Project 05: Functional and structural characterization of putative substrates of type I secretion systems (Supervising Investigator: Lutz Schmitt)

Background: Type I secretion systems (T1SS) are widespread in Gram-negative bacteria and secrete a large variety of proteins carrying a C-terminal secretion signal. In general, T1SS are composed of an ABC transporter, a membrane fusion protein and an outer membrane factor that are organized in an operon. Furthermore, many substrates contain nonapeptide repeats (so-called GG repeats) that bind Ca^{2+} in the extracellular space and trigger folding of the substrate. The repeats form the RTX (repeats in toxins) domain, which coined the terms RTX protein family. The paradigm of T1SS is the hemolysin A (HlyA) T1SS. Here, HlyA, a 1024 amino acids sized pore-forming toxin is secreted in a step from the cytosol to the extracellular space, where it binds, folds and forms pore in the plasma membrane of human target cells.

Own previous work: Based on the operon organization and the presence of the RTX domain, we have selected 10 putative RTX proteins. For MbxA from *Moraxella bovis*, we have established the heterologous expression and secretion by the HlyA T1SS. The LC_{50} (lethal concentration at which 50% cells are dead) of epithelial and T-cells was within the low nanomolar range. We could also demonstrate these cells lysed within minutes through the appearance of membrane blebs.

Aim of the project: The project aims at identifying the cell surface receptor of MbxA by biochemical and cell biology approaches. Additionally, the capability of the HlyA T1SS to heterologously secrete other RTX proteins will be analyzed by biochemical means. Successful candidates will be analyzed with respect to cellular target(s) and mode of killing. In parallel, the structure of MbxA and other RTX proteins will be determined by X-ray crystallography and / or single particle cryo electron microscopy (EM).

Work program:

- Secretion analysis
- Receptor identification by for example precipitation analysis followed by MS
- Generation of receptor-less cell lines
- Protein purification
- Structure determination by X-ray crystallography and / or single particle cryo-EM