

Identification and 3D visualisation of filamentous structural components of synaptic active zones in locust visual systems

D. Kolb¹, M.A. Pabst¹, J. Neumüller², M. Pavelka², F.C. Rind³, P.J. Simmons³, S. Masich⁴, O. Shupliakov⁵, and G. Leitinger^{1,6}

1. Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria. 2. Center for Anatomy and Cell Biology, Department of Cell Biology and Ultrastructure Research, Medical University of Vienna, Austria. 3. School of Biology, Ridley Building, Newcastle University, Newcastle upon Tyne, UK. 4. Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden. 5. Department of Neuroscience, DBRM, Karolinska Institutet, Stockholm, Sweden. 6. Core Facility Ultrastructure Analysis, Center for Medical Research (ZMF), Medical University of Graz, Austria

Dagmar.kolb@medunigraz.at

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In insect synapses, the active zones consist of a presynaptic bar to which vesicles are attached by thin filaments.

Here, we investigated the 3D organisation of the presynaptic bar in visual synapses in the locust to reveal how the molecular components of this specialisation are organised. We show that the presynaptic bar is composed of a base with branched extensions that are interconnected by filaments. Furthermore, we identified the localisation of one of the molecular components of the filaments, the locust ortholog of the “Bruchpilot” protein, which has been proposed to be a structural component of the active zone in *Drosophila* synapses [1].

A pre-embedding immunogold staining procedure was applied to these synapses to determine the subcellular localisation of the Bruchpilot ortholog using the NC82 antibody, which recognizes an epitope near the C-terminal end of the Bruchpilot protein in *Drosophila* [2]. This antibody also labelled the presynaptic bar in the locust synapses selectively.

To determine the 3D architecture of the structural component labelled by the antibody, we applied electron tomography. For this, the specimen was tilted with respect to the beam and series of 2D images at different projection directions were recorded. From this data set a three dimensional volume was reconstructed by weighted backprojection. 3D models of immunogold-labeled structures were depicted by surface rendering and thresholding using the AMIRA Visualization Package.

Using electron tomography of immunostained preparations, we determined that NC 82 is associated with a part of the filamentous structure between the vesicles. The structural components labelled with the immunogold granules were closer to the vesicles than to the cell membrane. Our analysis allows suggesting that the C-terminal part of the Bruchpilot ortholog in the locust visual synapses is involved in the organisation of the pool of synaptic vesicles attached to the presynaptic bar.

1. R.J. Kittel et al., Science 312(5776) p1051.
2. D.A. Wagh et al., Neuron 49(6) p833.
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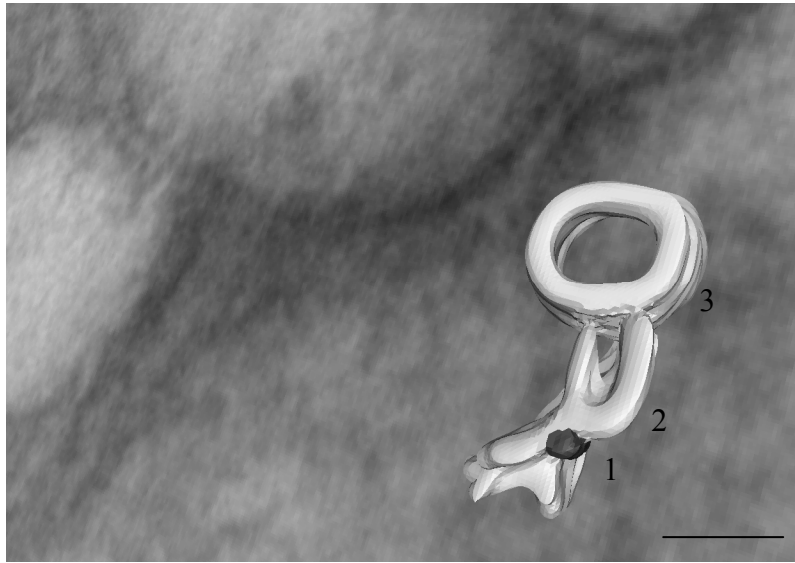


Figure 1. Bruchpilot staining (1) at filaments (2) close to a synaptic vesicle (3) in a presynaptic profile in a visual system of locust (bar= 30nm)