doi:10.1017/S1431927611001103
Microsc. Microanal. 17 (Suppl 2), 2011

Watching the Exocytosis of Secretory Vesicles in Live Cells

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When cells wish to export cargo, they first package it into secretory vesicles. Next, the membrane surrounding a vesicle fuses with the membrane surrounding the cell (plasma membrane). Thereafter, the cargo is free to diffuse into the external space. The membrane fusion and the molecular events preceding it are collectively called exocytosis. In live cells, electrophysiology has been a powerful method to study the fusion step, but this technique does not directly report events that precede fusion.

TIRF has become a useful alternative that can be used in live cells adhering to a glass coverslip. The cell surface is illuminated by the evanescent field generated at the glass-cell interface by total internal reflection (TIR), most often applied through an oil immersion objective of high numerical aperture [1, 2]. Filled with fluorescent cargo, individual secretory vesicles are readily seen in TIRF, and exocytosis is detected as cargo diffuses from the vesicle. The first such recordings were made from cells whose vesicles were labeled with organic dyes [3-5]. Later work used transfected cells that expressed vesicle markers fused to fluorescent proteins [6-11].

For viewing cell surfaces in two colors, an optical attachment (e.g. Dual View by Photometrics Inc) is used to split images into two colors. One color is used to label an organelle close to the cell surface, and another to label one of several proteins important for the organelle's function. For example, actin and dynamin were observed as they were recruited and released from clathrin-coated pits undergoing endocytosis [12]. Two-color TIRF also made it possible to measure fluorescence resonance energy transfer (FRET) between proteins participating in specific cell surface events [13]. TIRF is now extensively used for this purpose (e.g. [14, 15]).

Most recently we have begun to image the molecular preparations a vesicle must make before fusion [16, 17]. Three proteins form the heart of the fusion machinery and are collectively called SNAREs. VAMP-2 resides in the membrane of the secretory vesicle, and Syntaxin-1a and SNAP-25 reside in the plasma membrane. Before a vesicle can fuse with the plasma membrane, it must recruit Syntaxin-1a and SNAP-25. Indeed, when cells expressed syntaxin or SNAP-25 at low levels, both proteins were seen to cluster in the plasma membrane subjacent to the vesicle site. In any given second, about half the vesicles visible in TIRF were associated with a syntaxin cluster, indicating that half the vesicles had molecularly docked. Vesicles with syntaxin clusters were competent to undergo exocytosis, those without clusters were not. After exocytosis, syntaxin clusters disassembled. Even before exocytosis, clusters beneath vesicles reversibly assembled and disassembled. Tracking single syntaxin and SNAP-25 molecules in the plasma membrane revealed that most syntaxin and SNAP-25 molecules were freely mobile in the plasma membrane, but minority populations of each protein were strongly hindered in their mobility. Syntaxin molecules could be observed as they were captured and released by vesicles. Evidently vesicles bear a receptor for syntaxin. When the surface density of syntaxin was varied, it appeared that one vesicle could

accommodate maximally 90 molecules of syntaxin, hence there are about 90 syntaxin receptors per vesicle. By combining single molecule recording and biochemistry, we determined that the average docked vesicle in an untransfected cell assembles 50-70 molecules of each, syntaxin and SNAP-25. It appears that TIRF can readily image the interaction of organelles with a small number of molecules, including single molecules.

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https://doi.org/10.1017/S1431927611001103 Published online by Cambridge University Press