Determination of Complexes formed during protein secretion through Type III secretion system in Enteropathogenic *E. coli*

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The Type III secretion system (T3SS) is used by many Gram-negative pathogenic bacteria to deliver effector proteins directly into eukaryotic host cells (1). The T3SS machinery exports virulence factors from the cytoplasm through both the inner and the outer membrane to the external milieu. The proteins that are being secreted through this unique nanomachine are essential for the pathogenicity of the bacteria (2). Although T3SS is very common in gram-negative bacteria, little are known about its function and the precise pathway that proteins follow towards their secretion. In order to shed more light on the molecular mechanism of the T3SS, all the protein complexes that are formed during secretion through this specific secretion system need to be identified and correlated in a time dependent manner.

Towards this direction, we use in the present study the enteropathogenic *Escherichia coli* (EPEC) as a model organism. EPEC causes diarrhea disease and is lethal for children (3). In the present study, we analyzed the T3SS-related functional complexome of EPEC. In a global approach, cytosolic protein complexes were isolated and fractionated by two complementary approaches: Native polyacrylamide gel electrophoresis and size exclusion chromatography. In a targeted approach, selected His-tagged T3SS-proteins were used as baits for the selective isolation of T3SS- protein interactors. Protein subunits of the complexes were identified by "bottom-up" proteomics using an LTQ-Orbitrap. Protein quantification was performed using label-free approaches. Several potential T3SS pre-secretion protein complexes and interactions were thus identified in the EPEC cytosol against a background of the house-keeping complexome.

A series of complexes were predicted by computer-assisted pairwise and statistical analysis and were validated with immuno-detection with specific antisera and single gene knocked-out mutants. The accumulation of specific T3SS complexes in the cytoplasm was correlated in a time-dependent manner to the orderly secretion of specific sub-sets of secreted T3S proteins identified in the extracellular milieu. Our data reveal a dynamic exchange between T3SS components depending on the secretion state of the EPEC cell. Our pipeline is generally applicable to the dissection of cellular sub-complexomes in any cell.

References

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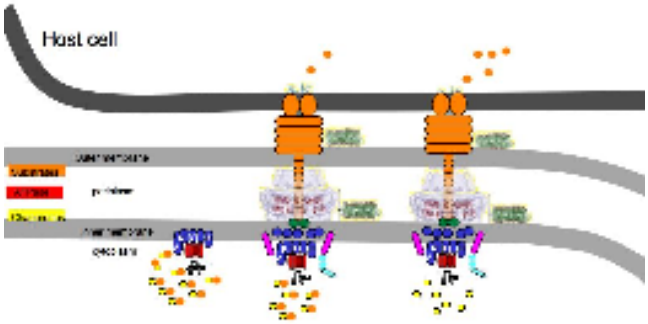

Figure: Protein complexes formed during secretion through T3SS