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The changing nature of the *Brucella*-Containing Vacuole

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Summary

Bacteria of the genus *Brucella* are intracellular vacuolar pathogens of mammals that cause the worldwide zoonosis brucellosis, and reside within phagocytes of infected hosts to promote their survival, persistence and proliferation. These traits are essential to the bacterium's ability to cause disease and have been the subject of much investigation to gain an understanding of *Brucella* pathogenic mechanisms. Although the endoplasmic reticulum-derived nature of the *Brucella* replicative niche has been long known, major strides have recently been made in deciphering the molecular mechanisms of its biogenesis, including the identification of bacterial determinants and host cellular pathways involved in this process. Here I will review and discuss the most recent advances in our knowledge of *Brucella* intracellular pathogenesis, with an emphasis on bacterial exploitation of the host endoplasmic reticulum-associated functions, and how autophagy-related processes contribute to the bacterium's intracellular cycle.

Bacteria of the *Brucella* genus are the causative agent of brucellosis, a zoonotic disease of worldwide distribution that affects both animals and humans and inflicts economical and public health burden in endemic areas (Pappas *et al.*, 2005). While brucellosis causes abortion and sterility in animals, the human disease is characterized by recurrent fever and debilitating musculoskeletal, cardiac and neurological complications at the chronic stage of the infection. *Brucella* primarily infects professional phagocytes such as macrophages or dendritic cells (Archambaud *et al.*, 2010) at the onset of infection. These act both as a survival/replication niche and as vectors for systemic dissemination to other organs. Bacteria subsequently infect cells of myeloid lineage, including macrophages in the spleen and liver, and persist within granulomatous lesions, or infect and proliferate within placental trophoblasts in pregnant animals (Atluri *et al.*, 2011). These pathological aspects of brucellosis emphasize the importance of the bacterium's intracellular cycle to the disease's development and progression, and rationalize the need to understand the underlying mechanisms of its intracellular cycle. As bacteria that have undergone a long evolution with mammalian hosts, *Brucella* spp. have selected a sophisticated intracellular cycle that ensures their survival, immune evasion, proliferation and persistence within the host, portraying an exquisite model of pathogen subversion of host cell organelles and functions. Extensive studies of the *Brucella* intracellular cycle have revealed that this pathogen controls the

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conversion of its intracellular compartment, the *Brucella*-containing vacuole (BCV), from an endosomal (eBCV) to a replicative (rBCV) vacuole derived from the endoplasmic reticulum (ER) (Pizarro-Cerdá, Moreno, *et al.*, 1998; Pizarro-Cerdá, Méresse, *et al.*, 1998; Comerci *et al.*, 2001; Delrue *et al.*, 2001; Celli *et al.*, 2003; Salcedo *et al.*, 2008; Starr *et al.*, 2008; Salcedo, Chevrier, *et al.*, 2013), then the subsequent biogenesis of an autophagy-related compartment (aBCV) (Starr *et al.*, 2012) (Fig. 1). Here I will review our current knowledge of how *Brucella* modulates these sequential changes in its vacuole, with an emphasis on subversion of the host endoplasmic reticulum (ER) and its associated functions.

The endosomal BCV: playing with fire

A wealth of knowledge of the *Brucella* intracellular cycle has been garnered using of a variety of murine and human macrophage or monocyte *in vitro* models. Additional host cells such as the non-phagocytic HeLa cell line have also been used (Pizarro-Cerdá, Moreno, *et al.*, 1998; Pizarro-Cerdá, Méresse, *et al.*, 1998; Comerci *et al.*, 2001; Delrue *et al.*, 2001; Archambaud *et al.*, 2010) and shown to faithfully recapitulate *Brucella* intracellular cycle in macrophages (Celli *et al.*, 2003; Celli *et al.*, 2005; Starr *et al.*, 2008; Atluri *et al.*, 2011; Starr *et al.*, 2012). This epithelial cell line is more amenable to manipulation and microscopy, providing a useful tool for cell biological studies of host factors required for *Brucella* intracellular pathogenesis.

