Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4621–4624, May 1994

Review

Regulated vesicular fusion in neurons: Snapping together the details

I. Christina Bark and Michael C. Wilson

Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

ABSTRACT **In the past year major strides have been made toward our understanding of the molecular mechanisms involved in regulated vesicle fusion and exocytosis in neurons and neuroendocrine cells. Much of this advance has come from the identification of proteins participating in these events and of their potential roles mediated by interactions with each other, the constituent membranes, and, in some cases, Ca^{2+} signaling. The involvement of vesicle fusion in elongation of neuronal processes during development and release of transmitters and neuromodulatory peptides in the mature nervous system indicates, however, that refinements in the fusion machinery may be required for each of these acts. For many of the participants in synaptic membrane fusion, variant isoforms have been identified that exhibit modifications that might alter interactive properties of these proteins. We discuss the idea that diversification of isoforms, as illustrated by the expression of alternatively spliced variants of SNAP-25, is likely to be an important component in providing the detail necessary to differentiate the physiology of regulated fusion of different classes of vesicles employed in development, neurotransmission, and secretion.**

Constitutive trafficking and subsequent fusion of vesicles to incorporate components into the cell surface and intracellular organelles is a process common to all cells. In neural and endocrine cells, however, vesicular fusion and transport take on an added regulated dimension. In addition to providing material for growth cone expansion and neurite extension during development, vesicular fusion is performed in these cells for regulated secretion of neurotransmitters and modulatory peptides (1, 2). Not surprisingly, there are characteristic differences between the vesicles as well as other components that participate in these distinct docking and fusion events. One particularly fruitful avenue in characterizing these differences has been to identify proteins associated with nerve terminals (3, 4). In a remarkable convergence of alternative approaches to the problems of vesicular trafficking and neurotransmitter release, recent advances have now not only established the importance of a number of these characterized proteins but also implicated their specific functions in these tasks in neurons and endocrine cells (see ref. 5). For many of these proteins, these functions can be distinguished independently by their homology to proteins shown to be involved in trafficking in yeast (6). The physiological diversity of vesicular storage and fusion in these cell types, however, suggests that a yet further degree of specialization is required from these proteins for selective targeting, protein, and membrane interactions. This requirement could be resolved by variant isoforms of participating proteins displaying subtle modification of domains that provide such functions.

Classes of vesicles can be distinguished by morphology, content, and probability of release in response to Ca influx. During neurite outgrowth, plasmalemma precursor vesicles (PPVs) fuse at the leading plasma membrane in a Ca-dependent, possibly ATP independent, manner to deliver membrane components but appear not to discharge neurotransmitters (7). In contrast, neurosecretion is mediated by two types of vesicles (for review, see refs. 1, 2, and 8): large dense core vesicles (LDCVs) or secretory granules (SGs) typically associated with neuropeptides; and small clear synaptic vesicles (SVs) present in neurons or synaptic-like microvesicles (SLMVs) of neuroendocrine cells that contain classical neurotransmitters such as glutamate, γ-aminobutyric acid (GABA), and acetylcholine. Within the presynaptic terminal, docking and fusion of LDCVs primarily occurs extrajunctionally, whereas SVs selectively fuse at the active zone immediately apposing the synaptic cleft (9, 10). Similarly, Ca channels may be clustered at the active zone and therefore be intimately associated with SVs (see ref. 11); however, it is not known if this same close relationship exists with other classes of vesicles. The spatial compartmentalization of fusion sites and Ca signaling is likely to play a part in determining distinct rates of secretion in response to stimulation: from <1 msec for SVs to tens of milliseconds for SGs (12).

Players in Vesicle Docking and Fusion

In a model for vesicular fusion at the nerve terminal, Rothman and colleagues (13) have proposed that the integral SV protein synaptobrevin/VAMP (Syb) and plasma membrane proteins syntaxin (Sytx) and synaptosomal-associated protein of 25 kDa (SNAP-25) serve as membrane receptors, termed SNAREs, for a core fusion apparatus comprised of *N*-ethylmaleimide-sensitive factor (NSF) and three isoforms of soluble NSF-associated proteins (α-, β-, and γ-SNAPs) (see Fig. 1). That Syb, Sytx, and SNAP-25 each, in fact, do play a central role in exocytosis is demonstrated by their identification as specific substrates for botulinum neurotoxins (BoNTs) that selectively block neurotransmission (reviewed in ref. 5). More recent evidence suggests that Syb, Sytx, SNAP-25, and an additional integral membrane protein of vesicles, synaptotagmin (Sytg), first form a precomplex, presumably bridging vesicular and plasma membranes, where Sytg is displaced by α-SNAP prior to entry of NSF into the complex (14). Because Sytg contains two regions of a sequence homologous to the phospholipid and Ca ion binding C2 domains of protein kinase C (16), it has been suggested to act as a Ca ion sensor (17). Interestingly, Sytg interacts with the plasma membrane protein Sytx, which has been found tightly associated with N-type Ca channels, and neurexins, a family of transmembrane proteins bearing a laminin-like extracellular domain (18–22). This suggests that Sytg may function as a clamp whose release is coupled to Ca influx (14).

