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Biogenesis of the lysosome-derived vacuole containing *Coxiella burnetii*

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Abstract

Coxiella burnetii utilizes a Type IV Secretion System (T4SS) to modify host endomembrane transport systems to form a unique lysosome-derived niche called the *Coxiella*-containing vacuole (CCV). Although the CCV has lysosomal properties, this organelle displays distinct characteristics such as homotypic fusion and a cholesterol enriched limiting membrane, in addition to robustly interacting with autophagosomes. This review describes recent advances in understanding CCV biogenesis and the mechanisms *C. burnetii* employs to maintain this unique compartment.

Keywords: *Coxiella burnetii*; Autophagy; Traffic; T4SS; *Coxiella*-containing vacuole

1. Introduction

Coxiella burnetii is a Gram-negative, intracellular, bacterial pathogen that causes the disease Q Fever [14]. Macrophages internalize *C. burnetii*, which results in the formation of a phagosome that matures canonically along the endocytic pathway. This compartment develops into a lysosome-derived organelle called the *Coxiella*-containing vacuole (CCV). The lumen of the mature CCV is acidic, contains active lysosomal proteases and the vacuole membrane contains lysosomal proteins such as the vacuolar ATPase, LAMP-1, and CD63 [19,29].

C. burnetii requires a Dot/Icm Type IVB Secretion System (T4SS) to replicate inside of the CCV. The *C. burnetii* T4SS translocates approximately 130 bacterial effector proteins across the CCV membrane and into the host cell [5,6,42,25,43]. *C. burnetii* strains containing loss-of-function mutations in essential *dot* or *icm* genes cannot replicate inside host cells [1,5]. It is assumed that some T4SS effectors modify traffic of host endomembrane transport vesicles because the CCV itself is a modified lysosome. Few *C. burnetii* T4SS effectors have been functionally connected to a host pathway, let alone assigned a specific biochemical activity [23,27,35,22,8]. Accordingly, many details of how *C. burnetii* alters membrane transport in the host to facilitate CCV biogenesis remain unclear.

Here, we review the contributions of host transport pathways including endocytosis, cholesterol transport, autophagy, and membrane fusion to CCV biogenesis, and *C. burnetii* T4SS effectors that modify these pathways (Fig. 1). We hope to underscore the unique nature of the CCV and how investigation of its specialized characteristics will improve our understanding of *C. burnetii* pathogenesis and fundamental cellular processes that control lysosome biogenesis.

2. Role of the endocytic pathway in initiating CCV biogenesis

Genetic and chemical approaches demonstrate that initial transport of *C. burnetii* to the lysosome is mediated by host endocytic machinery. Mutants deficient in Dot/Icm transporter function are phagocytosed by host cells and transported to lysosomes but cannot form mature CCVs because translocation of T4SS effectors is blocked. These mutants survive in tight-fitting phagolysosomes for several days after infection

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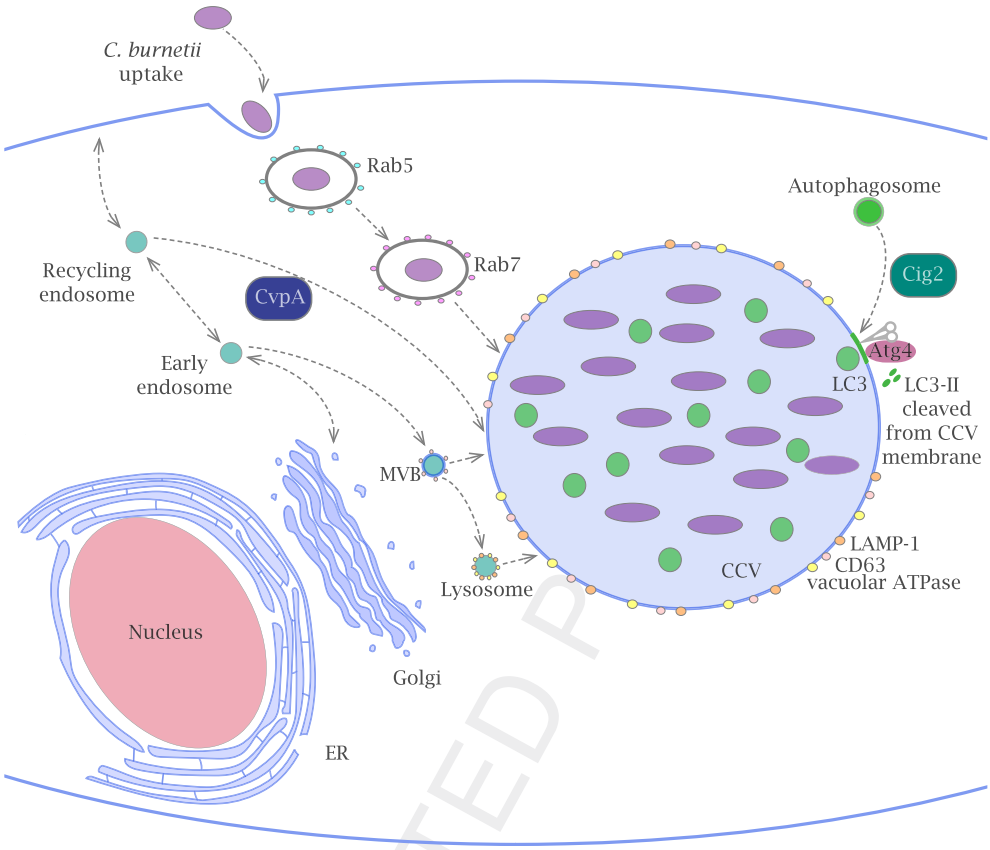