Following phagocytic uptake by macrophages or entry into non-phagocytic cells, *Brucella* resides within a membrane-bound compartment, the BCV. Acquisition of endocytic markers, such as Rab5, its effector EEA1 and the transferrin receptor TfR (Pizarro-Cerdá, Moreno, *et al.*, 1998; Pizarro-Cerdá, Méresse, *et al.*, 1998; Comerci *et al.*, 2001; Delrue *et al.*, 2001; Celli *et al.*, 2003; Bellaire *et al.*, 2005; Salcedo *et al.*, 2008; Starr *et al.*, 2008; Salcedo, Chevrier, *et al.*, 2013), during early maturation indicates its interaction with early endosomes. These interactions are transient and followed by acquisition of the late endocytic markers LAMP1, CD63 and Rab7 (Pizarro-Cerdá, Méresse, *et al.*, 1998; Celli *et al.*, 2003; Bellaire *et al.*, 2005; Starr *et al.*, 2008) and BCV acidification to pH ~4–4.5 (Porte *et al.*, 1999; Boschiroli, 2002; Starr *et al.*, 2008), suggesting a normal maturation process along the default degradative endocytic pathway. Early studies of BCV trafficking in HeLa cells and primary murine macrophages however failed to detect acquisition of the lysosomal hydrolase Cathepsin D (Pizarro-Cerdá, Méresse, *et al.*, 1998; Celli *et al.*, 2003), which led to the conclusion that *Brucella* avoids fusion with bactericidal lysosomes as a means of intracellular survival. Yet this model is not consistent with the enrichment of late endosomal/lysosomal markers on the maturing eBCV, or with its rapid acidification, which are considered correlates of fusion with lysosomes. Using live cell imaging techniques, Starr *et al.* showed significant delivery of a fluid phase marker chased to terminal lysosomes prior to infection, and directly visualized eBCV-lysosome fusion events, indicating that eBCVs indeed undergo fusion with lysosomes, albeit not to the extent of a phagosome containing an inert particle (Starr *et al.*, 2008). This apparent discrepancy in the extent of eBCV fusion with lysosomes likely stems from drastically reduced detection of soluble antigens in fixed and permeabilized samples, due to their leakage, whereas live cell imaging provides optimal sensitivity of detection of fluid phase markers (Drecktrah *et al.*, 2007; Starr *et al.*, 2008).

BCV maturation along the endocytic pathway appears deleterious to bacteria, as some are killed during the eBCV stage (Celli *et al.*, 2003). Nonetheless, inhibition of fusion with late endosomes/lysosomes via overexpression of a dominant negative allele of the small GTPase Rab7 (Rab7^{T22N}) impairs *Brucella*'s ability to generate its replicative niche (rBCV) and proliferate intracellularly (Starr *et al.*, 2008), indicating that the endosomal stage does not simply reflect a default bactericidal pathway, but serves a specific purpose. Indeed, eBCV acidification constitutes a signal for intracellular induction of *Brucella*'s major virulence determinant, the VirB Type IV secretion system (T4SS) (Sieira *et al.*, 2000; Boschiroli, 2002; O'Callaghan *et al.*, 2002), an apparatus essential for rBCV biogenesis and replication (Lestrate *et al.*, 2000; Comerci *et al.*, 2001; Delrue *et al.*, 2001; Celli *et al.*, 2003) that delivers effector proteins into the host cell. Hence, the eBCV stage seems to constitute a rite of passage for *Brucella* towards generating the replication-permissive rBCV.

