New and Notable

Are There Too Many or Too Few SNAREs in Proteoliposomes?

Leonid V. Chernomordik and Kamran Melikov

Section on Membrane Biology, Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-1855

Phospholipid vesicles referred to as liposomes allow reconstitution of protein function in the absence of other proteins. Liposomes have been widely used in exploring viral fusion proteins and, more recently, proteins that mediate intracellular fusion. An evolutionarily conserved family of proteins called SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) is critically important in membrane and protein trafficking in eukaryotic cells including docking and fusion of synaptic vesicle carrying v-SNARE VAMP with plasma membrane carrying t-SNAREs SNAP-25 and syntaxin (1). The specific role of SNAREs in intracellular fusion has been explored by many groups and in many experimental systems. However the important question whether pairing of v- and t-SNAREs directly mediates fusion or acts upstream of it remains under debate (1–5). One of the most important arguments that SNAREs might act as fusogens came from reports on fusion between SNARE-bearing proteoliposomes (6). Two articles in this and previous issues provide new insights into fusogenic activity of reconstituted SNAREs (7,8).

Do SNAREs represent minimal fusion machinery? According to one of the conventional definitions, a fusion protein is a protein that is critical for fusion in a biologically relevant context and is sufficient to promote merger of both outer and inner leaflets of the membranes in model systems. SNAREs satisfy this definition, as evidenced by fusion reported for SNAREs reconstituted in lipid bilayers (6,9–11) and expressed at cell surface (12). A stronger definition would additionally require this reconstituted fusion to be comparable with the biologically relevant one in rates of both lipid and content mixing under conditions that mimic physiological conditions; in particular, in dependency on the lipid and medium compositions; and in the numbers of the proteins required for fusion. The surface density of fusion proteins determines not only the rates of fusion but also the observed fusion phenotypes, with fewer proteins required for the merger of outer leaflets (hemifusion) than for complete fusion (10,13).

In many earlier articles, SNARE–mediated liposome fusion was studied at very high surface densities of SNAREs with protein/lipid ratios of v-SNARE VAMP as high as 1:20–50 (6,10) corresponding to 300–750 proteins per vesicle. This exceeds v-SNARE densities characteristic for synaptic vesicles, widely quoted as 30–100 v-SNAREs per vesicle (3) but might be as low as 10–15 v-SNAREs/vesicle (protein/lipid ~1:1000) as estimated in Dennison et al. (8). Two new articles explore the fusogenic activity of proteoliposomes with SNARE (synaptobrevin) surface density in the range from 1:950 to 1:120. Both groups formed proteoliposomes by a direct reconstitution method, where detergent-solubilized SNAREs were added to preformed liposomes rather than by a comicellization technique based on mixing of proteins and lipids in detergent. Whereas most of the earlier work on SNARE proteoliposomes was done on comicellization proteoliposomes (6,11) but see Lu et al. (10), proteoliposomes prepared by direct reconstitution are much more homogeneous in size and protein content (7) and thus are better suited for analysis of the effects of the surface density of reconstituted proteins. In contrast to studies using proteoliposomes with high surface densities of SNAREs (6,10), both Chen et al. and Dennison et al. report that at lower protein/lipid ratios of up to 1:120–250, fusion is very slow and inefficient (7,8), and does not exceed 4% even after 75 min (8). At these protein densities, fusion is not observed even when efficient docking of proteoliposomes is induced by subfusogenic concentrations of polyethylenglycol (PEG) (8), indicating that in artificial systems, a high density of SNAREs is needed not only to increase docking probability but for the actual fusion reaction as well. Interestingly, for the same average protein density, proteoliposomes formed by comicellization are more fusogenic. This might be explained by the heterogeneity of these liposomes containing a fraction of smaller and apparently more fusogenic vesicles that are enriched in SNAREs (7).

At high enough density, SNAREs mediate robust lipid mixing between proteoliposomes (7,10), and, even at lower densities, reconstituted SNAREs promote fusion driven by PEG (8). In both cases, SNARE-promoted fusion between liposomes proceeds by the same fusion-through-hemifusion pathway as diverse biological fusion reactions including intracellular fusion (13). Based on the analysis of the effect of SNARE proteins on the kinetics of PEG-induced fusion, Dennison et al. (8) conclude that SNAREs promote formation of a local hemifusion connection, referred to as a stalk intermediate (13). Finding that similar to many other fusion reactions (13), including SNARE-dependent fusion between yeast vacuoles (5), SNARE-dependent liposome fusion observed at high protein densities is inhibited by lysophosphatidylcholine (7) further strengthens this conclusion.

Although work on SNARE proteoliposomes, including the new studies, has already brought important insights into the mechanisms of the interactions

Submitted January 24, 2006, and accepted for publication February 27, 2006.

Address reprint requests to Leonid V. Chernomordik, E-mail: lchern@helix.nih.gov.

