Dissecting Type III Protein Secretion Mechanism in Enteropathogenic *E. coli* by Dynamic Complexome and Secretome Analysis

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The type III secretion system (T3SS) is a highly specialized bacterial protein secretory pathway. It plays an essential role in the pathogenesis of many Gram-negative bacteria including *Yersinia*, *Shigella*, *Salmonella, Bordetella*, *Pseudomonas*, and Enteropathogenic *E. coli* (EPEC). T3SS is encoded by the locus of enterocyte effacement (LEE) and is used to inject both LEE and non-LEE effector proteins into the host cell, where these effectors modulate host key cellular processes. Although a plethora of studies have expanded our knowledge on the structure and function of T3SS and its secreted effectors, the precise mechanisms of type III protein secretion and translocation and their co-ordination remain poorly understood. To better understand these mechanisms, a comprehensive analysis of the protein complexes during Type III protein secretion is required. Here, 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. Proteins 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 knock-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.

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