Fig. 1. *Coxiella burnetii* transport and interactions with host endosomal organelles. Upon uptake, *C. burnetii* resides in an endocytic vacuole that is transported to lysosomes by a process that requires the host endocytic machinery and Rab proteins (Rab5 and Rab7) that regulate early and late endosomal fusion events. Once in an acidified compartment, *C. burnetii* activates the Dot/Icm system and initiates translocation of an estimated 130 bacterial effector proteins into the host cell cytosol. The effector protein Cig2 promotes robust fusion of autophagosomes with the CCV, and locks the compartment in an autolysosomal state of maturation that is necessary for the process of CCV homotypic fusion. The interaction between the effector protein CvpA and the host clathrin adaptor AP-2 promotes interactions between the CCV and endocytic recycling vesicles. Cholesterol enrichment of the CCV is likely facilitated by fusion of cholesterol-rich organelles with the CCV, however, effectors that modulate cholesterol remain to be identified (compartments enriched in cholesterol are depicted in blue and include the plasma membrane, Golgi apparatus, ER, MVB, and CCV).

[1,28,33,5]. When expression of the *icmDJB* operon is induced one day after infection with a mutant having a transposon insertion in the *icmD* gene, the mutant regains the ability to create a vacuole that supports *C. burnetii* replication. This indicates that effector proteins delivered by the T4SS are not required for modulation of early endocytic processes that occur before bacteria are transported to a lysosomal compartment [1]. Similarly, inhibition of bacterial protein synthesis with chloramphenicol does not block *C. burnetii* transit to the lysosome but prevents translocation of effectors [18]. On the host side, inhibition of phagosome acidification with bafilomycin A1 also blocks bacterial replication and prevents Dot/Icm-mediated translocation of effectors [34]. Additionally, it takes several hours after infection until delivery of effectors into the host cell cytoplasm can be detected [34]. Multiple *C. burnetii* effectors produced constitutively from a plasmid have been analysed for translocation kinetics, and translocation for all was shown to be dependent on endocytic transport and vacuole acidification. These data indicate that the host endocytic machinery delivers *C. burnetii* to an acidified organelle by a process that does not require bacterial effector proteins, and that the Dot/Icm system begins to deliver effector proteins into the host cell after *C. burnetii* senses the environment of a late-endocytic organelle. Although these data do not rule out the possibility that there are specialized effectors that might be translocated before *C. burnetii* senses a late endocytic compartment, this seems unlikely as bacteria are not metabolically active in early endocytic compartments, which would limit their capacity to energize the T4SS.

Several members of the Rab GTPase family are required for transit of *C. burnetii* through the endocytic pathway. Rab GTPases associate with intracellular membranes and promote interactions between components of membrane transport machinery, such as SNARE proteins and coat components. Rab5 and Rab7 are essential regulators of membrane fusion with early and late endosomes, respectively. Rab5 associates with phagosomes containing *C. burnetii* within minutes of infection and promotes fusion with early endosomes. Next, Rab7 associates with the CCV to promote fusion with late endosomes and remains associated with the mature CCV [34]. Importantly, interfering with the function of these Rab proteins,

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