Analysis of secretory granule movement in wildtype and *munc18-1* null mutant chromaffin cells with evanescent wave microscopy

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Secretory vesicle exocytosis is a highly dynamic process that is spatially and temporally regulated via a cascade of protein-protein interactions. The sec1/munc18 (SM) protein family is involved in all membrane trafficking events and is essential for synaptic vesicle exocytosis. Deletion of Munc18-1 in mouse adrenal chromaffin cells results in a 90% decrease in the amount of docked vesicles, which correlates with the same decrease in vesicle release and *vice versa*, Munc18-1 overexpression enhances vesicle docking and secretion. This remarkable phenotype may be caused by a Munc18-1 dependent change in the kinetics of vesicle trafficking towards the membrane or a change in the degree of fixation of docked vesicles at the membrane. To discriminate between these two possibilities we visualized both vesicle trafficking towards and vesicle docking at the membrane in living cells using total internal reflection fluorescence microscopy (TIRFM). We show that in the absence of Munc18-1, vesicles near the membrane move faster and spend less time docked at the membrane whereas overexpression of Munc18-1 results in a much more restricted movement with an increase in time spend at the membrane. TIRF analysis of Munc18EGFP localization reveals Munc18 clusters at the cell membrane suggesting that Munc18 serves as a vesicle-docking platform that actively keeps vesicles docked at the membrane thereby facilitating vesicle fusion.

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