Focus of the PhD-Project (Prof. Dr. Richard Zimmermann)

## EF-hand proteins and calcium leakage from the human endoplasmic reticulum during membrane protein biogenesis

The initial step in the assembly of most plasma membrane proteins is their insertion into the membrane of the endoplasmic reticulum (ER). The ER also plays a central role in calcium storage. Protein insertion into the ER membrane involves a sophisticated machinery. In particular, it involves the protein translocase in the ER membrane, comprising Sec61a, Sec61b, and Sec61g as central components. In addition to these heterotrimeric Sec61 complexes, the Hsp70-type molecular chaperone BiP of the ER lumen is part of protein translocase and facilitates gating of the Sec61 complex. BiP is recruited to the Sec61 complex by one of the membrane integrated Hsp40-type co-chaperones, such as ERi1 and Sec63, and controlled by its nucleotide exchange factors, Grp170 and Sil1. We characterized the Sec61 complex as an ion conducting channel with a highly dynamic pore structure and a multitude of open channel states ranging from ~6 to 60Å. One of the central open questions in the field is related to the question of how cellular calcium homeostasis is maintained under conditions of Sec61 channel opening. The gating of the Sec61 channel from the closed to the open state for protein translocation is modulated by the ribosome, signal peptides of precursor poylpeptides, and Sec63 on the cytosolic face and by the ER-lumenal BiP. Gating from the open to the closed state involves the cytoslic EF-hand protein calmodulin and the ER lumenal BiP. Furthermore, Sec63 was found to be associated with the lumenal EF-hand proteins, calumenin and reticulocalbin. We expect this interaction to contribute to gating of the activated Sec61 complex, too. Therefore, the aim of the project is to characterize the interactions between calmodulin and the Sec61 complex. BiP and the Sec61 complex, and between Sec63 and calumenin both at the functional and structural level.

## Methods:

Design and purification of recombinant proteins; pull down assays; peptide spot assays; surface plasmon resonance spectroscopy; siRNA mediated gene silencing and plasmid rescue strategies in human cells; probing protein interactions in mammalian cells.

## Selected publications:

Tyedmers, J., Lerner, M., Bies, C., Dudek, J., Skowronek, M., Haas, I., Heim, N., Nastainczyk, W., Volkmer, J. and Zimmermann, R. (2000) Homologs of the yeast Sec complex subunits Sec62p and Sec63p are abundant proteins in dog pancreas microsomes. *Proc. Natl. Acad. Sci. USA* **97**, 7214-7219.

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Tyedmers, J., Lerner, M., Wiedmann, M., Volkmer, J. and Zimmermann, R. (2003) Polypeptide chain binding proteins mediate completion of cotranslational protein translocation into the mammalian endoplasmic reticulum. *EMBO rep.* **4**, 505-510.

Wirth, A., Jung, M., Bies, C., Frien, M., Tyedmers, J., Zimmermann, R. and Wagner, R. (2003) The Sec61p complex is a dynamic precursor activated channel. *Mol. Cell* **12**, 261-268.

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Dudek, J., Greiner, M., Müller, A., Hendershot, L. M., Kopsch, K., Nastainczyk, W. and Zimmermann, R. (2005) ERj1p plays a basic role in protein biogenesis at the endplasmic reticulum. *Nat. Struct. Mol. Biol.* **12**, 1008-1014.

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Zimmermann, R., Müller, L. and Wullich, B. (2006) Protein transport into the endoplasmic reticulum: mechanisms and pathologies. *Trends in Molecular Medicine* **12**, 567-573.

Zahedi, R. P., Völzing, C., Schmitt, A., Frien, M., Jung, M., Dudek, J., Wortelkamp, S., Sickmann, A. and Zimmermann, R. (2009) Analysis of the membrane proteome of canine pancreatic rough microsomes identifies a novel Hsp40, termed ERj7. *Proteomics* **9**, 3463-3473.

Erdmann, F., Schäuble, N., Lang, S., Jung, M., Honigmann, A., Ahmad, M., Dudek, J., Benedix, J., Harsmann, A., Kopp, A., Helms, V., Cavalié, A., Wagner, R. and Zimmermann, R. (2011) Interaction of calmodulin with Sec61a limits Ca<sup>2+</sup> leakage from the endoplasmic reticulum. *EMBO J.* **30**, 17-31.

Zimmermann, R., Eyrisch, S., Ahmad, M. and Helms, V. (2011) Protein translocation across the ER membrane. *Biochim. Biophys. Acta* **1808**, 912-924.

Lang, S., Schäuble, A., Cavalié, A. and Zimmermann, R. (2011) Live cell calcium imaging in combination with siRNA mediated gene silencing identifies Ca<sup>2+</sup> leak channels in the ER membrane and their regulatory mechanisms. *J. Visualized Experiments* DOI: 10.3791/2730.