Analysis of the secretion of type III secretion effectors of the human pathogen *Chlamydia trachomatis*

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Chlamydiales are a large group of highly related obligate intracellular bacteria that includes the human pathogens *Chlamydia trachomatis* and *Chlamydophila pneumoniae*. *C. trachomatis* is an agent of genital and ocular infections, and *Cph. pneumoniae* causes atypical pneumonias. Chlamydiae are characterised by a unique infectious cycle that includes two morphological forms: the infectious but metabolically inert elementary bodies (EBs), and the non-infectious but metabolically active reticulate bodies (RBs). Infection begins with the attachment of EBs to the surface of susceptible cells, normally epithelial cells. This results in localised actin reorganisation and internalisation of EBs. Once internalised, the EBs reside in a membrane-bound vacuole, termed an 'inclusion'. Within minutes after uptake, EBs start differentiating into RBs, which undergo repeated cycles of binary fusion. As the inclusion expands, bacterial replication yields both RBs and EBs, and ultimately EBs exit the host cell. The released EBs can then infect neighbouring cells.

Relatively little is known about the molecular and cellular mechanisms by which chlamydiae thwart host cells because they are intractable to genetic manipulation. However, all chlamydiae code for the core components of a type III secretion system (T3SS), a protein transport mechanism used by several pathogenic Gram-negative bacteria to translocate 'effector' proteins from the bacterial cytoplasm directly into the cytosol or membranes of eukaryotic host cells. Chlamydiae translocate at least ~ 60-90 type III secretion (T3S) effector proteins into host cells. These effectors are thought to play crucial roles thorough the chlamydial infectious cycle but not much is known about their functions. To identify and understand the function of the complete set of chlamydial T3S effectors is a tremendous challenge. It promises major breakthroughs in our knowledge of how chlamydiae, and intracellular pathogens in general, manipulate their hosts. Furthermore, it will certainly reveal novel and unique aspects of fundamental cell biology and might also help to combat microbial infectious diseases.

The putative chlamydial ~ 60-90 T3S effectors include the Inc proteins, which have a 40-60 residues-long bi-lobal hydrophobic domain that is a very good indicator that they localise to the inclusion membrane. In the proposed project, we will explore the ability of T3SSs to recognise substrates from heterologous systems and use *Yersinia enterocolitca* to test the hypothesis that all Inc proteins from *C. trachomatis* are type III secreted.

T3S substrates normally possess a non-cleavable secretion signal located within the first 20-30 amino acids. Therefore, hybrids consisting of a translational fusion between the N-terminal of candidate Inc proteins (excluding the hydrophobic domain) and a reporter protein [e.g. TEM-1 β-lactamase] will be constructed. Because of competition for the secretion machinery, endogenous T3S substrates of *Yersinia* could hamper secretion of Inc-reporter protein. Hence, a *Y. enterocolitica* effector poly-mutant strain and an isogenic T3S-deficient derivative will be constructed. It will then be tested if the N-terminal of the Inc proteins can direct the T3S of the chimeras in *Yersinia*. If relevant, this analysis could be expanded to other potential chlamydial T3S effectors. To provide insights on the nature of a possible 'universal' T3S signal and to help the identification of additional chlamydial T3S effectors, the efficiency

of secretion of the Inc chimeras will be compared to that of similar *Yersinia* effector hydrid proteins and of a *Yersinia* full-length T3S effector.

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