UNIVERSITÄT DES SAARLANDES

SFB 894 Seminars

SFB 894
"Ca^{2+} signals: Molecular Mechanisms and Integrative Functions"

Talk by Shigeki Watanabe, PhD
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Host: PD Ute Becherer
August 23, 2013, 13.00 pm
Building 60, Lecture Hall Human Genetics

"Synaptic vesicle endocytosis: catch me if you can"

Abstract

To sustain neurotransmission, synaptic vesicles must be recycled locally at synapses. To fully characterize synaptic vesicle cycle, synapses must be observed at the ultrastructural level because organelles and proteins involved in the process are small. These organelles and proteins are highly clustered in a very confined space, and thus individual structures and molecules cannot be resolved using conventional fluorescence microscopy.

However, electron microscopy, unfortunately, only captures snap-shots of synapses with no temporal information, and proteins are not visible in electron micrographs.

To understand how endocytosis takes place and how particular proteins are involved in the process, neurons must be stimulated and observed in a temporal sequence. Moreover, the locations of proteins relative to subcellular features such as active zones and endocytic pits must be visualized. For these reasons, we developed two techniques to capture synaptic vesicles and their associated proteins in action at the ultrastructural level: time-resolved imaging using 'flash-and-freeze' electron microscopy, and spatially-resolved correlative nano-resolution fluorescence electron microscopy (nano-fEM). 'Flash-and-freeze' electron microscopy combines optogenetics with electron microscopy to stimulate synaptic transmission and capture rapid events with ~10 ms temporal resolution. Nano-fEM couples super-resolution fluorescence microscopy with electron microscopy to localize proteins at nanometer resolution.

Using these two techniques, we found that, in contrast to classical models, endocytosis occurs within 50 ms at the edges of active zones.