A Conserved Membrane-spanning Amino Acid Motif Drives Homomorphic and Supports Heteromeric Assembly of Presynaptic SNARE Proteins*

Rico Laage‡, Jan Rohde, Bettina Brosig, and Dieter Langosch§

From the Department of Neurobiology, Universität Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

Assembly of the SNARE proteins synaptobrevin/VAMP, syntaxin, and SNAP-25 to binary and ternary complexes is important for docking and/or fusion of presynaptic vesicles to the neuronal plasma membrane prior to regulated neurotransmitter release. Despite the well characterized structure of their cytoplasmic assembly domains, little is known about the role of the transmembrane segments in SNARE protein assembly and function. Here, we identified conserved amino acid motifs within the transmembrane segments that are required for homodimerization of synaptobrevin II and syntaxin 1A. Minimal motifs of 6–8 residues grafted onto an otherwise monomeric oligoalanine host sequence were sufficient for self-interaction of both transmembrane segments in detergent solution or membranes. These motifs constitute contiguous areas of interfacial residues assuming α-helical secondary structures. Since the motifs are conserved, they also contributed to heterodimerization of synaptobrevin II and syntaxin 1A and therefore appear to constitute interaction domains independent of the cytoplasmic coiled coil regions. Interactions between the transmembrane segments may stabilize the SNARE complex, cause its multimerization to previously observed multimeric superstructures, and/or be required for the fusogenic activity of SNARE proteins.

Intracellular membrane fusion events, e.g. constitutive or- ganelle traffic or Ca2+-regulated neurotransmitter release, require conserved sets of membrane proteins, designated SNAREs. The best characterized SNAREs are those mediating exocytosis of synaptic vesicles in neurons (reviewed in Refs. 1–4). In detergent extracts from presynaptic nerve terminals, the single-span integral membrane SNAREs synaptobrevin (also referred to as VAMP) and syntaxin together with the peripheral membrane SNARE protein SNAP-25 form a stable ternary complex that is disassembled in vitro after binding of soluble α-SNAP by the ATPase NSF (5, 6). According to the original SNARE hypothesis (7), interaction between these SNARE partners bridges opposing vesicular and plasma membranes. Therefore, assembly and disassembly of ternary SNARE complexes would proceed in a trans-configuration that is regarded essential for vesicle docking, priming and/or fusion (8–12). On the other hand, SNARE complexes are also found on the vesicular (13, 14) as well as the plasma (15) membrane in a cis-configuration, i.e. side by side. Protein domains involved in the binary and ternary interactions leading to SNARE complex formation have been originally identified by in vitro binding studies using recombinant soluble fragments of synaptobrevin II, syntaxin 1A, and SNAP-25 as follows: (i) the cytoplasmic domain of synaptobrevin II; (ii) a carboxyl-terminal, membrane-proximal region of syntaxin 1A; and (iii) carboxyl-terminal plus amino-terminal regions of SNAP-25 (16–21). More recently, structural studies confirmed that previously predicted cytoplasmic coiled coil domains of synaptobrevin II (H1, H2) and syntaxin 1A (H3) form a tightly packed parallel four-helical bundle together with two helices derived from SNAP-25 (H4 and H5) in the SNARE complex (22, 23). In contrast, the role of the carboxyl-terminal TMSs located at one side of the SNARE complex (24–26) in these interactions has only been characterized in part.

Transmembrane domains are known to participate in oligomerization of many different integral membrane proteins. It is thought that TMS self-assembly is driven by a close packing of their characteristically shaped surfaces that are defined by their primary structures (reviewed in Ref. 27). We have previously shown that the homodimeric structure originally observed for native synaptobrevin (28–30) is preserved with recombinant synaptobrevin II, where it depends on a specific amino acid motif within the TMS (31). Furthermore, a direct interaction of syntaxin and synaptobrevin TMSs in synthetic proteoliposomes was recently reported (32). Surprisingly, we found that the synaptobrevin II homodimerization motif is conserved within the TMS of syntaxin 1A. This predicted the involvement of this motif in a homotypic interaction of syntaxin 1A as well as in its heterophilic binding to synaptobrevin II.

