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Science **306**, 813c (2004);
DOI: 10.1126/science.1102272

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Response to Comment on "Transmembrane Segments of Syntaxin Line the Fusion Pore of Ca²⁺-Triggered Exocytosis"

We welcome the opportunity to discuss the relative merits of the lipidic, stalk-pore model for Ca²⁺-triggered membrane fusion versus our model of a fusion pore formed by the syntaxin membrane anchor (1). We note that the most conservative interpretation stated in our paper, that lipid molecules could intercalate between syntaxin transmembrane segments to complete the pore structure, contains elements of a lipidic fusion pore. Nevertheless, the points raised by Szule and Coorssen (2) merit detailed consideration.

Szule and Coorssen cited their studies (3, 4) on sea urchin egg cortical vesicles showing no SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) requirements for Ca²⁺-triggered fusion. This position is at odds with a large body of work showing that Ca²⁺-triggered release of neurotransmitter and hormone requires SNAREs. For example, the Hua and Scheller (5) study, cited in the comment, showed inhibition of release in PC12 cells by the VAMP2 coil domain (a SNARE fragment). In contrast, recombinant VAMP2 containing this domain had no effect on cortical vesicle fusion (4). Thus, the molecular mechanisms in cortical vesicle fusion are likely to be very different. The mechanism of viral fusion diverges even more markedly and depends on proteins with no homology to those operating in Ca²⁺-triggered release. Szule and Coorssen suggest that our estimate of five to eight copies of syntaxin in the fusion pore disagrees with an estimate (5) that three SNARE complexes cooperate during fusion. This number was the exponent of the concentration dependence of inhibition by the above-mentioned VAMP2 fragment. However, cooperativity exponents such as this and the related Hill coefficient represent lower bounds to the number of elements in a

cooperative unit rather than the actual number. The Hill coefficient for oxygen saturation of hemoglobin, for example, is 2.7, but hemoglobin has four subunits. Furthermore, the value of three is for inhibition by an added reagent. Even if the actual number of bound inhibitors is three, the fusion complex could contain many more SNAREs. If the binding of three blocks release, then the exponent will be three. Our estimate of five to eight copies of syntaxin is consistent with the correct interpretation of three as a lower bound.

Szule and Coorssen (2) also assert that our model requires the syntaxin transmembrane domain to be highly conserved. However, fusion pores are diverse structures with conductances that vary by more than 10-fold (6, 7). These conductance variations could reflect variations in the syntaxin sequence at these positions. It is also likely that variations in syntaxin copy number contribute to the diverse values of the fusion-pore conductance.

That a bulky tryptophan side chain would stress the membrane and induce curvature is an interesting possibility. However, curvature-stress relates more to fusion-pore formation and stability than to what we measured, which was flux through an open pore. Furthermore, if this segment of syntaxin does indeed span the plasma membrane, tryptophan residues located in the inner and outer bilayer leaflets will produce curvature-stress with opposite orientations. However, tryptophan residues presumed to have positions close to the cytoplasmic face (position 269) and extracellular face (position 283) had the same effect on fusion-pore flux. In addition, other tryptophan mutations should induce curvature stress, and none of the others affected fusion-pore flux. Szule and Coorssen were also concerned about mutations that only inhibited flux, stating that "enhancement of flux would be

more indicative of a direct role for the syntaxin TMD in pore formation." The I283G mutation did enhance fusion-pore flux [figure 3E in (1)], as did two new mutations studied since our initial report (1). We have thus met one of their criteria for a direct role.

Tryptophans at positions 285 and 287 inhibited release, but this result has no power to discriminate models, because an inhibitory action has many possible interpretations: reduced docking, priming, triggering, etc. Overexpression studies may have shortcomings, as suggested in (2), but our experiments in which the wild-type and 11 mutant proteins were overexpressed without altering fusion-pore flux provide an adequate control for an explanation based on expression levels.

We emphasize that despite our results suggesting that the initial fusion pore is composed of protein, a lipidic fusion pore is likely to come into play in the ensuing steps of Ca²⁺-triggered exocytosis. After the formation of a gap junction-like channel, an expansion occurs in which lipid molecules insert between the protein subunits. The transition to this state and its subsequent evolution depend critically on the behavior of lipids. Investigation of these processes will require a deep understanding of the physical and mechanical properties of the structures and aggregates that lipids are capable of forming.

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References and Notes

1. X. Han, C.-T. Wang, J. Bai, E. R. Chapman, M. B. Jackson, *Science* **304**, 289 (2004).
2. J. A. Szule, J. R. Coorssen, *Science* **306**, 813 (2004); www.sciencemag.org/cgi/content/full/306/5697/813b.
3. J. R. Coorssen et al., *J. Cell. Biol.* **116**, 2087 (2003).
4. J. A. Szule et al., *J. Biol. Chem.* **278**, 24251 (2003).
5. Y. Hua, R. H. Scheller, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 8065 (2001).
6. M. Lindau, W. Almers, *Curr. Opin. Neurobiol.* **7**, 509 (1995).
7. V. A. Klyachko, M. B. Jackson, *Nature* **418**, 89 (2002).
8. We thank E. R. Chapman for comments.

2 July 2004; accepted 4 October 2004