## Interaction partners of neuronal calcium sensor-1 in the mouse

*Malte Stockebrand, Joanna Hermainski, Olaf Pongs*

Institute for Neural Signal Transduction, Center for Molecular Neurobiology Hamburg, University hospital Hamburg-Eppendorf, Germany. E-mail: malte.stockebrand@zmnh.uni-hamburg.de

The neuronal calcium sensor protein NCS-1 has been implicated in the modulation of synaptic efficacy and vesicular trafficking. The yeast NCS-1 orthologue Frq1 functions as a regulatory subunit of phosphatidylinositol 4-kinase Pik1, playing an important role in Golgi function and vesicle trafficking. Given the high sequence conservation of NCS-1 proteins across phyla, we hypothesized that mammalian NCS-1 also functions in vesicular trafficking.

To address this hypothesis, we generated transgenic mice expressing GFP-tagged NCS-1 (NCS-1-EGFP) in the forebrain. We prepared forebrain lysates and isolated NCS-1-EGFP containing protein complexes by size-exclusion chromatography followed by immunoprecipitation with anti-GFP antibodies. Immunoprecipitated proteins were separated by SDS-PAGE. For control, we used brain lysates from transgenic mice expressing only EGFP under control of the same promoter. Specific protein bands, not apparent in controls were isolated and characterized by mass spectrometry (LC-MS/MS).

The MS-analysis indicated several candidate interaction partners of NCS-1, but a mammalian Pik1 orthologue was not among them. Instead, we identified two novel candidate proteins, Bet3 and SNAP-47, which both have an albeit ill-defined role in vesicular trafficking in eukaryotic cells. Their interaction with NCS-1 could be confirmed by co-immunoprecipitation with anti-NCS-1 antibodies from brain lysate of wild type mice using NCS-1 knock out animals for control. Immunofluorescence analysis shows perinuclear co-localization of NCS-1 with Bet3 and SNAP-47. We propose that *in vivo* NCS-1 plays a role in $Ca^{2+}$-dependent regulation of intracellular vesicle trafficking.