5 Summary

SNARE proteins are thought to be key mediators of all intracellular fusion reactions ranging from yeast to man. The assembly of ternary SNARE complexes between the synaptic vesicle protein synaptobrevin and the plasma membrane proteins syntaxin and SNAP-25 initiates membrane fusion at the nerve terminal. The interaction between SNARE proteins is mediated by a stretch of approximately 60 amino acids referred to as the SNARE motif. The crystal structure of the synaptic SNARE complex revealed an extended four helix bundle with all helices aligned in parallel (two from SNAP-25, one from syntaxin, and one from synaptobrevin). Syntaxin contains an additional independently folded domain known as the Habc domain. An intramolecular interaction between this domain and the SNARE motif arrests syntaxin in a closed conformation. Only when there is no interaction between the two domains (open conformation) does the SNARE complex assemble. In this study, the conformational switching of syntaxin and the structures of SNARE intermediates and fully assembled SNARE complexes were investigated.

Fluorescence anisotropy measurements revealed that the Habc domain slows down SNARE complex formation by a factor of 5-7, implying a weak interaction between the Habc domain and the SNARE motif ($\Delta G \approx 1$ kcal/mol). The intramolecular distances between the two domains were measured by single molecule fluorescence resonance energy transfer. Only 15-30% of all syntaxin molecules are in a closed conformation. Conformational fluctuations between the open and closed states occur in a time range of ~0.7 ms. In order to arrest syntaxin in a closed conformation an additional protein (munc-18) is required.

Binary complexes between syntaxin and SNAP-25 (2:1 stoichiometry) are potential intermediates in SNARE complex assembly. Electron paramagnetic resonance data indicated that the binary complex is composed of a bundle of four α-helices with the syntaxin helices being in register. Furthermore, binary complexes are loosely structured at the very N-and C-terminal ends of syntaxin and the C-terminal ends of both SNAP-25 helices. Binding of synaptobrevin displaces one of the syntaxin molecules and thus completes SNARE complex formation. Assembly of binary and ternary complexes from individual SNAREs is accompanied by large structural changes. Isolated synaptobrevin

and SNAP-25 are unstructured. Syntaxin, too, is unstructured but oligomerizes at higher μM concentrations. Oligomerization is mediated by the SNARE motif of syntaxin and may compete with intramolecular binding of the Habc domain.

To find out whether helices in the SNARE complex require juxtaposing helices, a C-terminally truncated synaptobrevin was used. Helices remain completely intact upstream from the truncated site; helices downstream from the truncated site collapse. Interacting layers in the four-helix-bundle of SNARE complexes therefore appear to be independent from layers in nearby positions. This agrees with the proposed "zippering mechanism" of complex formation and provides a structural basis according to which partially assembled complexes form intermediates in the progression toward membrane fusion.

To test whether SNARE proteins interact via their transmembrane regions syntaxin and synaptobrevin were studied in proteoliposomes. Syntaxin formed specific dimers that might be precursors in binary complex formation. In addition a new interaction between the transmembrane domains of syntaxin and synaptobrevin was observed which might be important for a late stage in membrane fusion. An extension of the four-helix bundle into the transmembrane region might contribute to the formation of membrane continuity between proximal leaflets of the fusing membranes.

In conclusion, syntaxin participates in many molecular interactions and undergoes multiple conformational transitions until it is bound in a fully assembled SNARE complex.