While the eBCV stage represents a transitional step towards rBCV biogenesis, it is also accompanied by cell cycle transitions in the bacterium. Deghelt *et al.* have elegantly shown that the infectious form of *Brucella* is arrested in the G1 phase for up to 6 h post infection, but the bacterium resumes its cell cycle and chromosomal replication while still within the eBCV(Deghelt *et al.*, 2014). Bacterial division and replication eventually occurs in rBCVs (Deghelt *et al.*, 2014), indicating that bacteria become primed for proliferation within eBCV and further highlighting their importance in the *Brucella* intracellular cycle.

Brucella and the ER: biogenesis of the replicative BCV

Brucella residence and replication within the endoplasmic reticulum (ER) of host cells was initially described in seminal ultrastructural studies of infected goat and bovine placentas, indicating bacterial proliferation within ER cisternae of trophoblasts (Anderson *et al.*, 1986; Meador and Deyoe, 1989), together with *in vitro* studies in Vero cells (Detilleux *et al.*, 1990). These studies also suggested that bacteria were transferred from phagosomes to the ER to undergo replication. The transition of *Brucella* from an endosomal vacuole to the ER was confirmed by immunolocalization of markers for specific intracellular compartments (Pizarro-Cerdá, Méresse, *et al.*, 1998; Celli *et al.*, 2003). Additional evidence that rBCVs originate from eBCVs, and do not constitute independent populations of bacterial vacuoles with different intracellular fates, was the demonstration that rBCV biogenesis requires host functions associated with endosomal maturation, specifically vacuolar acidification (Porte *et al.*, 1999; Starr *et al.*, 2008) and the late endosomal small GTPase Rab7 (Starr *et al.*, 2008). It is now commonly accepted that *Brucella* controls the traffic of its intracellular vacuole from the endocytic to the secretory compartment to generate an ER-derived, replication permissive vacuole (rBCV).

An increasing number of bacterial pathogens undergo critical interactions with organelles of the host cell secretory compartment, such as the ER and the Golgi apparatus (recently reviewed in (Celli and Tsolis, 2014)), but only a handful including *Brucella* spp. and *Legionella pneumophila* unambiguously establish residence within a vacuole with functional characteristics of the ER (Pizarro-Cerdá, Méresse, *et al.*, 1998; Celli *et al.*, 2003; Robinson and Roy, 2006). While the molecular mechanisms of biogenesis of the ER-derived replicative vacuole of *L. pneumophila* are well understood (as recently reviewed in (Hubber

and Roy, 2010)), details on the mechanisms of rBCV biogenesis are only starting to emerge. Unlike *L. pneumophila*, which rapidly redirects its original phagosome to the secretory pathway by recruiting and triggering fusion of secretory vesicles via modulation of the host GTPases Rab1 and ARF1 (Nagai, 2002; Dorer *et al.*, 2006; Machner and Isberg, 2006; Murata *et al.*, 2006; Ingmundson *et al.*, 2007), rBCV biogenesis does not require ARF1-dependent vesicular trafficking between the ER and the Golgi apparatus (Celli *et al.*, 2005). Instead, eBCVs localize to ER exit sites (ERES) during their maturation and functional disruption of ERES, via inactivation of the small GTPase Sar1, inhibits rBCV biogenesis indicating that *Brucella* intercepts the early secretory pathway at the ER interface to promote eBCV to rBCV conversion (Celli *et al.*, 2005). Via a proteomic approach, Fugier *et al*. also identified Rab2 and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as essential for rBCV biogenesis and bacterial replication (Fugier *et al.*, 2009). Since both proteins also participate in vesicular trafficking between the ER and the Golgi apparatus, these findings lend additional credence to the concept of *Brucella* subversion of specific components of the early secretory pathway to gain access to the ER.