Here, we (i) identify the minimal TMS amino acid motif required for synaptobrevin II homodimerization, (ii) show that a similar motif mediates syntaxin 1A homodimerization, and (iii) demonstrate that TMSs and cytoplasmic coiled coil domains cooperate in synaptobrevin/syntaxin heterodimerization.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—Rat syntaxin 1A cDNA was amplified by polymerase chain reaction with VENT polymerase (Biolabs) from a plasmid template as described previously for rat synaptobrevin II (31) with...
primers containing NheI (sense primer) and BglII (antisense primer) restriction sites. The amplified fragment was cut with NheI and BglII and ligated into the pET 21d (Novagen)-based plasmid pSNiR, previously cut with NheI and BamHI. In the resulting constructs, the amino termini of synaptobrevin or syntaxin are fused in frame to the carboxyl terminal of a tripartite fusion moiety consisting of the coding sequence of the HA marker epitope in case of synaptobrevin or the c-myc epitope for syntaxin, Staphylococcus aureus nuclease A, and a linker-region coding for 9 amino acids.

Construction of plasmid pToxR-A16 was described previously (33). All other pToxR constructs were made by ligating synthetic oligonucleotide cassettes encoding the desired sequences into plasmid pHSG394/MaeII (34) previously cut with NheI and BamHI. All constructs were verified by dideoxy sequencing.

Site-directed Mutagenesis—All mutants were made by oligonucleotide-directed mutagenesis performed according to Kunkel et al. (35) on single-stranded templates (T7 Mutagen-e-kit, Bio-Rad). All mutations were verified by dideoxy sequencing.

ToxR Activity Assays—ToxR activities were determined in quadruplicates in 9–11 independent experiments as described (33) and are given in Miller units (mean ± S.E.). Western blot analysis was done as described (33). PD-28 cell growth assays were done as described (36), and the absorbance was read at 650 nm at a pathlength of 6 mm. To correct for slightly different membrane insertion efficiencies, the β-galactosidase activities elicited by the mutant sequences were normalized to the A$_{600}$ values obtained after 48 h of cell growth.

Expression and Radiolabeling of Recombinant Proteins—Proteins, encoded by pSNiR vectors, were expressed in Escherichia coli BL21(DE3)pLyS5 (Novagen) as described (31), solubilized with 2% (v/v) polyoxyethylene 9 lauryl ether (Sigma) for mild SDS-PAGE, or with 2% (w/v) CHAPS (Applichem, Darmstadt) in solubilization buffer (50 mM Hepes, pH 7.9, 1 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride) for all other applications. The usual concentration of recombinant protein in solubilization buffer was 1 mg/ml. Biosynthetic labeling was performed by addition of 7.5 μCi of [35S]methionine/cysteine (2.1 Ci/mmol) (Amersham Pharmacia Biotech) per ml of bacterial culture 15 min after induction of expression with isopropyl-β-D-thiogalactopyranoside.

Cross-linking—CHAPS-solubilized proteins (see above) were treated with DSS (Pierce) dissolved in dimethyl sulfoxide. Dimethyl sulfoxide never exceeded 2% (v/v) of the reaction volume. The samples were shaken for 5 min at room temperature, and reactions were then quenched with 100 mM Tris, pH 7.4, for 10 min. The samples were redissolved in SDS sample buffer (50 mM Tris-HCl, pH 6.8, 1% (w/v) SDS, 6 M urea, 50 mM dithiothreitol, 20% (w/v) sucrose), separated by SDS-PAGE (5 μg/lane), and visualized by Western blotting using the HA-mb Ab 12CA5 for HA-tagged synaptobrevin or the myc-mAb 9E10 for myc-tagged syntaxin 1A as described (31). Minigels were run at 4 °C, and samples were not boiled prior to electrophoresis.

RESULTS

The Minimal Homodimerization Motifs of Synaptobrevin II and Syntaxin 1A TMSs and neighboring cytoplasmic coiled coil domains. A, an alignment of the TMSs reveals conservation of the 6 residues previously found important for synaptobrevin II homodimerization in syntaxin 1A (boxed). B, TMS mutants where different combinations of the central 15 amino acids were mutated to alanine (in boldface). Positions corresponding to the minimal 6- or 8-residue dimerization motifs identified in this study are boxed (see text). Syx-cyt was made by deletion of the syntaxin 1A TMS starting from Lys-265. C, the TMS proximal cytoplasmic domains are characterized by specific heptad repeat patterns of hydrophobic amino acids (indicated by α and d). In syb-60/84 and syx-230/251, five of these amino acids were exchanged to alanine (in boldface).

