

Visualization and real-time monitoring of membrane trafficking in resting and activated human T lymphocytes with FM1-43 styryl dye.**Alla F. Fomina**^{1,3}, Thomas J. Deerinck², Mark H. Ellisman², Michael D. Cahalan³.¹Univ. California, Davis, Davis, CA 95616; ²Univ. California, San Diego, La Jolla, CA 92093; ³Univ. California, Irvine, Irvine CA, 92697.

Membrane trafficking plays an important role in regulating the surface expression of membrane proteins in T lymphocytes and provides an important mechanism to modulate immune responses. However, the origin and fate of endosomal compartments have not been studied extensively in T lymphocytes, and little is known about how lymphocyte activation affects membrane trafficking. We employed a membrane dye FM 1-43 to investigate real-time membrane trafficking dynamics in living T lymphocytes, and to visualize endocytic compartments with ultrastructural resolution. We established that both resting and PHA-activated T cells continuously internalize their plasma membrane. The rate of endocytosis is increased ~ 10 fold in activated T cells, compared to resting T cells. In both resting and activated T cells the rate of plasma membrane internalization is strongly temperature-dependent. The activation energies were 153 kJ/mol and 76 kJ/mol at low (14-24° C) and high (24-34° C) temperatures, respectively. Elevation of cytosolic free Ca²⁺ concentration significantly accelerated the rate of membrane turnover. Using a photo-conversion technique to visualize endocytic compartments with EM resolution, we established that constitutively internalized cargo is carried to different late endocytic compartments in resting and activated T cells – lysosome-like structures in resting T cells and multivesicular bodies (MVB) in activated T cells. We also found that externalization of exosomes from MVB occurred commonly in activated but not in resting T cells. T cell exosomes contained raft-associated CD3 proteins, GM1 glycosphingolipids, and expressed phosphatidylserine at the outer membrane leaflet. Thus, the present study describes the possible routes for membrane protein internalization/externalization and demonstrates the utility of FM1-43 as a real-time marker of membrane trafficking in human T cells.