The VirB type IV secretion system (T4SS) is essential for biogenesis of the replicative vacuole and intracellular replication (Lestrate *et al.*, 2000; Comerci *et al.*, 2001; Delrue *et al.*, 2001; Celli *et al.*, 2003). Loss-of-function studies have clearly established that inactivation of the VirB T4SS via deletion or insertional mutagenesis of *virB* genes sequesters the corresponding bacterial mutants in eBCVs, unable to sustain interactions with ERES and convert their endosomal vacuole into an rBCV (Comerci *et al.*, 2001; Delrue *et al.*, 2001; Celli *et al.*, 2003; Celli *et al.*, 2005). Given that the VirB T4SS is thought to deliver effector proteins across the BCV membrane into the host cell (De Jong *et al.*, 2008; de Barsy *et al.*, 2011; Ines Marchesini *et al.*, 2011; Myeni *et al.*, 2013; Salcedo, Marchesini, *et al.*, 2013), a reasonable hypothesis is that *Brucella* modulates host functions involved in the early secretory pathway via the action of these effectors to generate the rBCV. In support of this, the *Brucella* secreted protein RicA binds Rab2 (de Barsy *et al.*, 2011). Deletion of RicA in *B. abortus* reduces recruitment of GTP-locked $Rab2^{Q65L}$ on BCVs and potentiates the bacterium's trafficking to the ER and its replication, suggesting that RicA may have a downmodulating function in BCV trafficking. Although RicA preferentially binds GDP-bound Rab2, it does not exhibit any guanine nucleotide exchange factor (GEF) activity (de Barsy *et al.*, 2011), so whether it affects Rab2 activity remains unclear. Additional evidence that *Brucella* T4SS effectors modulate secretory trafficking to promote rBCV biogenesis comes from the identification of a series of effectors (BspA, BspB, BspC, BspD, BspF, BspK) that either target compartments of the early secretory pathway or impair secretory trafficking when ectopically expressed in HeLa cells (Myeni *et al.*, 2013). Furthermore, *Brucella* infection impairs secretory trafficking in a BspA-, BspB- and BspF-dependent manner (Myeni *et al.*, 2013). Inhibition of secretory trafficking in *Brucella*-infected cells is evidenced by retention of a secretory marker (VSV-G-GFP) within the Golgi apparatus and its decreased delivery to the plasma membrane (Myeni *et al.*, 2013). Since inhibition of host secretory trafficking occurs prior to bacterial replication in the ER, it appears to be temporally consistent with eBCV to rBCV conversion events. Deletion of *bspB* reduces bacterial replication in primary murine macrophages, an effect that is potentiated by additional deletions of *bspA* and *bspF* (Myeni *et al.*, 2013), yet whether these mutants

display altered BCV trafficking remains to be established. Nonetheless, these findings may reflect bacterial effector-mediated alterations of early secretory trafficking associated with rBCV biogenesis. Yet, it cannot be ruled out that these may also reflect changes in secretory trafficking that promote bacterial replication within rBCVs. Similarly, it could represent changes aimed at altering surface expression of immune molecules, consistent with the retention of MHC Class I molecules in the Golgi apparatus of *Brucella*-infected monocytes (Barrionuevo *et al.*, 2012), or secretion of pro-inflammatory cytokines. Altogether, further identification and characterization of VirB effector targets and mode of action will likely elucidate the exact contribution of early secretory trafficking in rBCV biogenesis, and also shed light onto the reasons and consequences of *Brucella* impairment of secretory trafficking.

