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Interaction between the active zone components Munc13-1 and RIM1: Definition of binding sites using a random mutagenesis approach

Members of the Unc-13/Munc13 protein family are components of the presynaptic active zone and essential vesicle priming factors in regulated exocytosis. They are thought to mediate vesicle priming by stabilizing the open conformation of the t-SNARE Syntaxin, thereby promoting SNARE complex assembly. The Unc-13/Munc13 protein family is evolutionarily highly conserved. However, comparison of the various Unc-13 homologs in different species demonstrates that the respective genes consist of two modules that have evolved differentially and may have independent functional roles. The carboxy-terminal two-thirds of the protein are highly conserved between drosophila, C. elegans, mouse, rat, and human whereas of the known mammalian Unc-13 homologs only Munc13-1 and ubMunc13-2 contain an amino-terminus with homology to the Unc-13 L region of C. elegans. RIM is an active zone protein that has been found to bind this conserved amino-terminus of Munc13-1 and ubMunc13-2. RIM has been proposed to act at multiple steps of the synaptic vesicle cycle, including docking/targeting, priming, and fusion of synaptic vesicles. In the present study, I used a random mutagenesis approach in combination with yeast two-hybrid technology to identify residues in the Munc13-1/ubMunc13-2 sequence that are critical for RIM binding. The ultimate aim of these studies is to develop the basis for subtle genetic interference with the RIM/Munc13-interaction in mice using a knock in strategy, which will allow a detailed functional characterization of this synaptic regulatory protein-protein interaction.