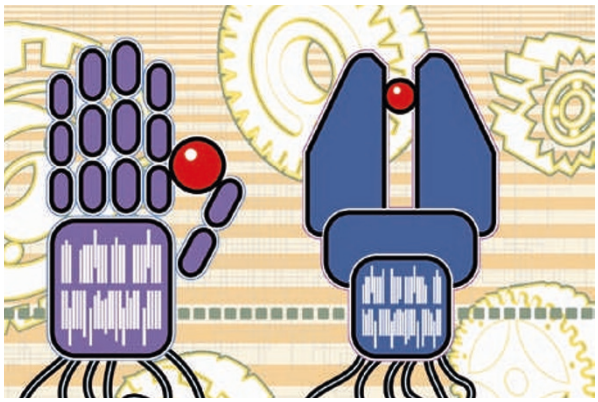
MEMBRANE DYNAMICS

Clamping complexin



CORBIS

The protein complexin interacts with the SNARE (soluble NSF-attachment protein receptor) complex and regulates neurotransmitter release. Two groups have analysed the molecular structure of complexin and conclude that its accessory helix acts as a fusion clamp (preventing fusion), whereas its amino-terminal domain acts as a fusion enhancer.

SNARE proteins are localized to synaptic vesicles and cell membranes, and their interaction attaches vesicles to the membrane. Cytosolic complexin binds through its central domain to the aligned α-helices of the SNARE complex and is displaced on Ca^{2+}-activated synaptotagmin binding prior to membrane fusion.

Giraudo *et al.* found that the sequence of the accessory helix of complexin is homologous to that of the membrane-proximal region of VAMP (also called synaptobrevin), one of the SNAREs, when the two sequences are aligned in an anti-parallel manner. They proposed that complexin competes with VAMP for the same binding site in the SNARE complex and thereby acts as a fusion clamp.

Using an *in vitro* fusion assay, they showed that membrane fusion was triggered when complexin and a recombinant VAMP peptide that was homologous to the complexin accessory helix were added at the same time in the absence of Ca^{2+} and synaptotagmin. When the VAMP peptide was added after complexin, fusion did not occur, supporting the hypothesis. Furthermore, mutations in the accessory helix that enhanced clamping rendered complexin more resistant to VAMP competition in the fusion assays. By contrast, mutations in this helix that reduced the clamping ability of complexin were dominant-negative and did not prevent fusion, confirming that it is the accessory helix of complexin that exerts the clamping function.

Maximov *et al.* studied the role of complexin in cultured mouse cortical neurons. Knocking down complexin with RNA interference increased spontaneous vesicle-fusion events but impaired evoked synaptic release. These effects were rescued by the addition of wild-type complexin but not complexin that lacked 40 amino acids at the N terminus (and that therefore lacked most of the accessory helix but not the central domain). A deletion mutant that lacked the N-terminal 27 amino acids but had an intact accessory helix was able to rescue the phenotype of spontaneous fusion events (that is, clamp spontaneous release), but not the impaired evoked response. These results suggest that the 27 amino acids of the N terminus are important for mediating membrane fusion events, and that different complexin domains mediate spontaneous and evoked release.

Furthermore, when a VAMP mutant that does not bind complexin but still forms SNARE complexes was introduced into cortical cultures from VAMP-knockout mice that lack spontaneous or evoked release, spontaneous fusion events were rescued and even elevated above those of cells expressing wild-type VAMP, whereas evoked events were decreased. This indicates that complexin executes its clamping function by binding to VAMP. Interestingly, a point mutation where VAMP inserts into the membrane (outside the SNARE complex) had the same phenotype, indicating that complexin acts on the force translation of SNAREs onto the membrane.

These results suggest that the accessory helix of complexin acts as a fusion clamp, by competing with VAMP to bind to the SNARE complex, and that the 27 amino acids of the N terminus of complexin are important for the activation of membrane-fusion events, revealing a previously unknown task division for the domains of complexin.

Claudia Wiedemann, Chief Editor,
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ORIGINAL RESEARCH PAPERS Giraudo, C. G. *et al.* Alternative zippering as an on-off switch for SNARE-mediated fusion. *Science* **323**, 512–516 (2009) | Maximov, A. *et al.* Complexin controls the force transfer from SNARE complexes to membranes in fusion. *Science* **323**, 516–521 (2009)