

Localized Plasma Membrane Topological Changes upon Exocytosis Visualized by Polarized- TIRFM

Arun Anantharam^a, Daniel Axelrod^b and Ronald W. Holz^a,

^aDepartment of Pharmacology, University of Michigan, Ann Arbor, MI 48109

^bDepartment of Physics and LSA Biophysics, University of Michigan, Ann Arbor, MI 48109

Total internal reflection fluorescence (TIRF) microscopy images the plasma membrane-cytosol interface and has allowed insights into the behavior of individual secretory granules before and during exocytosis. Much less is known about the dynamics of the other partner in exocytosis, the plasma membrane. We have implemented a TIRFM-based polarization technique (pTIRFM) to detect rapid submicron changes in plasma membrane topology as a result of exocytosis. The key to the technique is a polarization (p) perpendicular to the glass interface that is available in TIRF but not in epifluorescence (where only polarizations in the plane of the coverslip are possible) (Fig. 1). Together with polarization in the plane of the coverslip (s, also available in TIRFM), the technique detects the orientation of a plasma membrane fluorophore, diI, that orients its transition dipoles in the plane of the plasma membrane (Fig. 2). Successive images are taken of a sample with orthogonal excitation polarizations in TIR mode: p-pol and s-pol. Regions – even submicroscopic ones - in which the membrane deviates from parallelism with the glass coverslip are vividly highlighted by taking the ratio (p-pol)/(s-pol) (abbreviated P/S) of the membrane-embedded diI fluorescence images excited by the two polarizations. The larger the local deviation of the membrane from being parallel with the glass interface, the greater the P/S . Regions of local non-parallelism indicates increased curvature. The theoretical analysis indicates P/S is independent of diI concentration and that the sum $P+2S$ of the emissions reports the local fluorophore concentration convoluted with the exponentially decaying evanescent field for a high numerical aperture lens (NA=1.49). A third image is taken to image a granule marker (usually NPY) tagged with cerulean to locate granules and exocytosis. We originally imaged the sequence at 2 Hz, but now routinely image the sequence at 10 Hz. A theoretical analysis permits image simulations of predicted topologies of the post-fusion granule membrane-plasma membrane complex. We have applied the technique to adrenal chromaffin cells stained with diI and have obtained high temporal resolution imaging of submicron topological changes before, during and after fusion of individual granules with the plasma membrane. The submicron changes in plasma membrane topology at sites of exocytosis reflect fusion pore expansion and demonstrate both transient and persistent curvature at the sites of exocytosis. We have used this technique together with amperometry to investigate the regulation of the expansion. Our recent experiments demonstrate that the dynamin GTPase, a master regulator of membrane fission in endocytosis through its membrane sculpting ability, also regulates fusion pore expansion from milliseconds to many seconds after fusion.

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