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Host-pathogen reorganisation during host cell entry by *Chlamydia trachomatis*

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Abstract

Chlamydia trachomatis is obligate intracellular bacterial pathogen that remains a significant public health burden worldwide. A critical early event during infection is chlamydial entry into non-phagocytic host epithelial cells. Like other Gram-negative bacteria, *C. trachomatis* uses a type III secretion system (T3SS) to deliver virulence effector proteins into host cells. These effectors trigger bacterial uptake and promote bacterial survival and replication within the host cell. In this review, we highlight recent cryo-electron tomography that has provided striking insights into the initial interactions between *Chlamydia* and its host. We describe the polarised structure of extracellular *C. trachomatis* elementary bodies (EBs), and the supramolecular organisation of T3SS complexes on the EB surface, in addition to the changes in host and pathogen architecture that accompany bacterial internalisation and EB encapsulation into early intracellular vacuoles. Finally, we consider the implications for further understanding the mechanism of *C. trachomatis* entry and how this might relate to those of other bacteria and viruses.

Keywords: Chlamydia; Type III secretion; Entry; Cytoskeleton; Cryo-electron tomography

1. Introduction

Chlamydia trachomatis is an obligate intracellular bacterial pathogen. *Chlamydiae* cause diseases in humans and other animals, and in particular *C. trachomatis* remains the leading bacterial cause of sexually transmitted disease worldwide [1], while ocular infections cause blinding trachoma, which is designated as a neglected tropical disease by the World Health Organisation [2].

In common with other bacterial pathogens, a critical early step in chlamydial infection is the interaction of infectious but metabolically inactive extracellular elementary bodies (EBs) with the host cell plasma membrane. Adherent EBs trigger host actin reorganisation and membrane deformation, and rapidly internalise into endocytic vacuoles. These early bacteria-containing vacuoles then coalesce and traffic to the microtubule-organising centre, where they fuse to form a single specialised membrane-bound compartment termed an inclusion that remains segregated from the host endosomal pathway. Subsequently, EBs differentiate to form metabolically active reticulate bodies (RBs), which divide by binary fission before re-differentiating into EBs. Infectious EBs are then released from the host cell by inclusion extrusion or upon cell lysis [3]. In this review, we describe recent insights into EB structure and the morphological changes in pathogen and host that accompany EB internalisation. We discuss the implications for understanding the mechanism of *C. trachomatis* entry into host cells.

C. trachomatis EBs are atypically small Gram-negative cocci 0.3–0.4 μm in diameter. A long-recognised distinctive structural characteristic is their outer membrane, which is twice the normal thickness [4]. This is most likely due to the disulphide cross-linked network of major outer membrane proteins that confer the osmotic stability and rigidity essential

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for their extracellular lifestyle [5,6]. Both EBs and RBs possess type III secretion systems (T3SSs), envelope-spanning nanomachines conserved among diverse Gram-negative bacterial pathogens. T3SSs translocate virulence effector proteins directly into host cells, where they subvert cellular processes to promote bacterial entry, survival and replication [7]. Although it is not possible to selectively mutate T3SS-associated genes in *Chlamydiae*, chemical inhibition of T3SSs attenuates chlamydial entry and intracellular replication, arresting the bacterial lifecycle [8,9]. This demonstrates the importance of T3SS effectors at multiple stages of the chlamydial developmental cycle.

