

Focus of the PhD Project (Veit Flockerzi, MD/ Gabriel Schlenstedt, PhD)

## **YVC1 – structure, regulation and function of a calcium release channel in *Saccharomyces cerevisiae***

A variety of calcium-related gene products has been identified by the complete sequencing of the *S. cerevisiae* genome. We focus here on Yvc1, the only yeast gene product structurally related to the mammalian family of TRP (transient receptor potential) proteins. The mammalian TRP proteins comprise a diversity of related cation channels that play critical roles in processes ranging from sensory physiology to vasorelaxation and fertility. Yvc1 can serve a model protein for eukaryotic TRP channels responding to various environmental influences. Yvc1 is a 74-kDa protein located in the membrane of the vacuole, a large storage organelle that resembles vertebrate lysosomes. It underlies a cation conductance, which appears to be mechano- and osmo-sensitive. However, it is not known so far how the Yvc1-protein is regulated. The main goal of the project is to characterize the activation mode for Yvc1 and the regulation by interacting factors. The project includes: 1. Identification and characterization of Yvc1-interacting proteins by use of site-specific antibodies and yeast two-hybrid screens; 2. Functional expression of the channel complex and of mutants deficient in binding to interacting proteins; and 3. Characterization of the functional roles of Yvc1 in intracellular Ca-signaling in yeast.

### Methods:

Various molecular biology techniques (PCR, plasmid construction); screening of yeast two-hybrid libraries; yeast genetics (construction of mutant strains by homologous recombination); protein chemistry (purification of recombinant proteins, column chromatography); generation, purification and use of antibodies; protein interaction studies (pulldown assays, peptide spot binding method); cell culture (mammalian cell lines); functional expression of cDNAs; recording of Yvc1 currents (patch-clamp method), calcium imaging (using calcium indicators including the fluorescent dye FURA-2) and various other techniques to study Ca signaling.

### Selected publications:

#### Flockerzi and Schlenstedt

Chang, Y., Schlenstedt, G., Flockerzi, V. and Beck, A. (2010) Properties of the intracellular transient receptor potential (TRP) channel in yeast, Yvc1. *FEBS Lett.* **584**, 2028-2032.

## Flockerzi

Montell, C., Birnbaumer, L. and Flockerzi, V. (2002). The TRP channels, a remarkable functional family. *Cell* **108**, 595-598.

Wissenbach, U., Philipp, S.E., Gross, S.A., Cavalié, A. and Flockerzi, V. (2007) Primary structure, chromosomal localization and expression in immune cells of the murine ORAI and STIM genes. *Cell Calcium* **42**, 439-446.

Bödding, M., Wissenbach, U. and Flockerzi, V. (2007) Characterization of TRPM8 as a pharmacophore receptor, *Cell Calcium* **42**, 618-28.

Flockerzi, V. (2007) Introduction to TRP channels. In: Handbook of experimental pharmacology, vol. 179 (eds.: Flockerzi, V., Nilius B.), Transient receptor potential (TRP) channels, pp 1-19. Springer-Verlag Heidelberg.

Link, S., Meissner, M., Held, B., Beck, A., Weissgerber, P., Freichel, M. and Flockerzi, V. (2009) Diversity and developmental expression of L-type calcium channel beta2 proteins and their influence on calcium current in murine heart. *J. Biol. Chem.* **284**, 30129-30137.

Meissner, M., Weissgerber, P., Londoño, J.E., Prenen, J., Link, S., Ruppenthal, S., Molkentin, J.D., Lipp, P., Nilius, B., Freichel, M. and Flockerzi, V. (2011) Moderate calcium channel dysfunction in adult mice with inducible cardiomyocyte-specific excision of the *cacnb2* gene, *J. Biol. Chem.* **286**, 15875-15882.

## Schlenstedt

Cook, A., Fernandez, E., Lindner, D., Ebert, J., Schlenstedt, G. and Conti, E. (2005) The structure of the nuclear export receptor Cse1 in its cytosolic state reveals a closed conformation incompatible with cargo binding. *Mol. Cell* **18**, 355-367.

Caesar, S., Greiner, M. and Schlenstedt, G. (2006) Kap120 functions as a nuclear import receptor for ribosome assembly factor Rpf1 in yeast. *Mol. Cell. Biol.* **26**, 3170-3180.

Hahn, S., Maurer, P., Caesar, S. and Schlenstedt, G. (2008) Classical NLS proteins from *Saccharomyces cerevisiae*. *J. Mol. Biol.* **379**, 678-694.

Neuber, A., Franke, J., Wittstruck, A., Schlenstedt, G., Sommer, T. and Stade, K. (2008) The nuclear export receptor Xpo1/Crm1 is physically and functionally linked to the spindle pole body in budding yeast. *Mol. Cell. Biol.* **28**, 5348-5358.

Kim, K.-Y., Truman, A.W., Caesar, S., Schlenstedt, G. and Levin, D.E. (2010) Yeast Mpk1 cell wall integrity mitogen-activated protein kinase regulates nucleocytoplasmic shuttling of the Swi6 transcriptional regulator. *Mol. Biol. Cell* **21**, 1609-1619.

Hahn, S. and Schlenstedt, G. (2011) Importin  $\beta$ -type nuclear transport receptors have distinct binding affinities for Ran-GTP. *Biochem. Biophys. Res. Commun.* **406**, 383-388.