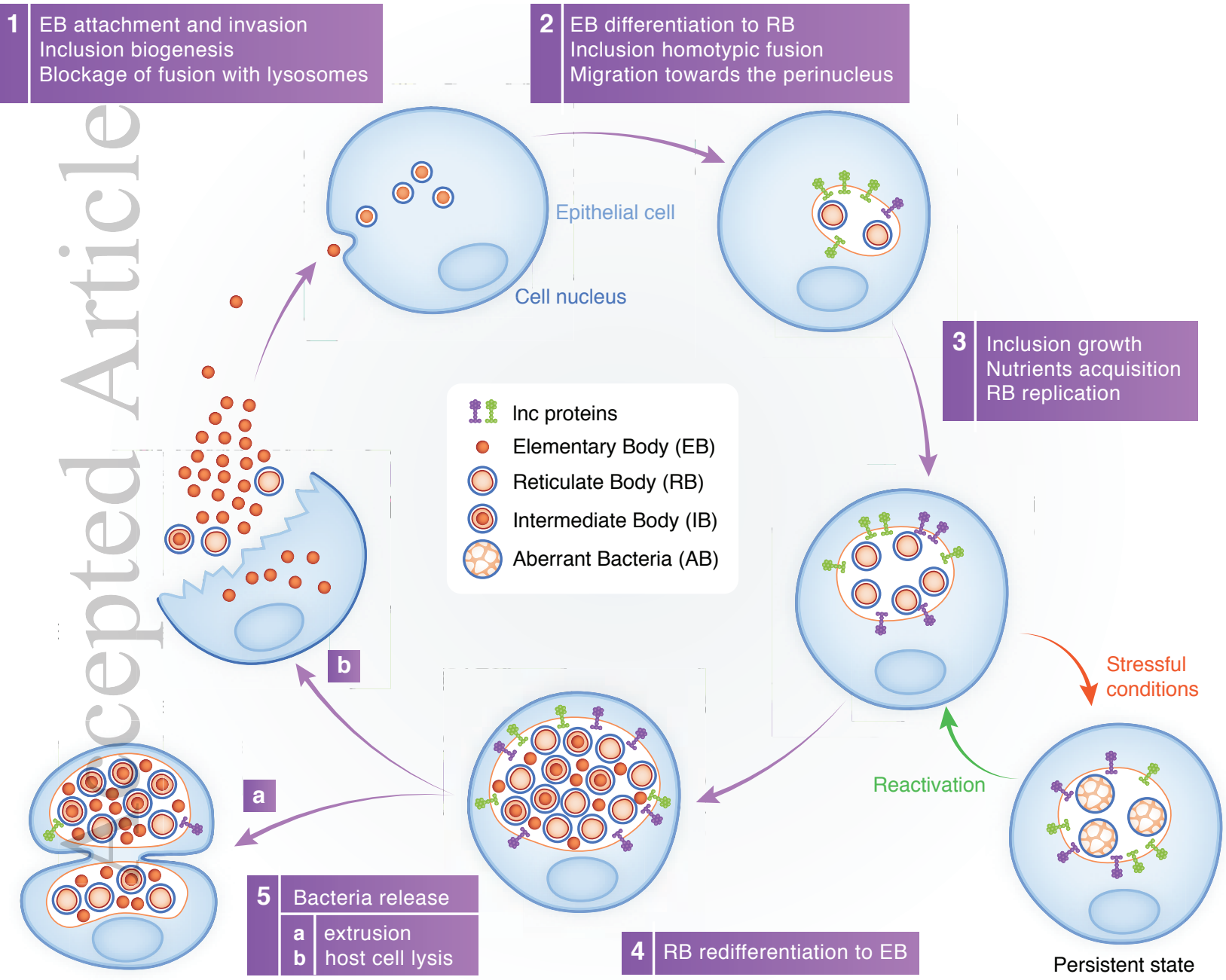
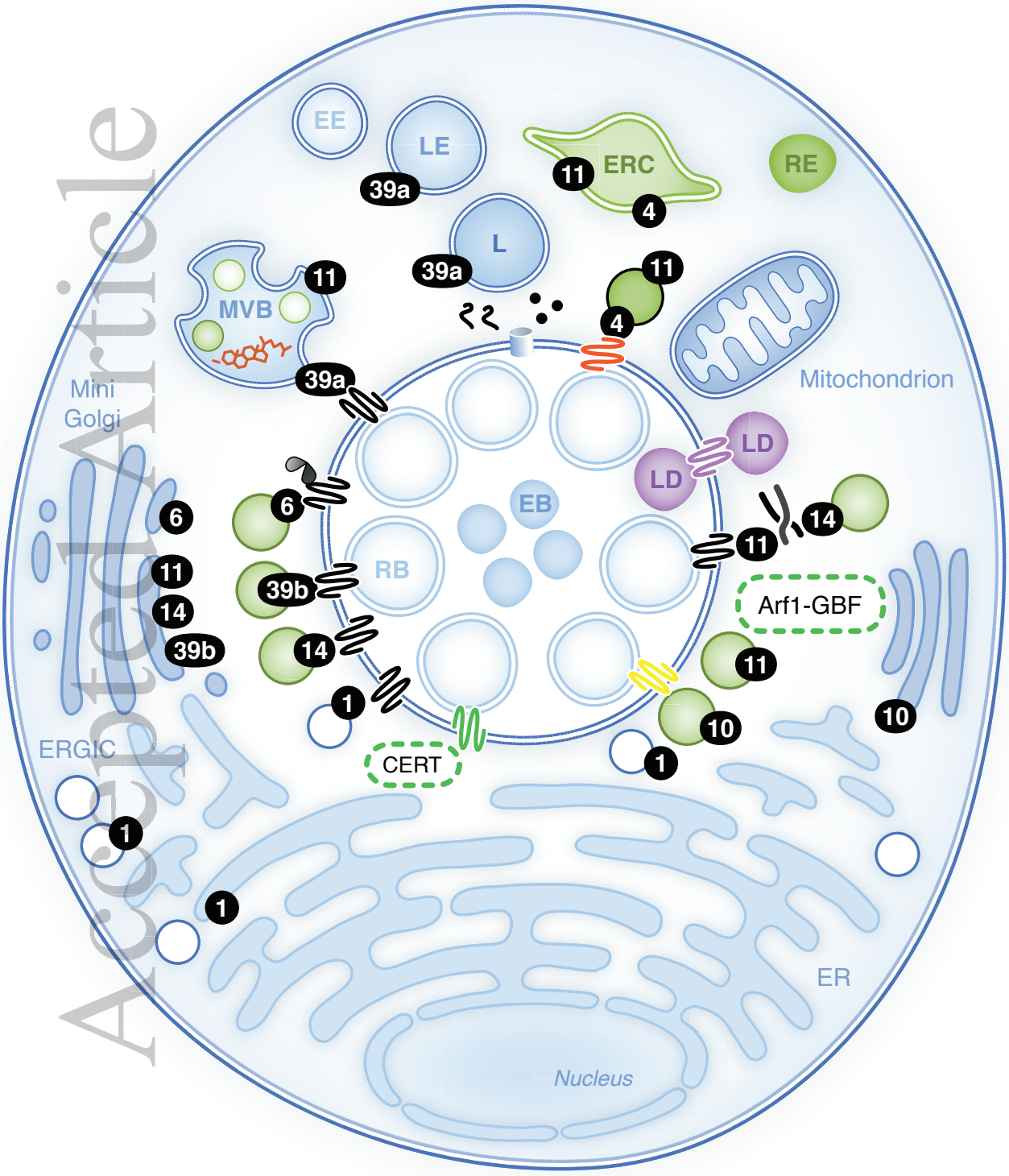
Rab	Subcellular localization	Main eukaryotic transport step	Recruitment to inclusions	Inc	Transport to inclusions
1	ER, Golgi	ER-to-Golgi intra-Golgi	*Ct (+)* *Cpn (+)*	nd Cpn0585	nd nd
4	EE, SE	PM-to-EE SE-to-PM	*Ct (+)* *Cpn (+)*	CT229 nd	nd nd
6	Golgi	Golgi-to-ER intra-Golgi EE-to-TGN	*Ct (+)* *Cpn (-)*	nd -	SM transport -
10	Golgi	ER-to-Golgi TGN-to-PM	*Ct (-)* *Cpn (+)*	- Cpn0585	- nd
11	RE, TGN	RE-to-PM TGN-to-EE TGN-to-PM	*Ct (+)* *Cpn (+)*	nd Cpn0585	SM transport nd
14	EE, TGN	TGN-to-EE TGN-to-PM RE-to-PM	*Ct (+)* *Cpn* nd	nd nd	SM transport nd

TABLE 1. Rab GTPases recruited to *C. trachomatis*- and *C. pneumoniae*- inclusions. Brief summary of the subcellular localization and the transport step regulate by certain Rabs in eukaryotic cells. In addition, Rab recruitment to chlamydial inclusions, Rab-interacting Incs and Rab function in chlamydial-infected cells. ER: endoplasmic reticulum; EE: early endosomes; SE: sorting endosomes; PM: plasma membrane; TGN: trans-Golgi network; SM: sphingomyelin; RE: recycling endosomes; *Ct*: *Chlamydia trachomatis*; *Cpn*: *Chlamydia pneumoniae*; nd: not determined.





Host proteins

- № Rab proteins
- FIP2
- BICD1

Bacterial proteins

- CT229
- Lda1/3
- IncD
- Cpn0585
- Unknown Inc

Host nutrients

- Cholesterol
- Peptides
- Amino acids
- Tf vesicle
- SM vesicle
- LBPA vesicle