MOI Project 5

Integrative structure-function analysis of the RTX toxin MbxA from *Moraxella bovis*

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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: We developed a method to secrete the RTX toxin MbxA from Moraxella bovis heterologously in E. coli. The animal pathogen MbxA induces infectious bovine keraconjunctivitis (IBK), which is the most common eye disease in cattle. For heterologous secretion, we used the hemolysin type I secretion system, which allowed us to purify an inactive and an active form of MbxA to homogeneity. Using this system, we were able to demonstrate that MbxA has a previously unknown lytic ability towards human cells. Cell death is preceded by blebbing of the plasma membrane. Interestingly, we demonstrated that at concentrations 10-fold above the LC_{50}, an internalization occurs that is accompanied by a massive increase in intracellular Ca^{2+} levels and an externalization of phospholipids of the phosphatidylserine family. These cellular responses imply that an apoptotic signaling pathway is induced leading to cell death. For some RTX toxins, however, pyroptosis has been identified as the actual mechanism, so that further physiological studies are required. Interestingly, internalization does not occur at concentrations around the LC_{50} value and implies a cellular defense mechanism. In addition, initial in vitro experiments showed that the active form of MbxA has an intrinsic affinity for phosphatidylcholine lipids and cholesterol, suggesting that a possible mechanism of action of MbxA could also be receptor-independent.

Aim of the project: The project aims at identifying the cell surface receptor of MbxA by biochemical and cell biology approaches. Additionally, the precise molecular mechanism of cell death will be analyzed. In parallel, the structure of active MbxA reconstituted in lipid nanoparticles will be determined by single particle cryo electron microscopy (EM).

Work program:

- Protein secretion and purification
- Receptor identification using human cell lines
- Single particle cryo-EM studies of the MbxA pore in lipid nanoparticles
- Super-resolution microscopy to study pore formation and cell death