While *Brucella* replication is commonly associated with biogenesis of its ER-derived vacuole, alternate replication niches have been documented. Immunoglobulin G (IgG)-opsonized *B. abortus* replicates in the human monocytic cell line THP1 within LAMP1-positive, non-acidic endosomal vacuoles that do not fuse with lysosomes (Bellaire *et al.*, 2005). Although this suggests that *Brucella* intracellular trafficking may be altered by opsonization and cause bacterial replication in a modified eBCV, these endosomal vacuoles may alternatively correspond to autophagic aBCVs (see below; (Starr *et al.*, 2012)), given the late time point analyzed, and their further characterization is warranted. Nonetheless, a recent investigation of *Brucella* spp. infection of human trophoblasts revealed that strains of *B. abortus* and *B. suis* replicate in large endosomal inclusions in extra-villous trophoblasts (EVTs) or in the EVT-like cell line JEG-3, although they seem to reach, and replicate within, the ER in trophoblast cell lines of other lineages (BeWo, JAR, HTR8 cells) and in syncytiotrophoblasts (Salcedo, Chevrier, *et al.*, 2013). This suggests that EVTs have the capacity to interfere with normal BCV trafficking and restrict bacteria to an endosomal vacuole, possibly the eBCV, where they nonetheless exhibit replication that does not depend upon the VirB T4SS (Salcedo, Chevrier, *et al.*, 2013). Interestingly, *B. melitensis* strains reach, and replicate within, the ER in EVTs (Salcedo, Chevrier, *et al.*, 2013), indicating that this species is more resistant to EVT trafficking restriction. EVTs therefore constitute an interesting model to dissect *Brucella* mechanisms of replication within an endosomal environment, and to tease out differences in the ability of *Brucella* species to achieve an optimal intracellular cycle.

Autophagy, the unfolded protein response and the *Brucella* intracellular cycle

Although significant strides have been made in identifying host factors that specifically contribute to rBCV biogenesis, how *Brucella* controls conversion of its endosomal eBCV into the ER-derived rBCV has largely remained elusive, in part because this process does not intuitively abide by known host cell trafficking pathways. Early studies of BCV intracellular trafficking proposed the involvement of the autophagy pathway in rBCV biogenesis (Pizarro-Cerdá, Moreno, *et al.*, 1998; Pizarro-Cerdá, Méresse, *et al.*, 1998). Autophagy invokes the capture of cytosolic components, damaged organelles, protein aggregates and intracellular microbes (whether cytosolic or vacuolar) into double membrane

vacuoles called autophagosomes. These then mature into degradative autolysosomes via interactions with late endocytic compartments and fusion with lysosomes, which allows degradation and recycling of autolysosome content. While originally identified as a process of nutrient recycling under starvation conditions, autophagy fulfills many homeostatic functions in eukaryotic cells and also contributes to innate immunity via its antibacterial function (Levine *et al.*, 2011). Based on multimembrane BCV structures, increased bacterial replication upon amino-acid starvation, and accumulation of the lysomotropic probe monodansylcadaverine (MDC) in BCVs, it was proposed that these vacuoles traffic via the autophagy pathway in HeLa cells (Pizarro-Cerdá, Moreno, *et al.*, 1998; Pizarro-Cerdá, Méresse, *et al.*, 1998), but this assumption requires validation using more specific tools and assays for autophagy that have since become available (Klionsky *et al.*, 2012). In an elegant genetic screen in *Drosophila* S2 cells for ER-associated proteins required for *Brucella* replication, Qin *et al*., identified the unfolded protein response (UPR) transmembrane sensor IRE1α as necessary for bacterial replication (Qin *et al.*, 2008). The UPR is an ER stress response activated under physiological conditions that overwhelm the ER protein folding capacity, leading to the accumulation of unfolded proteins. Altered gene expression, mRNA turnover, translation and protein folding capacity are initiated by activation of three UPR sensors IRE1α, ATF6 and PERK, which together resolve ER stress and restore cellular homeostasis (Walter and Ron, 2011). One of these responses is IRE1α-dependent induction of autophagy, which contributes to controlling ER expansion during the UPR (Yorimitsu *et al.*, 2006; Bernales *et al.*, 2006) and is independent of ATF6 or PERK (Ogata *et al.*, 2006). Since *Brucella* replication was not affected by ATF6 and PERK depletion, Qin *et al* proposed that IRE1α affects bacterial replication via activation of autophagy to promote eBCV to rBCV conversion, but this was not further examined (Qin *et al.*, 2008). In contrast to this model, depletion or deletion of various host proteins involved in the canonical cascade of autophagosome biogenesis, namely Beclin1, ULK1, LC3B, ATG5, ATG7, ATG16L1 and ATG4B, does not impair rBCV biogenesis in either HeLa cells or primary macrophages, arguing against a role of canonical autophagy in this step of the *Brucella* intracellular cycle (Starr *et al.*, 2012).