Adding further to the complexity of these envisioned interactions, additional proteins have been recently identified, but their roles in regulated vesicle fusion have yet to be so deftly assigned. The small vesicle GTP binding protein Rab3A, for example, is found in a complex of Syb, Sytx, and SNAP-25 that apparently lacks NSF and associated SNAPs (23), suggesting its participation in early steps of docking. Based on its binding to Sytx, a novel protein Munc-18 has been identified that bears significant similarity to the *Caenorhabditis elegans* gene *unc-18* (previously implicated in neurotransmission) as well as homology to *sec-1* of yeast, also thought to be involved in exocytosis (24). That both Munc-18 and SNAP-25 bind to distinct domains of Sytx (24), which also inter-

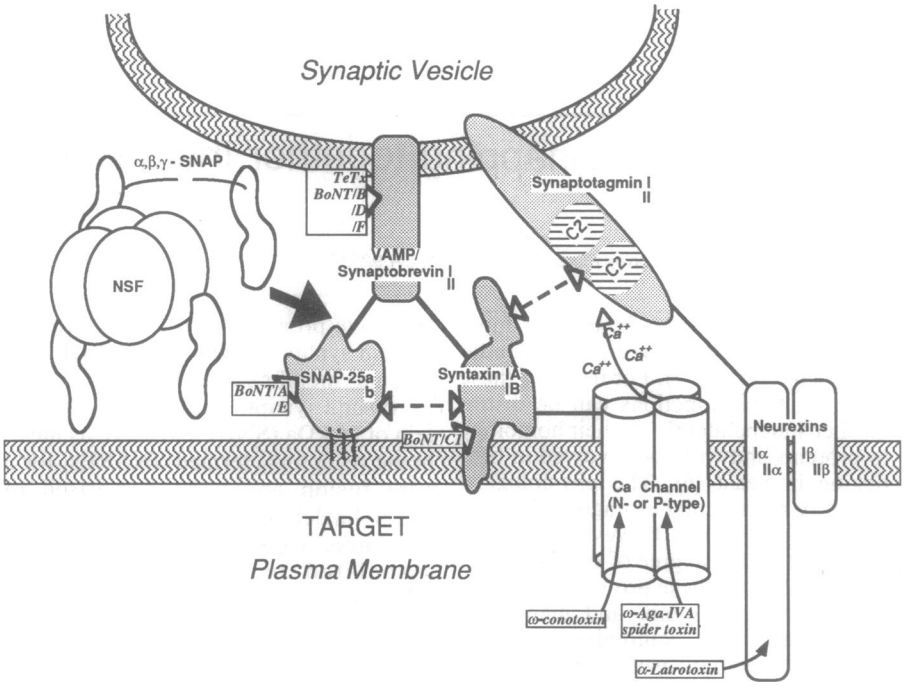

FIG. 1. Schematic representation of some of the protein interactions likely targeting synaptic vesicle fusion in neurons. Isoforms of targeting membrane proteins (indicated by shading) VAMP/synaptobrevin (Syb) and synaptotagmin (Sytg) and SNAP-25 and syntaxin (Sytx) are positioned on vesicular and plasma membranes of the presynaptic nerve terminals. As proposed by Rothman and colleagues (13, 14), tetrameric NSF and associated SNAP proteins (the β-SNAP isoform appears specific to neural systems) interact transiently with the ensemble of vesicle and plasma membrane targeting proteins. Upon entry into an initial complex comprised of Syb, Sytg, SNAP-25, and Sytx (solid arrow), α-SNAP is thought to dislodge Sytg (depicted by the doubleheaded arrow) and assemble with NSF to form a 20S particle with the remaining Syb, SNAP-25, and Sytx. Subsequently, NSF-catalyzed ATP hydrolysis is proposed to lead to disassociation of Sytx, SNAP-25, and Syb (doubleheaded arrow). The intermediary interactions postulated to be interrupted by the NSF fusion machine between Sytx and Sytg and between Sytx and SNAP-25 are indicated by dashed lines, whereas the association of Sytx with the ω-conotoxin binding α-subunit of the N-type, as well as other Ca ion channels, and Sytg with the α-subunit of neurexins is represented as solid lines. Botulinum neurotoxin serotypes (BoNT/A to BoNT/F), tetanus toxin (TeTX), and other neurotoxins that have been instrumental in distinguishing the participants and their roles are indicated within boxes aimed at their targets. While this model appears consistent with docking and setting the stage for fusion of synaptic vesicles, less is known of the scenario for LDCVs. For example, peptidergic exocytosis from LDCVs, in contrast to transmitter release from SVs, is not stimulated by α-latrotoxin at neuromuscular junctions (15), suggesting that dispersed LDCVs either do not interact with neurexins when targeted to the membrane for fusion or employ a different isoform localized away from the active zone and insensitive to the toxin. In growth cones, beyond the involvement of SNAP-25 that is likely to be performed by the a isoform, the participation of the other represented proteins in fusion of PPVs for plasmalemma expansion remains an open question.

acts directly with Syb (25), underscores the apt christening of Sytx from the Greek (18) that anticipated its importance in assembling multiple proteins at the plasma membrane. The characterization of the so-called cysteine string proteins (CSPs) of *Torpedo* and *Drosophila*, distinguished by their unusual motif of 10 or 11 consecutive cysteine residues, as a synaptic vesicle protein that affects N-type Ca channels (26, 27) and is required for effective synaptic transmission (28), indicates that this protein may provide a further link in coupling the control of Ca ion influx with vesicle fusion, perhaps by signaling docking of vesicles. Although several questions concerning the actual sequence of events and proper protein partnering during exocytosis remain unresolved, these observations already suggest that these membrane-associated proteins are critical components for determining the specificity and physiological characteristics of regulated vesicular fusion in neurons and endocrine cells.

