Crystallisation and Functional Analysis of Procarytic and Eukaryotic Rhomboid Proteases and Hsp70 Chaperones

Christian Körner, Martin Sichting, Michael Groll, *Institute of physiological cemistry, University of Munich. Butenandtstr. 5, 81377 Munich, Germany.* E-mail: christian.koerner@bio.med.uni-muenchen.de

Protein degradation and processing is an essential cellular process, which is performed by various intracellular proteases. Most proteases are localized in the cytosol. However, there exist also few examples of membrane proteases, which differ in their architecture, mechanism, regulation and function. Recently a new family of transmembrane proteases termed rhomboid proteases was discovered. They cleave their substrates within their transmembrane domains. Members of this family belong to the class of serine proteases. They are distributed among all three kingdoms of life and are located to the inner membrane of bacteria, to the membrane of the Golgi apparatus and to the inner membrane of mitochondria. All rhomboid proteases possess multiple transmembrane domains. We try to purify and crystallise the rhomboid protease from various bacteria and archaea to reveal its proteolytic mechanism. Additionally we are developing an in vitro assay to identify possible substrates of rhomboid proteases and look for knock out phenotypes.

The second main subject of our work is revealing the three dimensional structure and possible mechanism of prokaryotic and eukaryotic members of Hsp70 chaperones. Although the structure of different domains of Hsp70 proteins have been already published, no crystal structure of the whole protein exists so far. The orientation and spacial arrangement of the N-terminal nucleotide binding domain and the C-terminal substrate binding domain in the structure of the whole molecule will provide insights for the mechanism of action of this class of proteins.

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