Three recent studies have however shed new light on the potential roles of autophagy and the UPR in rBCV biogenesis and *Brucella* replication (de Jong *et al.*, 2013; Smith *et al.*, 2013; Taguchi *et al.*, 2015). First, they all showed that *Brucella* infection of macrophages or HeLa cells induces activation of only IRE1α in the case of *B. abortus* (de Jong *et al.*, 2013; Taguchi *et al.*, 2015), and IRE1α, ATF6 and PERK in the case of *B. melitensis* (Smith *et al.*, 2013). While the discrepancy in the UPR pathways activated by different *Brucella* species needs to be reconciled, these findings further implicate IRE1α in *Brucella* replication. These studies did not determine whether UPR activation precedes rBCV biogenesis, or results from bacterial replication in the ER. Yet, Taguchi *et al.* detected IRE1α activation as early as 4 h post infection, which argues for UPR induction at the eBCV stage(Taguchi *et al.*, 2015). Indeed, phosphorylation of IRE1α upon *B. abortus* infection occurs via Yip1A, a host protein that binds to the COPII coat components, Sec23 and Sec24, and localizes to ERES. Both IRE1α and Yip1A are required for rBCV biogenesis and *Brucella* replication(Taguchi *et al.*, 2015). Interestingly, Yip1A-dependent activation of IRE1α was associated with an upregulation of COPII components and the GTPase Sar1 (Taguchi *et al.*, 2015), suggesting

an enhancement of vesicle budding at ERES, consistent with the requirement for Sar1 in rBCV biogenesis (Celli *et al.*, 2005). Moreover, Yip1A-dependent activation of IRE1α also triggered formation of large vacuoles, whose occurrence also depended upon the autophagy-associated proteins ATG9 and WIPI (Taguchi *et al.*, 2015). Depletion of either ATG9 or WIPI, but not the autophagy-associated protein DFCP1, inhibited rBCV biogenesis and restrained *B. abortus* in eBCVs(Taguchi *et al.*, 2015). The emerging picture from these findings is that IRE1α activation upon *Brucella* infection occurs at ERES and leads to the formation of vacuoles of autophagic origin that may promote eBCV to rBCV conversion, a scenario consistent with ERES being a site of autophagosome biogenesis (Carlos Martín Zoppino *et al.*, 2010; Graef *et al.*, 2013; Wang *et al.*, 2014). ATG9 and WIPI requirement for this process provides a strong argument for a role of autophagy in rBCV biogenesis, but it will be important to further define if other autophagy-associated proteins are required, since many key complexes involved in initiation and elongation of canonical autophagosomes have been ruled out (Starr *et al.*, 2012). It is therefore possible that *Brucella* subverts a subset of autophagy-associated proteins, including ATG9 and WIPI, to promote accretion of ER-derived membranes on the eBCV and its conversion into an rBCV, in a process that may functionally differ from canonical autophagy.

Whether *Brucella* infection triggers a complete or partial UPR remains to be established. Studies using *B. abortus* have only observed activation of IRE1α and linked it to host cell sensing of VirB T4SS-mediated delivery of the effector, VceC (de Jong *et al.*, 2013). By contrast, Smith *et al.* have observed activation of all three arms of the UPR in *B. melitensis*-infected macrophages, via delivery of the *Brucella* TIR-domain containing protein TcpB (Smith *et al.*, 2013), and also proposed that UPR activation benefits bacterial replication, since counteracting it with the chemical chaperone tauroursodeoxycholic acid (TUDCA), which facilitates protein folding, affects *B. melitensis* intracellular growth (Smith *et al.*, 2013). While this requires confirmation by more targeted means of inhibiting the UPR, it also remains to be determined whether this requirement of the UPR for bacterial replication only reflects activation of IRE1α, possibly as a sole means to promote rBCV biogenesis, or additional signaling that may benefit bacterial growth within the rBCV. It is worth noting that additional effectors (BspC, BspG, BspH, BspK) also trigger ER stress upon ectopic expression in HeLa cells (Myeni *et al.*, 2013), suggesting that the mechanisms of UPR induction by *Brucella* are more complex than currently envisioned.

