Comment on “Transmembrane Segments of Syntaxin Line the Fusion Pore of Ca\textsuperscript{2+}-Triggered Exocytosis”

**Ca\textsuperscript{2+}-triggered** membrane fusion is the defining stage of exocytosis, proceeding by an as-yet-unknown molecular mechanism. Han et al. (1) described a model in which multiple syntaxin transmembrane domains (TMDs) act as subunits of a proteinaceous fusion pore. Although intriguing, the interpretations do not address sequence conservation or other data in the field, and the results are equally consistent with the well-established stalk-pore model for membrane fusion (2). As disruptions induced by the mutations used in (1) will include profound effects on local membrane curvature stress, the results of these studies should also be considered in terms of a lipidic fusion-pore model.

The model of syntaxin TMDs forming the fusion pore (1) is consistent with the demonstrated lack of critical SNARE (soluble N-ethylmaleimide–sensitive factor attachment protein receptor) cytoplasmic domain interactions during the Ca\textsuperscript{2+}-triggered fusion steps (3, 4). The fusion pore was characterized as a gap junction–like channel (5), and small molecule fluxes might thus be affected by changes in pore-lining constituents, as in ion channels (6, 7). Within the parameters of this model, Han et al. mutated amino acids in the TMD of syntaxin, a protein essential for exocytosis. Tryptophan replacement of isoleucine (I269W), glycine (G276W), and isoleucine (I283W) in the TMD decreased transmitter flux, leading the authors to model isoleucine (I283W) in the TMD of syntaxin, a protein essential for Ca\textsuperscript{2+} flux, as aligning to the putative pore-lining residues. Based on the rationale that introduction of large tryptophan moieties “blocked” the fusion pore, the molecular volume of pore-lining amino acids should be highly conserved. To test this, we aligned the TMD of syntaxin homologs (Fig. 1) and compared the amino acid residues corresponding to positions 269, 276, and 283 of rat syntaxin 1A [as used in (1); see Table 1]. There is no clear conservation of molecular volumes (14). This lack of correlation appears regardless of the source or type of vesicle (Fig. 1).

Interestingly, these seven residues that interchangeably occur at positions 269, 276, and 283 (listed in Table 1) are considered to be among the most hydrophobic (15). Tryptophan, used to test the model (1), is much more hydrophilic than any of the naturally occurring amino acids at these positions. Langosch et al. (16) noted that the TMDs of syntaxin homologs contain an overrepresentation of isoleucine and valine (the two most hydrophobic residues) as compared with other tail-anchored membrane proteins. These residues would thus contribute substantially to the hydrophobic volume of the bilayer added by syntaxin TMD, which would strongly affect membrane curvature stress (17, 18). Local spontaneous curvature is a crucial parameter for membrane merger.

**Table 1.** Residue incidence aligned to the putative pore-lining residues of rat syntaxin 1A (1). A total of 72 syntaxin sequences [18 syntaxin homologs (1A, 1B, 1B2, 1C, 2, 3, 4, 4A, 5, 5A, 6, 7, 8, 10, 12, 13, 16, 17)] from human, rhesus monkey, rat, mouse, bovine, sheep, chicken, rainbow trout, zebrafish, squid, *Limulus*, urchin, snail, *Aplysia*, yeast, *Arabidopsis*, and soybean origins were analyzed from sequence data available in the protein databank (www.ncbi.nlm.nih.gov). Residues are arranged in order of decreasing relative molecular volume, as indicated.

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<tr>
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<th>% Residue Conservation</th>
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<tr>
<td>Aligned to position</td>
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<tr>
<td>269</td>
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<td>276</td>
<td>2.8</td>
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<td>283</td>
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**Fig. 1.** The sequence homology of the transmembrane domains of (A) syntaxin 1A homologs across species and (B) syntaxin homologs across a list of common model species. The gray boxes highlight the putative pore-lining residues as aligned to the indicated amino acid positions of rat syntaxin 1A. For syntaxin 1A homologs (A): 75% are I, 12.5% are L, and 12.5% are V at position 269; 75% are G, 12.5% are I, and 12.5% are V at position 276; 37.5% are V, 25% are I, 25% are L, and 12.5% are F. For other syntaxin homologs (B): 75% are L and 25% are A at the position corresponding to 269 of rat syntaxin 1A; 50% are I, 37.5% are L, and 12.5% are V at the position corresponding to 276 of rat syntaxin 1A; 37.5% are I, 37.5% are L, 12.5% are V, and 12.5% are A at the position corresponding to 283 of rat syntaxin 1A.
as described by the stalk-pore hypothesis (2). At positions 269, 276, and 283, tryptophan residues might well alter the anchoring of the TMD within the bilayer (19), affecting the hydrophobic volume contributed, altering the local spontaneous curvature stress, and thereby interfering with the formation/stability of a lipidic pore, detected as decreased flux through the fusion pore (1). Such a local effect would be particularly marked in this study (1) in which the density of mutated syntaxins was ~10-fold that of the native protein. Notably, tryptophan mutations at positions 285 and 287 produced the most potent inhibition of secretion (1), yet these residues do not lie on the putative pore-lining face of syntaxin.

Making unambiguous interpretations of the data presented by Han et al. (1) is difficult, as the mutations produced only inhibitory effects; enhancement of flux would be more indicative of a direct role for the syntaxin TMD in pore formation. This, then, may well represent a more general shortcoming of overexpression approaches in studies of membrane functions. Because specific membrane microdomains are known to be critical mechanistic elements, alterations to local curvature stresses by the overexpression of excess exogenous proteins/peptides can disrupt the integrity of these micromdomains, leading to bilayer instability or other unintended effects on local functions. This caveat must be considered when using such approaches in vivo or in vitro.

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References and Notes
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