A conservation of the synaptobrevin II homo-dimerization motif in syntaxin 1A

B TMS mutants

C cytoplasmic mutants

# Fig. 1. Amino acid sequences of synaptobrevin II and syntaxin 1A TMSs and neighboring cytoplasmic coiled coil domains. A, an alignment of the TMSs reveals conservation of the 6 residues previously found important for synaptobrevin II homodimerization in syntaxin 1A (boxed). B, TMS mutants where different combinations of the central 15 amino acids were mutated to alanine (in boldface). Positions corresponding to the minimal 6- or 8-residue dimerization motifs identified in this study are boxed (see text). Syx-cyt was made by deletion of the syntaxin 1A TMS starting from Lys-265. C, the TMS proximal cytoplasmic domains are characterized by specific heptad repeat patterns of hydrophobic amino acids (indicated by α and d). In syb-60/84 and syx-230/251, five of these amino acids were exchanged to alanine (in boldface).
synaptobrevin II (Fig. 1A). Therefore, we examined the potential role of this motif in syntaxin 1A homodimerization. Analysis of recombinant syntaxin 1A by SDS-PAGE under mild conditions indeed revealed its partial homodimerization. Dimer formation was almost completely abrogated when the TMS had been deleted (syx-cyt); its central 15 hydrophobic amino acids were replaced by the oligoalanine sequence (syx-A15) or mutated in three positions (syx-mult). Grafting the motif homologous to the synaptobrevin II homodimerization motif (Met-267, Ile-270, Cys-271, Leu-275, Ile-278, and Ile-279) onto the oligoalanine sequence (syx-A8) resulted in syntaxin homodimerization that was even stronger than that of the wt protein (Fig. 2B). Thus, syntaxin 1A also forms homodimers by sequence-specific self-interaction of its TMS based on an amino acid motif almost identical to that previously identified in synaptobrevin II.

To examine whether the cytoplasmic coiled coil domains of synaptobrevin and syntaxin are involved in homodimerization, multiple mutations were made in positions relevant for binary and ternary SNARE protein interactions (Fig. 1C) (Refs. 18, 19, 23, and see below). These mutations had no detectable effect on homodimerization of both proteins, indicating that the cytoplasmic coiled coil domains do not self-interact (results not shown, but see Fig. 4).

Self-interaction of SNARE TMSs in Membranes—To examine self-interaction of the SNARE TMSs in the absence of the cytoplasmic domains and incorporated into membranes, we used the ToxR transcription activator system. We previously established this system as a sensitive tool to study TMS-TMS interactions using the structurally well characterized glycoporphin A TMS dimer for reference (36, 38). The ToxR protein is anchored by a single TMS of choice within the inner membrane of expressing E. coli cells where it is thought to exist in a monomer/dimer equilibrium. The dimeric form binds to the ctx promoter thus initiating lacZ transcription in the indicator cells.

**FIG. 2.** Homodimerization of synaptobrevin II and syntaxin 1A. Wild type and mutated full-length proteins were separated by SDS-PAGE and visualized by Western blotting. A, the 66-kDa homodimer of syb-wt (synaptobrevin/nuclease A fusion = 33-kDa monomer) is disrupted upon different TMS mutations (syb-A15 and syb-mult) but preserved with a 6-residue TMS motif (syb-A8). B, the 100-kDa homodimer of syx-wt (syntaxin/nuclease A fusion = 50-kDa monomer) is disrupted upon TMS-deletion (syx-cyt) or mutation (syx-A15 and syx-mult) but appears even stronger with a conserved 6-residue TMS motif (syx-A8).

**FIG. 3.** Self-assembly of SNARE TMSs in membranes as probed with the ToxR system. A, functional organization of ToxR chimeric proteins. The cytoplasmic ToxR domain is linked via a TMS of choice to the periplasmic MalE moiety. Upon dimerization, ToxR binds to the ctx promoter thus initiating lacZ transcription in the indicator cells. OM, outer membrane; IM, inner membrane. B, different levels of β-galactosidase (β-gal) activity reveal self-assembly of wt TMSs (syb-wt and syx-wt) which is decreased by the mult mutations but preserved with motifs of 8 amino acids (syb-A6 and syx-A6). Significant differences (p < 0.05) are marked with * and highly significant differences (p < 0.001) with ** (Student’s t test). The TMS sequences expressed within the context of ToxR proteins correspond to those given in Fig. 1B. The activity elicited by the oligoalanine sequence (A16) reflects the background signal of the system (marked with a dashed line). C, the expression levels of the ToxR proteins with the SNARE TMSs in FHK12 cells were similar, whereas the A16 construct was overexpressed (33) as revealed by Western blotting. D, all constructs, except the negative control with the deleted TMS (ATM), supported the growth of MalE-deficient PD28 cells to similar degrees indicating their similarly efficient membrane integration. MU, Miller units.
expression is therefore diagnostic of ToxR self-assembly in the membrane.

