Characterization of Ca\textsuperscript{2+}-dependent vesicle trafficking steps in ribbon synapses of the retina

Ribbon synapses of the retina are specialized tonically active synapses with a particularly high vesicle turnover. Ribbon synapses can release large numbers of synaptic vesicles within a very short timescale and maintain fast vesicle exocytosis for long periods of time. The synaptic ribbon is a specialized presynaptic specialization in the active zone of ribbon synapses that speeds synaptic vesicle trafficking and provides the ribbon synapse with a large pool of release ready vesicles. How the ribbon exactly works at a molecular level is unknown. RIBEYE is the major component of synaptic ribbons (Schmitz et al., 2000; Magupalli et al., 2008) and likely determines the function of synaptic ribbons and ribbon synapses to a large extent. RIBEYE builds the scaffold of synaptic ribbons and recruits functionally important proteins to synaptic ribbons (Alpadi et al., 2008). Interestingly, using yeast-two-hybrid- and related techniques, we obtained data which suggest that synaptic ribbons are linked to the regulation of intracellular Ca\textsuperscript{2+}-levels and are associated with distinct Ca\textsuperscript{2+}-binding proteins (unpublished data). These ribbon-associated proteins are likely important for the regulation of the synaptic vesicle cycle in ribbon synapses.

In the present project, the composition of these synaptic ribbon-associated protein complexes involved in the regulation of intracellular presynaptic Ca\textsuperscript{2+} will be analyzed in detail using various molecular techniques (Yeast-Two-hybrid- and related techniques), biochemical techniques (immunoprecipitations, „pulldown“ analyses) as well as morphological assays. We will use Ca\textsuperscript{2+}-imaging techniques to analyze the importance of synaptic ribbons and synaptic ribbon-associated proteins for the regulation of intracellular Ca\textsuperscript{2+}-levels and Ca\textsuperscript{2+}-dependent signalling cascades. The physiological importance of this Ca\textsuperscript{2+}-regulating, ribbon-linked protein network for distinct steps of the synaptic vesicle cycle will be determined with various physiological approaches (imaging techniques with FM4-64, TIRF) and patch-clamp analyses. Bipolar cell cultures from the mouse retina as well as organotypic retina cultures will be used as model systems for the physiological analyses. These model systems can be manipulated by recombinant viruses to characterize the importance of distinct proteins for distinct physiological processes (exocytosis, endocytosis) in the ribbon synapse. The effects of these manipulations on intracellular Ca\textsuperscript{2+} and vesicle trafficking will be characterized by various techniques. A protein link of synaptic ribbons to presynaptic Ca\textsuperscript{2+}-channels will be analyzed in details using a variety of biochemical and molecular methods. We expect from these analyses insights on how the ribbon works at a molecular level and how synaptic ribbon proteins are involved in vision. These analyses will also help to better understand diseases of the visual system.

Methods for the project:
Protein-protein interaction analyses („pulldown“ analyses with retinal fractions, immunoprecipitation, Yeast-Two-Hybrid-techniques); Ca\textsuperscript{2+}-imaging techniques; General morphological techniques and immunoelectron microscopic techniques (fluorescence microscopy, conventional transmission electron microscopy and immunogold electron microscopy), organotypical cultures of the retina, mouse bipolar
cell culture, recombinant virus techniques, FM4-64 labelling of vesicle trafficking, RNAi.

Selected publications:


