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Review article

Crossing the border - Solute entry into the chlamydial inclusion

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ABSTRACT

Chlamydiales comprise important human and animal pathogens as well as endosymbionts of amoebae. Generally, these obligate intracellular living bacteria are characterized by a biphasic developmental cycle, a reduced genome and a restricted metabolic capacity. Because of their metabolic impairment, *Chlamydiales* essentially rely on the uptake of diverse metabolites from their hosts. *Chlamydiales* thrive in a special compartment, the inclusion, and hence are surrounded by an additional membrane. Solutes might enter the inclusion through pores and open channels or by redirection of host vesicles, which fuse with the inclusion membrane and release their internal cargo. Recent investigations shed new light on the chlamydia-host interaction and identified an additional way for nutrient uptake into the inclusion. Proteome studies and targeting analyses identified chlamydial and host solute carriers in inclusions of *Chlamydia trachomatis* infected cells. These transporters are involved in the provision of UDP-glucose and biotin, and probably deliver further metabolites to the inclusion. By the controlled recruitment of specific solute carriers to the inclusion, the chlamydial resident thus can actively manipulate the metabolite availability and composition in the inclusion. This review summarizes recent findings and new ideas on carrier mediated solute uptake into the chlamydial inclusion in the context of the bacterial and host metabolism.

1. Introduction

The *Chlamydiales* order exclusively comprises obligate intracellular bacteria living in eukaryotic cells (Everett, 2000). *Chlamydia trachomatis* and *Chlamydia pneumoniae* represent the major human pathogens of the well-known family *Chlamydiacea*e (Mandell et al., 2010; Sachse et al., 2015). *C. trachomatis* is a leading cause of sexually transmitted diseases worldwide and genital tract infections may result in inflammations, infertility or ectopic pregnancy. Moreover, eye infections with *C. trachomatis* are still an important problem in many parts of the developing world and repeated, untreated Trachoma can lead to damages of the cornea and even blindness. *C. pneumoniae* may infect the respiratory tract and be responsible for community-acquired pneumonia but also seems to be associated with a wide range of chronic diseases (Mandell et al., 2010; Roulis et al., 2013). The remaining members of the *Chlamydiaceae* family rather infect animals (Everett, 2000) and comprise several pathogenic strains of veterinary importance (Longbottom and Coulter, 2003; Wheelhouse and Longbottom, 2012). However, some of these chlamydiae are occasionally transmitted to human and may cause zoonotic diseases (mainly *C. psittaci*) (Longbottom and Coulter, 2003; Wheelhouse and Longbottom, 2012).

During the past years, more and more *Chlamydia*-related species have been identified. These so-called “environmental chlamydiae” were detected in a comparatively diverse range of host cells, including amoebae, fish, insects, reptiles and mammals (Horn, 2008; Wheelhouse and Longbottom, 2012). Their considerable and previously highly underestimated variability led to the proposal of many new families of the order *Chlamydiales* (Everett, 2000; Kuo and Stephens, 2011; Lagkouvardos et al., 2014). It is necessary to mention, that the commonly used terms “pathogenic chlamydiae” for *Chlamydiaceae* and “environmental chlamydiae” for the remaining *Chlamydiales* may be misleading. Some *Chlamydiaceae* species apparently exhibit a broader host range (Horn, 2008); *C. pneumoniae* for example can even survive in amoebae (Essig et al., 1997). Moreover, an increasing number of observational studies, such as the detection of *Waddlia chondrophila* (*Waddliaceae*) in association with miscarriage in pregnant women (Baud et al., 2007; Baud et al., 2014) or of *Simkania negevensis* (*Simkaniaceae*) in different clinical samples (Vouga et al., 2017), led to the suggestion that also certain “environmental chlamydiae” might represent emerging human pathogens (Ammerdorffer et al., 2017; Corsaro and Greub, 2006). The in depth analysis of representative *Chlamydia*-related species helps to identify characteristic features of the individual organisms and might provide new insights into the evolution of pathogenicity.