Here, we replaced the ToxR TMS by the synaptobrevin II or syntaxin 1A TMSs to study their self-interaction. The transcripational activities of these chimeric ToxR proteins indicated that both SNARE TMSs self-interacted similarly well in the membrane. The degrees of interaction were comparable to that of a previously characterized membrane-spanning leucine zipper (33) and below that of the glycophorin A TMS (36, 38). Since the signals were reduced by the mult mutations to statistically significant degrees (Fig. 3B; two-tailed Student’s t test, p < 0.05), the interactions are sequence-specific and involve the same faces of the transmembrane helices as determined for full-length proteins in detergent solution. The weaker effects of the mult mutations found here as compared with detergent solution is assumed to result from higher protein concentrations and/or preorientation of the interacting domains in the membrane (39). The minimal interaction motifs were determined using an oligoalanine host sequence (A16) previously shown to partially partition into the membrane where it stays largely monomeric (33). The 6-residue motifs, which were sufficient for homodimerization of full-length SNAREs in detergent (Fig. 2), partially restored self-interaction in the membrane. To obtain wild-type levels of homodimerization, however, these motifs had to be expanded by two additional conserved isoleucine residues completing the contiguous areas of interfacial residues (Figs. 3B and 7). To exclude that different concentrations of the ToxR chimeric proteins in the membranes distorted the signals, we ascertained their similar expression levels by Western blot analysis (Fig. 3C). Furthermore, we assessed the efficiency of the ToxR constructs to integrate into the inner bacterial membrane by testing their ability to functionally complement the maltose-binding protein (MaltE) deficiency of PD28 cells. Due to a deletion in MaltE, this strain is unable to grow in minimal medium with maltose as the only carbon source (40). In cells expressing correctly inserted ToxR membrane proteins, however, the MaltE domain allows maltose uptake and thus cell growth (36). All constructs complemented MaltE deficiency to comparable degrees, thus confirming their similarly efficient membrane integration; a ToxR protein with deleted TMS (∆TMS) did not support cell growth (Fig. 3D) as expected (36).

In sum, both SNARE TMSs are capable of self-assembling in membranes in the absence of the cytoplasmic domains. Conserved motifs of 8 amino acids are sufficient to mimic these homotypic interactions.

Heterodimerization of Synaptobrevin II and Syntaxin 1A—Conservation of the self-interacting TMS motifs suggested that TMS-TMS interactions may also be important for heterodimerization of both proteins. This is in line with a recently reported contribution of the TMSs to synaptobrevin/syntaxin interaction (32). On the other hand, synaptobrevin II and syntaxin 1A are known to interact via cytoplasmic coiled coil domains in binary as well as in ternary complexes including SNAP-25 (16, 18–20, 22, 23). The hallmark of coiled coil structures is that the a and d positions within repeated abcdefg motifs form the hydrophobic core of the helix-helix interfaces. Mutating a and d positions of the H3 region of syntaxin previously resulted in loss of ternary as well as binary interactions (18).

To compare the contribution of the cytoplasmic coiled coil domains and the TMSs to heterodimerization, we generated mutations in either part of synaptobrevin II and syntaxin 1A. To test these proteins (tagged with HA or myc epitopes, respectively) for their ability to form homo- and heterodimers, they were co-incubated, cross-linked with DSS, and analyzed by SDS-PAGE followed by immunoblotting. Wild-type proteins formed homodimers plus an additional band of intermediate apparent molecular weight that was detected with both antibodies and thus identified as the heterodimer. This result suggests that homodimerization competes with heterodimerization. Mutating five a and d positions in the coiled coil domains of synaptobrevin (syb-60/84) or syntaxin (sxy-230/251) (Figs. 1C and 7) strongly reduced heterodimerization, as expected (Fig. 4). In comparison, the TMS mult mutations had a detectable but somewhat less pronounced effect on heterodimerization as assayed by DSS cross-linking or SDS-PAGE analysis under mild conditions (data not shown).