0006-3495/06/04/2657/02 $2.00

doi: 10.1529/biophysj.106.081133

between v- and t-SNAREs and between SNAREs and other components of the machinery (8,11,14), we still do not know enough to fully apply these results to SNARE-dependent biological fusion. Note that even when densities of SNAREs are high enough to promote PEG-dependent fusion, this promotion does not require formation of trans-SNARE complexes (8). In addition, matching surface densities of SNAREs in proteoliposomes to those in synaptic vesicles likely oversimplifies the comparison. In contrast to proteoliposomes, where SNAREs might be randomly distributed over the surface, in a biological setting SNAREs along with other proteins are likely concentrated in a prefusion complex with a specific lipid composition. This can be important for the assembly of SNARE aggregates and coordinated function of multi-protein machinery (2,15).

In different reconstituted systems, SNAREs-mediated fusion alternatively proceeds fast and efficiently (9), requires thermal activation (3) or induction by PEG (8), or fails completely (7). What might be the basis for such variability? Viral fusion proteins such as influenza virus hemagglutinin both catalyze formation of early intermediates in the fusion pathway and provide the driving force for fusion by making the postfusion state energetically more favorable than the prefusion state (13). Assuming that the intracellular fusion machinery performs both of these jobs, one can suggest that SNAREs promote the earliest fusion intermediates. These intermediates effectively advance to become observables such as lipid mixing and content mixing only under the tension (13,16) that in biological reactions might be generated by other fusion proteins involved in SNARE-dependent fusion. The two new articles (7,8) describe well-defined reconstituted systems where SNAREs are insufficient for fusion. These data are consistent with the work in native membranes emphasizing the importance of proteins other than the SNAREs. In the absence of these proteins, the driving force necessary for fusion might come from the intrinsic properties of a particular model system. Comparative analysis of SNARE-dependent fusion in different well-defined model systems will hopefully help in understanding the particular role that SNAREs play in the fusion event in those systems and ultimately in vivo.

REFERENCES

1. Jahn, R., T. Lang, and T. C. Sudhof. 2003. Membrane fusion. *Cell*. 112:519–533.
2. Jackson, M. B., and E. R. Chapman. 2006. Fusion pores and fusion machines in Ca^{2+}-triggered exocytosis. *Annu. Rev. Biophys. Biomol. Struct.* (Epub ahead of print).
3. Brunger, A. T. 2005. Structure and function of SNARE and SNARE-interacting proteins. *Q. Rev. Biophys.* 38:1–47.
4. Coorssen J. R., P. S. Blank, F. Albertorio, L. Bezrukov, I. Kolosova, X. Chen, P. S. Backlund Jr., and J. Zimmerberg. 2003. Regulated secretion: SNARE density, vesicle fusion and calcium dependence. *J. Cell Sci.* 116:2087–2097.
5. Reese, C., and A. Mayer. 2005. Transition from hemifusion to pore opening is rate limiting for vacuole membrane fusion. *J. Cell Biol.* 171:981–990.
6. Weber, T., B. V. Zemelman, J. A. McNew, B. Westermann, M. Gmachl, F. Parlati, T. H. Sollner H, and J. E. Rothman. 1998. SNAREpins: minimal machinery for membrane fusion. *Cell*. 92:759–772.
7. Chen, X., D. Araç, T-M. Wang, C. J. Gilpin, J. Zimmerberg, and J. Rizo. 2006. SNARE-mediated lipid mixing depends on the physical state of the vesicles. *Biophys. J.* 90:2062–2074.
8. Dennison, S. M., M. E. Bowen, A. T. Brunger, and B. Lentz. 2005. Neuronal snares do not trigger fusion between synthetic membranes but do promote PEG-mediated membrane fusion. *Biophys. J.* 90: 1661–1675.
9. Liu, T., W. C. Tucker, A. Bhalla, E. R. Chapman, and J. C. Weisshaar. 2005. SNARE-driven, 25-millisecond vesicle fusion in vitro. *Biophys. J.* 89:2458–2472.
10. Lu, X., F. Zhang, J. A. McNew, and Y. K. Shin. 2005. Membrane fusion induced by neuronal SNAREs transits through hemifusion. *J. Biol. Chem.* 280:30538–30541.
11. Schuette, C. G., K. Hatsuzawa, M. Margittai, A. Stein, D. Riedel, P. Kuster, M. Konig, C. Seidel, and R. Jahn. 2004. Determinants of liposome fusion mediated by synaptic SNARE proteins. *Proc. Natl. Acad. Sci. USA*. 101:2858–2863.
12. Hu, C., M. Ahmed, T. J. Melia, T. H. Sollner, T. Mayer, and J. E. Rothman. 2003. Fusion of cells by flipped SNAREs. *Science*. 300:1745–1749.
13. Chernomordik, L. V., and M. M. Kozlov. 2005. Membrane hemifusion: crossing a chasm in two leaps. *Cell*. 123:375–382.
14. Zhang, Y., Z. Su, F. Zhang, Y. Chen, and Y. K. Shin. 2005. A partially zipped SNARE complex stabilized by the membrane. *J. Biol. Chem.* 280:15595–15600.
15. Rickman, C., K. Hu, J. Carroll, and B. Davletov. 2005. Self-assembly of SNARE fusion proteins into star-shaped oligomers. *Biochem. J.* 388:75–79.
16. Zimmerberg, J., F. S. Cohen, and A. Finkelstein. 1980. Micromolar Ca^{2+} stimulates fusion of lipid vesicles with planar bilayers containing a calcium-binding protein. *Science*. 210:906–908.