The SNAP-25 linker is an integral regulator of exocytosis

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The formation of membrane-bridging *trans*-SNARE complexes is a key step in Ca^{2+}-triggered exocytosis. While the transmembrane domain-anchored SNARE proteins Syntaxin-1A and Synaptobrevin-2 each insert a single SNARE motif into the SNARE complex, SNAP-25 is anchored by palmitoylation and contributes two SNARE motifs (Q_b and Q_c) to the assembly. Here, we have investigated whether the integrity and length of the linker between Q_b and Q_c as well as the position of the palmitoylation site are of mechanistic relevance for exocytosis. Thus, we expressed linker mutants in SNAP-$25^{-/-}$ chromaffin cells and characterized secretion by membrane capacitance measurements and amperometry. Co-expression of separated Q_b and Q_c motifs or expression of a mutant whose linker was substituted by a flexible G/S-containing peptide failed to rescue secretion, stressing the functional importance of the linker. While insertion of up to 28 residues near the linker middle did not affect secretion, insertion of G/S-peptides between Q_b and palmitoylation site slowed down triggering and altered fusion pore behavior. This linker extension also further decelerated secretion of palmitoylation mutants, suggesting a stabilizing effect of the normal linker on C-terminal SNARE assembly and membrane interaction. In summary, we show here that the SNAP-25 linker is a positive regulator of SNARE complex formation and membrane fusion.