Ultrafast membrane trafficking at synapses

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Neurons can fire at extremely high rates. To sustain neurotransmission, synaptic vesicles must be recycled locally at synapses. Two models for synaptic vesicle endocytosis have been put forward based on the morphological studies in frog neuromuscular junctions. Heuser and Reese proposed that endocytosis occurs via a slow mechanism using clathrin scaffolds. Ceccarelli and his coworkers proposed a fast mechanism, kiss-and-run. Since then, many studies have sought to identify the mechanism for synaptic vesicle endocytosis. However, instead of resolving the issue, conflicting evidence has accumulated over the years. The molecular studies have suggested clathrin and clathrin-associated proteins are essential. However, the kinetics studies have suggested that both forms co-exist. Our data identify a third pathway that is fast, but requires clathrin to regenerate vesicles.

To investigate how endocytosis takes place, we developed a method, 'flash-and-freeze' that couples optogenetic stimulation with rapid high-pressure freezing and captures endocytosis at millisecond temporal resolution. To our surprise, vesicle membrane is recovered via ultrafast endocytosis within 100 ms following a single stimulus. The large endocytic vesicles then fuse to form an endosome and are resolved by clathrin into synaptic vesicles in 5-6 s. When experiments are performed at 20ºC instead of 37ºC, ultrafast endocytosis fails, and clathrin regenerates synaptic vesicles directly from plasma membrane. These results suggest that recycling of synaptic vesicles is normally a rapid two-step process: ultrafast endocytosis that removes excess membrane from the surface and then clathrin-mediated biogenesis of synaptic vesicles from endosomes. We are currently studying the molecular mechanisms underlying each step of the pathway. I will present our recent progress.