Although recent studies revealed a high diversity among *Chlamydiales*, all characterized members seem to share a similar developmental cycle (AbdelRahman and Belland, 2005; Hatch, 1999; Pilhofer et al., 2014). It is generally biphasic and starts with the

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elementary body (EB), which is the infectious and non-dividing form. The small and comparatively robust EB is apparently well equipped to cope with unfavorable conditions during transmission. After attachment to and uptake by the host cell, the internalized EB remains within the host-derived vacuole, termed inclusion. In this specialized compartment, the EB differentiates into the reticulate body (RB). The RB is generally larger and more fragile than the EB and can multiply by binary fission. After several rounds of replication, the RB differentiates into the EB that exits the host cell and initiates another cycle. Under certain stress conditions, the RBs of the *Chlamydiaceae* members and of *Simkania negevensis* do not differentiate into EBs but alternatively enter a viable but non-culturable state (Hogan et al., 2004; Vouga et al., 2017). Whether this persistent stage is an adaptation to unfavorable environmental conditions or a strategy to overcome host cell immune mechanisms or plays a different role *in vivo* is not completely clarified yet.

A physiologically very important common characteristic of *Chlamydiales* is their limited metabolic capacity (Omsland et al., 2014). Members of the *Chlamydiaceae* family generally possess the smallest, most reductive genomes (Collingro et al., 2011). The considerable genome size reduction is associated with the deletion of various biosynthetic pathways (Omsland et al., 2014). To compensate for the corresponding physiological deficits, *Chlamydiaceae* tap the metabolite pool of the host cell and import the missing compounds directly. This high level of metabolic rationalization results in a strict dependence on the host and was probably supported by the constant environment and stable milieu in the mammalian cell.

Analyses of representative “environmental chlamydiae” suggest that non-*Chlamydiaceae* possess larger and quite divergent genomes (Collingro et al., 2011). Moreover, they generally exhibit extended biosynthetic capacities and hence seem to be more flexible and metabolically less dependent on the host. However, they still rely on the uptake of various metabolites, such as specific amino acids, nucleotides and cofactors, from the host cell (Omsland et al., 2014).

Recent investigations provided interesting insights into developmental-stage specific differences of the chlamydial metabolism (König et al., 2017; Omsland et al., 2012; Saka et al., 2011). Although the EB was initially considered as metabolically inert, it exhibits host-free metabolic activity under appropriate conditions (Omsland et al., 2012; Sixt et al., 2013). The EB generally exhibits lower and more limited metabolic activity when compared to the RB; however, it is clearly capable of carbohydrate catabolism and ATP production. Cellular reproduction involves increased synthesis of biomolecules and consumption of metabolites. Consequently, the RB shows not only significantly higher metabolic activity than the EB but also a metabolic shift towards exploitation of specific host solutes, including the energy currency ATP (König et al., 2017; Omsland et al., 2012; Saka et al., 2011). Moreover, the EB of most characterized *Chlamydiales* exhibits a high degree of disulfide-crosslinking of the outer membrane, which decreases during EB to RB differentiation and thus results in a higher fluidity and increased permeability of the outer membrane of the RB (Hatch et al., 1986; Omsland et al., 2014; Aistleitner et al., 2015).

2. *Chlamydiales* rearrange the inclusion according to their specific demands

Intracellular establishment, differentiation and proliferation of chlamydiae are associated with several molecular, morphological and physiological alterations of their host cells (Elwell et al., 2016). Rapidly upon contact with the host cell the EB already secrets invasion-related effectors (via its type III secretion system) presumably inducing host signaling and profound alterations at the host plasma membrane (Mueller et al., 2014; Peters et al., 2007). Subsequent to invasion, the protein composition of the nascent inclusion becomes substantially modified by insertion of specific secreted effectors, the inclusion membrane proteins (Incs) (Rockey et al., 2002; Collingro et al., 2011). Inc proteins are supposed to act as structural components, to fulfill functions in biogenesis of the inclusion and to interact with host cell components (Mital et al., 2013). They mediate the recruitment of different host proteins and vesicles (e.g. exocytic vesicles) to the inclusion and participate in different processes, such as the interaction with the host cytoskeleton, the formation of ER-inclusion contact sites, lipid acquisition, the homotypic fusion of inclusions or the prevention of their fusion with lysosomes (Elwell et al., 2016). Therefore, Inc proteins play an important role in the establishment of an intracellular niche that renders survival and efficient proliferation of chlamydiae in the host cell possible.