The recent findings that autophagy-associated proteins are required for rBCV biogenesis have highlighted the possibility that *Brucella* subverts specific components of host cell membrane trafficking pathways to undergo its intracellular cycle. In agreement, Starr *et al.* observed that, by 48h post infection, following extensive replication in the ER bacteria become enclosed in multi-membrane structures consistent with autophagosomes (Starr *et al.*, 2012). Named aBCVs for autophagic BCVs, these vacuoles form both in macrophages and epithelial cells, contain one to many bacteria, and functionally differ from rBCVs as they do not display ER markers, but instead acquire late endosomal features consistent with a maturing autophagosome (Starr *et al.*, 2012). rBCV to aBCV conversion requires a subset of autophagy-associated proteins, since depletion of the autophagy initiation proteins Beclin1, ULK1 and ATG14L, but not of the autophagy elongation proteins ATG5, ATG7, LC3B,

ATG16L1 or ATG4B, abolishes aBCV formation (Starr *et al.*, 2012). These findings further exemplify the ability of *Brucella* to exploit specific membrane trafficking complexes involved in autophagy to promote its intracellular cycle. Importantly, aBCVs correlate with bacterial release and cell-to-cell spread, since blocking aBCV biogenesis reduced formation of infection foci in HeLa cells (Starr *et al.*, 2012), arguing that aBCVs contribute to completion of the *Brucella* cycle following its proliferation in the ER. How aBCVs form without requiring canonical autophagy elongation complexes remains to be established but is not inconsistent with reports of non-canonical, ATG5- and LC3-independent autophagy processes (Collins *et al.*, 2009; Nishida *et al.*, 2009). Altogether, these recent studies highlight complex interactions of BCVs with autophagy-associated membrane trafficking processes. Future studies need to address the molecular details of these critical trafficking steps, including the identification of bacterial factors that modulate the functional evolution of BCVs.

Concluding remarks

Major advances in our understanding of the *Brucella* intracellular cycle have recently been made regarding the evolutive nature of the BCV and host factors that are required for its functional transition from an endosomal to an ER-derived organelle to an autophagic vacuole. These findings highlight the complexity of the bacterium's cycle within mammalian cells and how studying this bacterium constitutes, beyond the need to understand its pathogenesis, a rich model of bacterial subversion of cell biological processes. Future challenges in this field are to identify the bacterial effectors that modulate the host processes involved in BCV trafficking, and characterize their mode of action to comprehend *Brucella* molecular mechanisms of its pathogenesis.

