Development in Visualization and Analysis of Membrane Dynamics by Two-photon Microscopy

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Multi-photon excitation process is a process in which a molecule transits to a higher electronically excited state by a simultaneous absorption of multiple photons. A new optical microscopy based on multi-photon excitation process (two-photon microscopy) has become widely used in the various fields of biological and medical sciences. One of the reasons is that two-photon microscopy can visualize living cells in near intact states for a long time within deeper layers of organs. By taking such advantages of two-photon microscopy including multicolor excitation capability, we have established a less-invasive imaging method for quantifying intracellular Ca^{2+} and simultaneously visualizing a single episode of fusion pore opening during exocytosis. In intact pancreatic acini, we have found a sequential compound exocyotsis for the first time. Recently, we have demonstrated the appearance and the transport of synaptic-like vesicles during exo- and endocytosis. Further exploration revealed dynamics and physiological roles of actin cytoskeleton, fusion-pores, and SNARE proteins. In addition, we have succeeded in the observation of intact neurons in the layer deeper than 0.9 mm from the surface in the brain neocortex of anesthetized mice. In the same preparation, we can determine dendritic spines and axon terminals morphologically, suggesting that their long-term changes can be chased in a living mouse. Two-photon microscopy will thus advance the researches on dynamics of synapses in the cerebral cortex, and mechanisms of exocytosis in neurons and secretory cells, in intact organs or brains *in vivo*. In the present lecture, we will discuss these properties of two-photon microscopy and new insights on synapses and exocytosis.