UMEÅ UNIVERSITET

# Coordinating type III secretion system biogenesis in *Yersinia pseudotuberculosis*

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## Akademisk avhandling

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**Abstract**
Various Gram-negative bacteria utilize type III secretion system (T3SS) to deliver effectors into eukaryotic host cells and establish mutualistic or pathogenic interactions. An example is the Ysc-Yop T3SS of pathogenic *Yersinia* species. The T3SS resembles a molecular syringe with a wide cylindrical membrane-spanning basal body that scaffolds a hollow extracellular needle with a pore-forming translocon complex crowned at the needle tip. Together they form a continuous conduit between bacteria and host cells that allow delivery of effector proteins.

Dedicated actions of cytoplasmic chaperones, regulators and components of the cytoplasmic complex orchestrates hierarchical assembly of T3SS. On the basis of secretion hierarchy, proteins can be categorized as 'early' needle complex proteins, 'middle' translocators and 'late' Yop effectors. However, how the system recognizes, prepares and mediates temporal delivery of T3S substrates is not fully understood. Herein, we have investigated the roles of YscX and YscY (present specifically in the Ysc family of T3SS), as well as YopN-TyeA (broadly distributed among T3SS families) to provide a better understanding of some of the molecular mechanisms governing spatiotemporal control of T3SS assembly.

Despite reciprocal YscX-YscY binary and YscX-YscY-SctV ternary interactions between the member proteins, functional interchangeability in *Yersinia* was not successful. This revealed YscX and YscY must perform functions unique to *Yersinia* T3SS. Defined domain swapping and site-directed mutagenesis identified two highly conserved cysteine residues important for YscX function. Moreover, the N-terminal region of YscX harboured an independent T3S signal. Manipulating the YscX N-terminus by exchanging it with equivalent secretion signals from different T3S substrates abrogated T3S activity. This was explained by the need for the YscX N-terminus to correctly localize and/or assemble the 'early' SctI inner adapter and SctF needle protein. Therefore, N-terminal YscX performs dual functions; one as a secretion signal and the other as a structural signal to control early stage assembly of T3SS.

In Ysc-Yop T3SS, YopN-TyeA complex is involved in the later stage of T3SS assembly, inhibiting Yops secretion until host cell contact is achieved. Analysis of the YopN C-terminus identified a specific domain stretching 279-287 critical for regulating Ysc-Yop T3SS activity. The regulation was mediated by specific hydrophobic contacts between $W_{279}$ of YopN and $F_8$ of TyeA.

In conclusion, this work has provided novel molecular mechanisms regarding the spatiotemporal assembly of T3SS. While the N-terminal region of YscX contributes to the early stage of T3SS assembly, the C-terminal region of YopN is critical for regulating Ysc-Yop activity at a later stage of T3SS assembly.

**Keywords**
*Yersinia pseudotuberculosis*, type III secretion system, YscX, YscY, YopN, TyeA, domain swapping, site-directed mutagenesis, N-terminal region, secretion signal

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