To evaluate quantitatively the roles of coiled coil domains and TMSs in heterophilic interaction, we developed an overlay assay. Equal amounts of wt and mutant proteins were separated by SDS-PAGE, blotted onto nitrocellulose, and probed with 35S-labeled wt-binding partners. By quantifying bound radioactivity, different degrees of heterodimerization were determined for wt and mutants. The results confirmed the importance of both coiled coil domains for heterophilic binding (Fig. 5A). Importantly, the multiple TMS mutations (syb-mult and sxy-mult) or replacement of the TMSs by the oligoalanine sequence (sxy-A15 and syb-A15) also significantly decreased binding to their respective wt SNARE partner (Fig. 5). To identify the minimal amino acid motifs responsible for heterophilic TMS-TMS interaction, we tested the proteins with the minimal homodimerization motifs. In case of syntaxin 1A, the motif of 6 residues (sxy-A6) was sufficient for wt level of heterodimerization with wt synaptobrevin II, whereas the 8-residue motif that completely restored homodimerization in the ToxR system (sxy-A8) was required in the reverse configuration.

By using an independent experimental approach, we investigated the influence of the mutations on the ability of synaptobrevin to co-precipitate 35S-labeled wt syntaxin from detergent solution. Upon co-incubation, we immunoprecipitated the synaptobrevin proteins and quantitated co-precipitated syntaxin upon SDS-PAGE. In agreement with the overlay assay (Fig. 5), the coiled coil mutant syb-60/84 as well as the TMS mutants syb-mult and syb-A15 co-precipitated significantly less syntaxin compared with wt synaptobrevin, whereas syb-A6 with the 8-residue TMS motif was as efficient as the wt protein (Fig. 6).
Together, these findings show that heterophilic interaction of synaptobrevin II with syntaxin 1A is not only dependent on soluble coiled coil structures but also on specific interactions of their TMSs. Conservation of the minimal TMS motifs mediating homo- and heterodimerization indicates that both types of interaction involve the same faces of the α-helical interaction domains.

**DISCUSSION**

Our data reveal that conserved amino acid motifs within the TMSs are crucial for homo- and heterodimerization of synaptobrevin II and syntaxin 1A, two natural binding partners in the presynaptic nerve terminal. Previously, we had shown that homodimerization of synaptobrevin II, originally observed for the native protein (28–30), depends on a specific amino acid motif in its TMS (31). Interestingly, this motif is almost completely conserved in the syntaxin 1A TMS. In analogy to synaptobrevin II, we show here that mutating or deleting the TMS nearly abolished homodimerization of syntaxin 1A. Furthermore, within full-length proteins, sets of 6 residues form the minimal TMS dimerization motifs when grafted onto an oligoalanine host sequence. By using the ToxR system, we demonstrate that both TMSs self-assemble even in the absence of the cytoplasmic domains in a membrane environment. Here, the amino acid motifs sufficient for wild-type level homodimerization contain two additional conserved isoleucine residues that complete contiguous interfacial areas modeled onto α-helical surfaces (Fig. 7).
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except arginine, lysine, glutamate, histidine, aspartate, and proline in order to retrieve selectively uninked TMSs. We allowed for up to two mismatches to detect closely related motifs. The search identified human, rat, mouse, and bovine synaptobrevin II (0 mismatches) and Xenopus laevis synaptobrevin II, rat synaptobrevin IIB, synaptobrevins from zebrafish and Japanese pufferfish, mouse syntaxin 1A (1 mismatch), rat endobrevin, human syntaxin 1A, and mouse syntaxin 3B and 3C (2 mismatches). Thus, it appears that the rat synaptobrevinII/syntaxin 1A motif is partially conserved in other SNARE protein family members, especially in orthologs.

Homodimerization of the TMS motifs reflects their spatial self-complementarity that allows tight packing of amino acid side chains. The near identity of the motifs in synaptobrevin II and syntaxin 1A therefore predicted that the TMSs also support heterophilic interaction of both proteins. Our data confirm this prediction since heterodimerization competes with homodimerization and is also reduced by mutating either TMS. Furthermore, the minimal homodimerization motifs suffice for a wild-type level of heterophilic interaction. Assuming a-helicity of both TMSs, these minimal motifs wrap around their surfaces in a right-handed fashion (Fig. 7). This suggests that the helices are tilted relative to each other in the dimer with negative packing angles in order to maximize side chain packing which is reminiscent of the glycoproph A transmembrane helix-helix pair (41).

In agreement with previous data (18, 19, 22, 23), our present results show that mutating a and d positions within the coiled coil domains also reduced heterodimerization without, however, affecting homodimerization. Thus TMS-TMS interactions appear as the main driving forces for homodimerization, whereas heterodimerization requires two distinct assembly domains, the cytoplasmic coiled coils and the TMSs.