2. The polarised architecture of *C. trachomatis* EBs

Early electron microscopy studies of chlamydial EBs in the absence of host cells by Matsumoto identified surface projections and surface complexes termed 'rosettes' [*e.g.* Ref. [10]]. Although these were later proposed to be T3SSs [11], the rosettes visualised by negative staining of the isolated *Chlamydia psittaci* envelope were also suggested to be outer membrane protein complexes [12]. Recently we have applied cryo-electron tomography to examine the structure of EBs in greater detail, both in isolation and during their entry into host cells [13]. This revealed that EBs have an inherently polarised architecture (Fig. 1). One bacterial hemisphere is characterised by a pronounced widening of the periplasmic space (~29 nm compared to ~14 nm on the opposite pole) that accommodates a semi-ordered array of 14–20 trans-periplasmic complexes with an average spacing of 56.5 nm ± 1.0 nm. Each complex originates at a distinct concave deformation of the inner membrane and contains a short ~30 nm needle-like filament that protrudes from the rigid bacterial outer membrane. The overall size and shape of these complexes are consistent with the T3SS, and labelling of the *Chlamydia* T3SS needle-forming protein (CdsF) [14] by immuno-gold electron microscopy demonstrated a similarly polarised distribution, confirming these complexes as T3SSs for the first time [13]. To date, this polarised battery of T3SSs is unique to *Chlamydia*, as other Gram-negative bacterial pathogens typically distribute their T3SSs evenly around the entire bacterial surface [*e.g.* Ref. [15]]. This specialised localisation might permit *Chlamydia* to concentrate the delivery of translocated effectors into the host cell cytosol, potentially enhancing the speed and efficacy of downstream effects such as actin polymerisation, membrane deformation, or the subversion of other host signalling pathways central to its intracellular lifestyle.

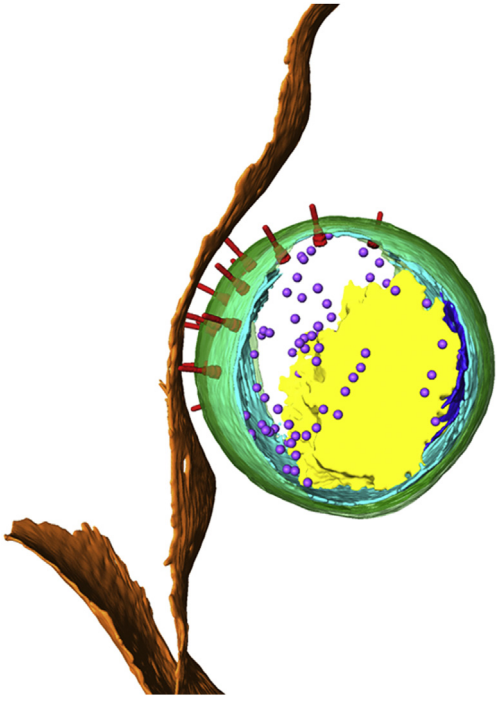

Fig. 1. **Polarised structure of the *Chlamydia trachomatis* elementary body in contact with the host cell**. Three-dimensional surface representation of a *Chlamydia trachomatis* elementary body in contact with the host cell, generated from segmentation of a cryo-electron tomogram. Cellular plasma membrane (orange), bacterial outer membrane (green), inner membrane (cyan), inner membrane invagination (blue), T3SS (red), ribosomes (purple) and DNA nucleoid (yellow) are shown.

The opposite pole with a narrower periplasmic space contains additional complexes of distinct morphology but unknown composition. These comprise trans-periplasmic bands of density with an average spacing of 14.5 nm ± 2.8 nm. In addition, an invagination of the inner membrane is present. This is an elongated membrane tubule in the native state, and can adopt a spherical topology after EBs are stressed by freeze-thaw, indicating a degree of morphological plasticity. In both states this is a significant structure, as the surface area is equivalent to 10–12% of the total inner membrane [13]. Although its function remains unknown, the invagination is perhaps reminiscent of the complex organelle-like membrane structures present in other members of the *Planctomycetes-Verrucomicrobia-Chlamydiae* [16].

3. EB-host interactions during early stages of cell entry

When visualised in the presence of host cells, all the EBs including those not directly adjacent to a host cell, oriented their T3SS array towards the host plasma membrane [13]. Whether this positioning and ensuing T3SS needle contact requires additional engagement of host receptors or polysaccharides implicated in chlamydial adhesion remains to be determined, although it is tempting to speculate that bacterial outer membrane adhesins such as OmcB and the family of polymorphic membrane proteins (Pmps) might also be polarised on the EB surface [17,18]. Strikingly, needles of the T3SS were frequently captured in direct contact with the host plasma membrane, providing a first view of the initial events that occur during effector translocation (Fig. 1) [13].

Our cryo-electron tomography also captured an unexpected diversity of early entry structures including phagocytic cups that tightly zipper around individual EBs. Distinct loops of membrane, from which actin filaments emanate, pinch away from these phagocytic cups, potentially providing one of the driving forces necessary for EB internalisation [13]. *C.*