Biogenesis, function and regulation of the type III secretion translocon of *Yersinia pseudotuberculosis*

Salah I. Farag

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till försvaret i Hörsal 933 Unod B 9, Norrlands universitetssjukhus, Fredag den 06 December, kl. 09:00.

Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor, Luís Jaime Mota, Lissabons nya universitet, Portugal.

Department of Molecular Biology
Biogenesis, function and regulation of the type III secretion translocon of *Yersinia pseudotuberculosis*

**Abstract**

Many Gram negative bacteria use type III secretion systems to cross-talk with eukaryotic cells. Type III secretion system assembly and function is tightly regulated. It initiates with assembly of a basal body-like structure, and is followed by a cytoplasmic-located substrate sorting and export platform that first engages with early substrates required for needle assembly. At the needle tip, a translocon is formed upon eukaryotic cell contact to allow the translocation of effector proteins to the host cell. The focus of this thesis is on understanding aspects of biogenesis, regulation and function of the translocon and its interaction with the host cell. Research questions are addressed in enteropathogenic *Yersinia pseudotuberculosis* model.

Prioritising the secretion of translocon components before effector proteins is a task given partly to the InvE/MxiC/HrpJ family of proteins. In *Yersinia*, homology to this protein family is partitioned over two proteins; YopN and TyeA. Certain *Yersinia* strains naturally produce a single YopN/TyeA polypeptide hybrid. To understand the implications of hybrid formation towards type III secretion control, a series of mutants were engineered to produce only a single hybrid peptide. Using in vitro assays revealed no difference in substrate secretion profiles between parent and mutants. Moreover, no obvious prioritisation of secretion between translocator and effector substrates was observed. Although these in vitro studies indicate that the YopN-TyeA single polypeptide is fully functionally competent, these mutants were attenuated in the mouse infection model. Hence, natural production of YopN and TyeA as a single polypeptide alone is unlikely to confer a fitness advantage to the infecting bacteria and is unlikely to orchestrate hierarchical substrate secretion.

The YopB and YopD translocon components form a pore in the host cell plasma membrane to deliver the effectors into the host cell. To better understand how YopD contributes to the biogenesis, function and regulation of the translocon pore, a series of mutants were constructed to disrupt two predicted α-helix motifs, one lying at the N-terminus and the other at the C-terminus. Based upon phenotypes associated with environmental control of Yop synthesis and secretion, effector translocation, evasion of phagocytosis, killing of immune cells and virulence in a mouse infection model, the mutants were grouped into three phenotypic classes. A particularly interesting mutant class maintained full T3SS function in vitro, but were attenuated for virulence in a murine oral infection model. To better understand the molecular basis for these phenotypic differences, the effectiveness of RAW 264.7 cells to respond to infection by these mutants was scrutinised. Sixteen individual cytokines were profiled with mouse cytokine screen multiplex analysis. Signature cytokine profiles were observed that could again separate the different YopD mutants into distinct categories. The activation and suppression of certain cytokines that function as central innate immune response modulators correlated well with the ability of mutant bacteria to modulate programmed cell death and antiphagocytosis pathways. Hence, the biogenesis of sub-optimal translocon pores alters host cell responsiveness and limits the ability of *Yersinia* to fortify against attack by both early and late arms of the host innate immune response.

The amount of bacteria now resistant to multiple antibiotics is alarming. By providing insights into a common virulence process, this work may ultimately facilitate the design of novel broad-acting inhibitors of type III secretion, and thereby be useful to treat an array of bacterial infections.

**Keywords**

*Yersinia pseudotuberculosis*, type III secretion system, translocation, translocon, regulation, bacteria-eukaryotic cell contact, cytokine profiling