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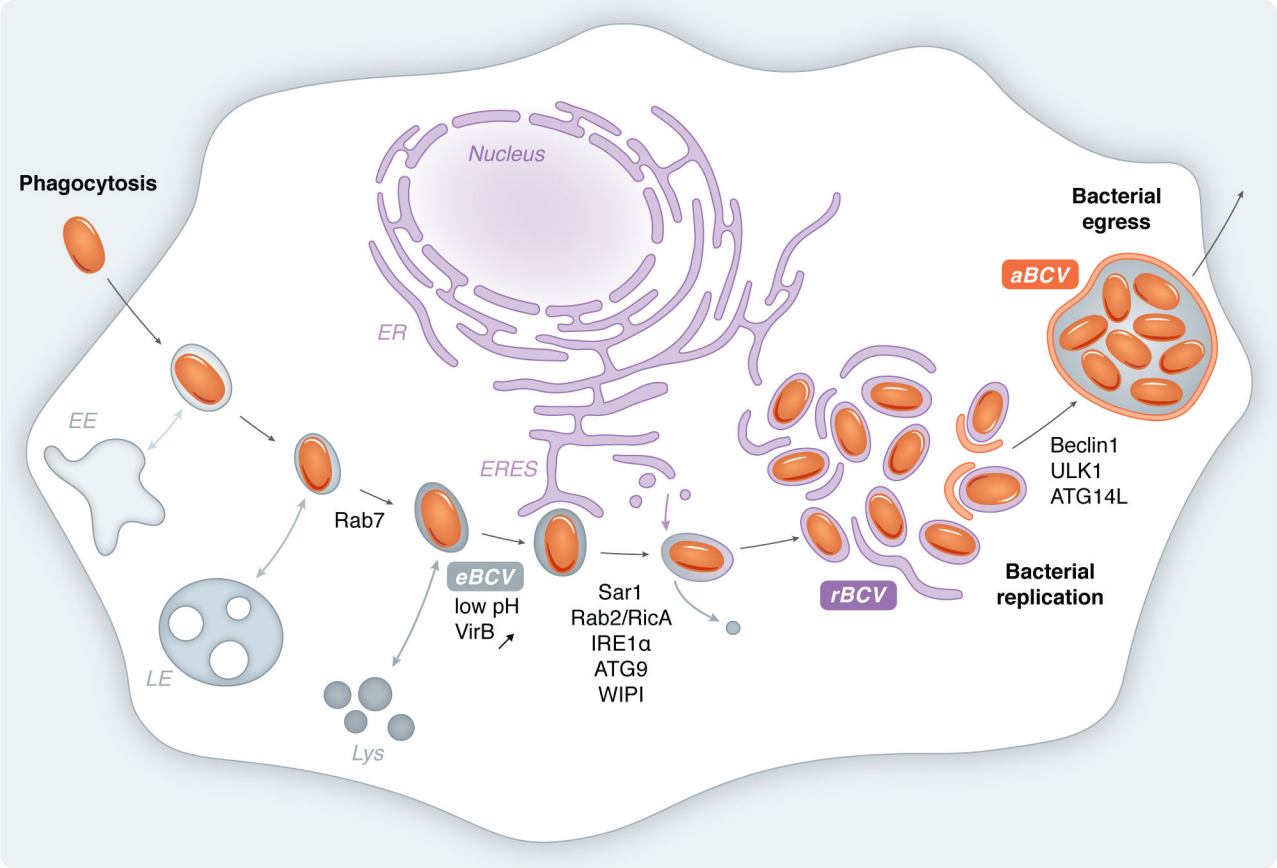

Figure 1. Model of *Brucella* intracellular trafficking in mammalian cells

Upon phagocytosis, *Brucella* resides within the *Brucella*-containing vacuole (BCV), which undergoes interactions with early endosomes (EE), late endosomes (LE) and partially fuse with lysosomes (Lys) to become the eBCV. The eBCV provides cues for induction of the VirB Type IV secretion system (T4SS), which deliver effector proteins that control eBCV interactions with ER exit sites (ERES). Activation of the UPR sensor IRE1α triggers formation of autophagic vesicles in a ATG9 and WIPI-dependent manner, that are thought to fuse with eBCV to promote rBCV biogenesis via accretion of ER-derived membranes and exclusion of endosomal membranes. The small GTPases Sar1 and Rab2 (via its interaction with the *Brucella* effector RicA) are required for rBCV biogenesis and subsequent bacterial replication in rBCVs. Following replication in the ER, rBCVs are converted into autophagic aBCVs via a process involving the autophagy initiation proteins Beclin1, ULK1 and ATG14L. aBCVs promote completion of the *Brucella* intracellular cycle by facilitating bacterial egress.