Although the inclusion provides some kind of shelter, it can also be considered as a metabolite barrier (Heinzen and Hackstadt, 1997). However, to guarantee efficient proliferation and survival, chlamydiae have to get access to diverse metabolites and this has to happen in accordance with the respective physiological demands of the bacterium. Selective interception of Golgi-derived vesicles and multivesicular bodies as well as the recruitment of lipid droplets and host lipid transfer proteins may deliver host lipids to the inclusion (Elwell and Engel, 2012). Membrane fusion events between the inclusion and host-derived vesicles (possibly rerouted from the secretory pathway) were suggested to provide also soluble nutrients to the bacterium (Saka and Valdivia, 2010). However, the cargo composition of many intracellular vesicles is still unknown and it is questionable whether their hijacking provides the bacterium with all required metabolites. Alternatively, the inclusion membrane might be equipped with transport proteins that mediate the selective passage of different solutes. In fact, proteome studies identified host-derived and bacterial transport proteins in the inclusion membrane (Aeberhard et al., 2015; Herweg et al., 2016; Herweg et al., 2015; Saka et al., 2011). Moreover, first studies indicate that host-derived solute transporters are involved in the uptake of specific metabolites into the *C. trachomatis* inclusion (Fisher et al., 2012; Gehre et al., 2016).

3. Nucleotide sugar uptake into the chlamydial inclusion

Glycogen is a multi-branched glucose polysaccharide and acts as an important energy storage compound in animals and humans, as well as in bacteria. *Chlamydia trachomatis*, *C. muridarum* (hamster, mouse) and *C. suis* (swine) possess the unique capacity to accumulate glycogen in the inclusion lumen (Everett, 2000; Gordon and Quan, 1965; Rogers et al., 1996).

Very recently, the luminal glycogen metabolism in the *C. trachomatis* inclusion was clarified (Gehre et al., 2016). Microscopic analyses indicate that the RB lacks intra-bacterial glycogen but apparently initiates the formation of extra-bacterial glycogen in the inclusion lumen. This glucose reservoir seems to be tapped to build up the intra-bacterial glycogen stock detectable in the EB (Gehre et al., 2016). Utilization of host cells (siRNA) and bacteria (mutagenesis) with defects in glycogen synthesis, microscopic and physiological studies suggest that two different mechanisms contribute to the accumulation of the luminal glycogen. A minor amount is probably translocated in bulk (via vesicles) from the host cytoplasm into the inclusion. The majority however is synthesized *de novo* involving the bacterial glycogen synthase (GlgA) and the corresponding branching enzyme (GlgB), which are secreted into the inclusion (Gehre et al., 2016; Lu et al., 2013).

GlgA produces the linear chain of α1,4-linked glucose molecules by introducing the glucose moiety from nucleotide sugars. Bacterial glucan synthases generally utilize ADP-glucose and release ADP whereas in animals and humans, UDP-glucose represents the precursor and hence UDP is released. Surprisingly, the chlamydial enzyme for ADP-glucose synthesis, the ADP-glucose phyrophosphorylase (GlgC), is apparently not secreted (Gehre et al., 2016). Therefore, it was not immediately clear how GlgA could obtain its substrate. Interestingly, GlgA from *C. trachomatis* accepts UDP-glucose as an additional substrate. Moreover, host-derived UDP-glucose was identified to represent the precursor for