The C-terminal membrane anchor of Syntaxin4 affects Munc18c-supported SNARE assembly *in vitro*

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Membrane fusion is essential for human health, playing a vital role in processes as diverse as neurotransmission and blood glucose control. Two protein families are indispensible: 1) the Sec1p/Munc18 (SM) proteins; and 2) the soluble N-ethylmaleimide-sensitive attachment protein receptor (SNARE) proteins. Whilst the essential nature of these proteins is irrefutable, questions remain as to the precise role of SM proteins. In particular, whether SM proteins promote and/or inhibit SNARE-complex formation required for membrane fusion remains controversial. Crystal structures of SM proteins alone and in complex with partners have provided some insight (reviewed in Archbold *et al.*, 2014). However, these structures lack the transmembrane spanning regions of the SNARE proteins and may not accurately reflect the native state. Moreover, the literature on the regulatory role of Munc18 proteins is confounded by use of different SNARE constructs, and varying experimental methods (reviewed in Rehman *et al.*, 2014; Archbold *et al.*, 2014). What is becoming increasingly clear is that the 3-domain Munc18 proteins are highly dynamic: individual domains can rotate by 50-180° to access a wide range of molecular shapes (see for example Hu *et al.*, 2007,2011; Christie *et al.*, 2012) and there are at least three independent binding sites for partner proteins.

Here we investigated the Munc18:SNARE system required for insulin-regulated delivery of glucose transporter 4 (GLUT4) to the cell surface. Two proteins, Munc18c and Syntaxin4 (Sx4), are essential. Sx4 in its native form is C-terminally anchored to the cell membrane by a transmembrane helix. We show using pulldown and fluorescence anisotropy experiments that the reported inhibitory role of Munc18c may be a consequence of experimental design. Our results suggest that SNARE proteins require C-terminal anchoring, for example through a transmembrane domain, to form a functional SNARE fusion complex in the presence of Munc18c. Without this anchor, Munc18c inhibits SNARE complex formation; with the C-terminal anchor there is no inhibition. Our findings reconcile contradictory reports on the role of Munc18c in SNARE assembly and have important implications for other SNARE membrane fusion systems. Specifically, they call into question the common practice of removing transmembrane helices to study protein interactions when the interacting region of the protein adjoins the transmembrane helix. Understanding the native interactions between Munc18 and Sx proteins is critical for unraveling the molecular basis of membrane fusion and its dysfunction in diseases like type II diabetes.

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