Identification of type III secretion effectors of *Chlamydia trachomatis*

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Chlamydiae are a large group of obligate intracellular bacteria that cause a wide range of diseases in both animals and humans. Of particular concern, infections with *Chlamydia trachomatis* are the most prevalent cause of preventable infectious blindness in developing countries and the major bacterial cause of sexually transmitted infections worldwide.

There is no established method to genetically manipulate chlamydiae and, therefore, the knowledge of the mechanisms used by these bacteria to thwart host cells is poor. However, all chlamydiae express an active type III secretion system (T3SS) throughout the infectious cycle. T3SS are used by many bacterial pathogens to manipulate eukaryotic host cells by translocating effector proteins into the host cell cytoplasm. The identity of the complete set of chlamydial T3S effectors is elusive but studies using other bacteria as surrogate hosts suggest that *C. trachomatis* might encode for over 70 effectors. We are currently developing *Yersinia enterocolitica* as a surrogate host to specifically and reliably identify chlamydial T3S effectors.

The aim of this project is to identify new T3S effectors of *C. trachomatis*. About 20 putative effectors will be expressed as appropriate hybrid proteins in *Y. enterocolitica* and their capacity to engage on the T3S pathway will be analysed. The candidate T3S effectors identified will be expressed as His-tagged or GST-tagged proteins in *Escherichia coli* and the more relevant one(s) will be purified by affinity chromatography. The purified protein(s) will be used to raise antibodies. These antibodies will allow testing the subcellular localisation of the candidate effector(s) in human epithelial HeLa cells infected with *C. trachomatis*. In parallel, candidate T3S effectors will be ectopically expressed in mammalian cells and their subcellular localisation and possible phenotype will be analysed. Technically, this will involve PCR, DNA cloning into plasmid vectors, SDS-PAGE, immunoblotting, protein expression and purification, mammalian cell culture, DNA transfection of mammalian cells, and immunofluorescence microscopy.