Various lines of previous evidence indicate that both domains are important for the function of SNARE proteins. Caenorhabditis elegans mutants with impaired neurotransmission contained synaptobrevin (snb-1) or syntaxin (une64) homologs with point mutations at a or d positions in the coiled coil region, a frameshift midway in the snb-1 TMS (42), or an un64 TMS truncated to 16 hydrophobic residues (43). For both TMS mutants, the residual hydrophobic domains should be sufficient to function as membrane anchors (44), which is supported by the apparent localization of the snb1 mutant to synaptic vesicles (43). The notion that the function of SNARE TMSs extends beyond their role as membrane anchors is underscored by the existence of syntaxin isoforms in C. elegans (43) and vertebrates (45, 46) distinguished only by their TMSs. Furthermore, alternatively spliced variants of synaptobrevin I differing only in their TMSs have been reported to localize to distinct subcellular compartments (47). It is therefore tempting to speculate that the structures of, and presumably interactions between, SNARE protein TMSs are important for their function.

TMS-TMS interactions may play a role at different stages of SNARE protein function. First, heterophilic TMS-TMS interaction may stabilize the SNARE complex. The original SNARE hypothesis postulated that synaptobrevin as a v-SNARE of synaptic vesicles interacts with the t-SNAREs syntaxin and SNAP-25 of the plasma membrane in a trans-configuration (5–7). On the other hand, ternary SNARE complexes are also present in synaptic vesicles (13, 48, 49) and the plasma membrane (15) in a cis-configuration allowing for TMS-TMS interactions. Indeed, the presence of the TMSs increased the thermal stability of a trypsin-resistant core SNARE complex (20). Furthermore, Margittai et al. (32) reported increased stability of the SNARE complex including the TMSs against disassembly mediated by α-SNAP and NSF as well as the appearance of the sub/syx-heterodimer upon partial disassembly. Evidence that co-localization of v- and t-SNAREs in the same membrane might be of functional relevance comes from yeast vacuole fusion (14), where synaptobrevin and syntaxin homologs form a pentameric cis-complex in one vacuole (50).

Second, Poirier et al. (20) provided evidence for another potential role of TMSs interactions. Strong multimerization of SNARE complexes was observed with both TMSs present; it was less pronounced when the TMS of either synaptobrevin II or syntaxin 1A was deleted and absent without TMSs, suggesting that multimerization is due to inter-SNARE TMS-TMS interactions. In line with this, electron microscopy revealed dimers and multimers of native or recombinant SNARE complexes that associated at the sites of the TMSs; multimerization was absent with SNARE complexes composed of the cytoplasmic domains only (26). Although the function of SNARE complex multimerization is currently not clear, it may play a role in the fusion reaction. This is supported by the observation of multimeric superstructures that appear to be functional intermediates of certain fusogenic viral membrane proteins. Enveloped viruses fuse with target membranes by way of viral glycoproteins that, in analogy to SNAREs, consist of carboxy-terminal TMSs and soluble coiled coil domains that assemble to rod-shaped trimeric helical bundles (51). Interestingly, the concerted action of at least three hemagglutinin trimers is required for fusion of influenza virus with the endosomal membrane (52). Likewise, fusion mediated by baculovirus surface glycoprotein 64 involves the assembly of transient multimeric complexes assembled by lateral association of stable glycoprotein 64 trimers (53). Upon multimerization of these fusogenic viral or SNARE protein complexes, the membrane-spanning domains may form a scaffold for fusion pores shown to precede virus-mediated bilayer fusion (54) or detected prior to catecholamine release from chromaffin granules (55), or serotonin release from leech neurons (56).

Third, SNARE TMS-TMS interactions may be directly involved in membrane fusion. Since the formation of the parallel coiled coil may proceed from the amino termini of SNARE proteins interacting in trans, the fusion of vesicular and plasma membranes may result from a successive “zippering up” of cytoplasmic domains that closely juxtaposes vesicle and target membranes (9, 22). The interaction of the TMSs may then be the final step completing membrane merger. As noted above, the arrangement of residues within the minimal TMS motifs suggests negative packing angles of the interacting α-helices. Since the cytoplasmic coiled coil domains assemble with positive packing angles (22), both assembly domains may be separated by a flexible linker region. In agreement with this, insertion of proline residues intended to disrupt a potential helical continuity between coiled coil domains and TMSs did not abolish SNARE protein-mediated liposome fusion (57). Thus, both interaction domains are considered to be largely independent of each other which may allow for the structural flexibility required for fusion.

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