ASSEMBLY OF THE TYPE III SECRETION SYSTEM

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Background: The Type III Secretion (T3S) System is a complex molecular nanomachine used by bacterial pathogens to transfer effector proteins into the cytosol of target host cells.

The assembly and function of the T3S machinery, the so-called injectisome, is a highly ordered and regulated process with more than 25 proteins involved. Although the global structure of the apparatus is known, the assembly of the system and its regulation remain largely elusive. Most importantly, data about structure, composition, and function of the transmembrane core is hard to obtain.

Objectives: Our aim is to elucidate the structure and order of assembly of the Type III Secretion System of Yersinia enterocolitica.

Methods: We applied two approaches:

1) Fluorescent reporter constructs were engineered to visualize the outer-membrane ring, the MS-ring and the C-ring of the injectisome. All three constructs were functional and combined by pair to validate the fact that they allow to visualize injectisome formation by fluorescence microscopy. They were then combined with an array of deletion mutations, which permitted to determine the prerequisites for the assembly of these three ring structures within the bacterial membrane.

2) Various substructures of the assembling injectisome were isolated, purified, and subsequently examined by Mass Spectrometry and Western Blot analysis.

Conclusions: Our results show the stepwise assembly of the T3S injectisome in the bacterial membrane. Importantly, the studies of early intermediary structures provide insight into the first steps of injectisome formation, that have been largely unknown so far. Evaluation of the proteins participating in assembly and structure of discrete subcomplexes of the injectisome further gives evidence for their functions in T3S.

These new findings concerning the assembly of the Type III Secretion System could be used to discern general rules that govern the formation and function of bacterial multimeric membrane protein complexes.