BAR domain scaffolds in clathrin-independent endocytosis of synaptic vesicles

Supervisor/s:

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Project A01

Collaboration with:

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Abstract:

Project A01 deals with the role of endocytic protein scaffolds in the endocytosis and recycling of synaptic vesicles (SVs) at central synapses. Within the last and current funding periods of Sfb958 we found that the internalization of SV membranes occurs predominantly by clathrin-independent endocytosis (CIE) mediated by actin and associated BAR domain scaffolds including endophilin (1,2). In further unpublished studies we have screened - using smart pool siRNA - a large cohort of dynamin-associated BAR domain proteins for their possible function in presynaptic CIE in hippocampal neurons in culture. These studies have revealed putative roles of CIP4/FBP-17 family members in CIE (Fig. 1A). Moreover, we identified an amphipathic helix within the vesicular glutamate transporter 1 (VGLUT1), a SV protein which binds to the BAR domain protein endophilin to regulate neurotransmission (3) and SV recycling (4), that may facilitate CIE of SV membranes (Fig. 1B; consistent with ref. 5) by aiding actin/ BAR protein-dependent membrane remodelling.

The proposed project aims at (i) dissecting the nanoscale localization of CIP4/FBP17-subfamily proteins at central synapses by super-resolution imaging, (ii) the functional characterization of CIP4/ FBP and endophilin BAR domain family proteins in presynaptic CIE by carrying out lentiviral knockdown/ rescue experiments in hippocampal neurons in culture paired with pHluorin imaging and electron microscopic studies, and, finally, (iii) analyzing the role of the conserved amphipathic helix within VGLUT1 and related vesicular neurotransmitter transporters in SV recycling. These works will be accompanied by (iv) a thorough characterization of the biophysical and cell biological properties of wild-type and mutant VGLUT1 and chimeras derived from it in vitro and in transfected non-neuronal cells as well as within neurons.

The proposed work will benefit from intense collaborations within the Sfb958 with the groups of Stephan Sigrist (on VGLUT and CIP4/FBP-17 at Drosophila NMJs), Christian Rosenmund (on endophilin and VGLUT1 in CIE) and with Christian Freund (on the molecular properties of CIP4/FBP-17 and VGLUT1 complex formation with other endocytic proteins such as dynamin and endophilin, respectively).

Requested funding: *One PhD student stipend for 12 months (07/2018-06/2019)*

Publication/s:

Own papers related to the proposed project:

(1) Soykan, T., Kaempf, N., Sakaba, T., Vollweiter, D., Goerdeler, F., Puchkov, D., Kononenko, N.L., Haucke, V. (2017) Synaptic vesicle endocytosis occurs on multiple timescales and is mediated by formin-dependent actin assembly. *Neuron*, **93**, 854-866

(2) Kononenko, N.L., Puchkov, D., Classen, G.A., Walter, A., Pechstein, A., Sawade, L., Kaempf, N., Trimbuch, T., Lorenz, D., Rosenmund, C., Maritzen, M., Haucke, V. (2014) Clathrin/ AP-2 mediate synaptic vesicle reformation from endosome-like vacuoles but are not essential for membrane retrieval at central synapses. *Neuron*, **82**, 981-988

Other related papers:

(3) Weston MC, Nehring RB, Wojcik SM, Rosenmund C (2011) Interplay between VGLUT isoforms and endophilin A1 regulates neurotransmitter release and short-term plasticity. *Neuron*, **69**, 1147-59

(4) Voglmaier SM, Kam K, Yang H, Fortin DL, Hua Z, Nicoll RA, Edwards RH (2006) Distinct endocytic pathways control the rate and extent of synaptic vesicle protein recycling. *Neuron,* **51**, 71-84.

(5) Pan PY, Marrs J, Ryan TA (2015) Vesicular glutamate transporter 1 orchestrates recruitment of other synaptic vesicle cargo proteins during synaptic vesicle recycling. *J Biol Chem*., **290**, 22593-60

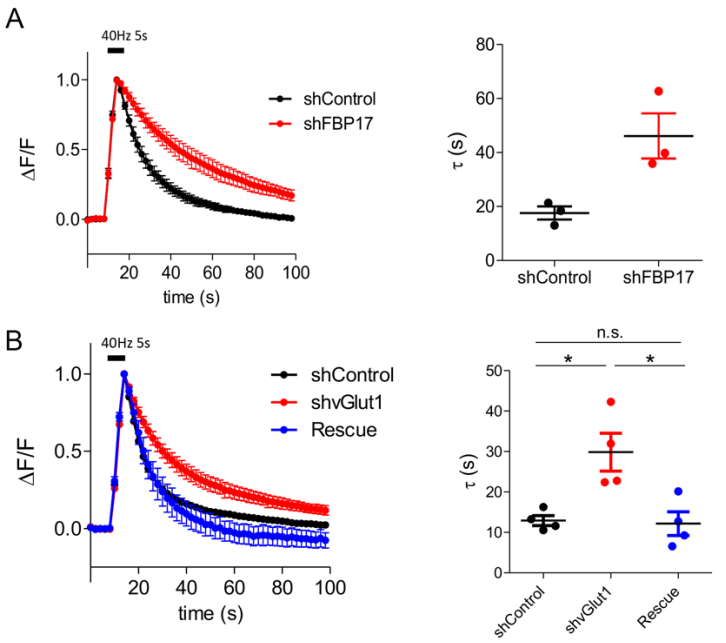

Figure 1: FBP-17 and VGLUT1 facilitate CIE of SV membranes