Isoforms to Vary the Theme

The prime candidates for leading roles in the initial targeting of vesicles to the plasma membrane, Sytg, Syb, SNAP-25, Sytx, and neurexins, are expressed as variant isoforms encoded either by different genes—e.g., Syb (29–32), Sytg (33, 34), Sytx (35), and neurexins I and II (20)—or, as SNAP-25 (36, 37) and neurexins (20), by alternative splicing. Importantly, the alternative isoforms of these proteins are differentially expressed. While some isoforms are specific to neuronal and neuroendocrine cells, others are expressed by other tissues, supporting the idea that these proteins function in distinct membrane fusion events possibly both regulated and constitutive in nature. Moreover, while Sytg is associated with all secretory vesicles, other vesicle proteins are not detected on LDCVs and thus appear specific to SVs (38). This raises the issue of whether the composition of the cast of individual components and their respective isoforms, drawn from the repertoire of vesicle and plasma membrane proteins, directs the specificity of vesicle fusion complexes.

The regulated expression of SNAP-25 presents an interesting example that may provide novel insight into how subtle variations in sequence between isoforms could contribute to different vesicle fusion events. SNAP-25, as other presynaptic proteins, is highly evolutionarily conserved with identical sequence in mouse (39), human (37), and chicken (40) and limited divergence in goldfish (41), *Torpedo*, and *Drosophila* (42). Based on the mammalian sequence, several structural domains have been postulated that are largely conserved through *Drosophila* and thus may reflect functionally important regions of the protein. At the amino terminus, the periodicity of hydrophobic residues predicts an amphipathic helix with a high potential for forming a coiled-coil structure that is likely to be involved in protein–protein interactions (39). Indeed, SNAP-25 specifically interacts with the carboxyl portion of Sytx (24), which also comprises a highly probable coiled-coil domain. Near its carboxyl terminus, SNAP-25 contains a short sequence, IDRI, identical to Sytx. Endopeptidergic cleavage either within this sequence or 17 residues further toward the carboxyl terminus of SNAP-25 by BoNT/E or BoNT/A (43, 44) effectively blocks exocytosis, thereby demonstrating functional importance of this end of the protein possibly in protein recognition. The idea that such interactions, in particular between the coiled-coil domains of SNAP-25 and Sytx, might engage to promote dynamic conformational changes in protein complexes at the plasma membrane leading to fusion and exocytosis (14), as proposed for influenza hemagglutinin in viral fusion (45), is certainly enticing and deserves further investigation.

The region that differs between SNAP-25 isoforms, and therefore implicated in recruiting distinct vesicle fusion events, is the domain that likely provides for plasma membrane attachment. In contrast to the other synaptic membrane proteins, SNAP-25 lacks a sufficient hy-

drophobic stretch for insertion in the membrane but, instead, is membrane associated, at least in part, through hydrophobic interactions provided by thioester-linked long chain fatty acylation of a centrally located cluster of four (or five in *Torpedo*) cysteine residues (46). Because protein palmitoylation is a dynamic, post-translational process, it provides an attractive mechanism for regulating the interaction of SNAP-25 with the plasma membrane and consequently with other proteins involved in vesicle fusion. For example, exposure of regenerating neurons to nitric oxide in culture, which rapidly leads to collapse of growth cones and inhibition of neurite extension, also inhibits the palmitoylation of SNAP-25 as well as other proteins (47). Since nitric oxide can act by nitrosylation of free cysteine thiol groups, this suggests that ongoing turnover of protein fatty acylation may be required for vesicle fusion in plasma membrane expansion and motility. In SNAP-25 this site of palmitoylation lies precisely within the domain that is encoded by duplicated, but divergent, exons (36, 37). Through alternative splicing of these exons, the arrangement and sequence context of this "cysteine quartet" motif is altered between the variant SNAP-25a and SNAP-25b isoforms. The regulated expression of the two isoforms, moreover, implicates these differences between alternative exon encoded sequences in modifying the role of SNAP-25 during brain development and in neurons and neuroendocrine cells (ref. 48; unpublished data). In neonatal rodent brain, where the low level of SNAP-25 mRNA predominantly consists of the SNAP-25a isoform, the protein chiefly accumulates in cell bodies and fiber tracts (49). In contrast, during synaptogenesis and brain maturation, the dramatic induction of SNAP-25 occurs principally by the increased and preferential expression of SNAP-25b mRNA, which correlates with a shift in localization of the protein to nerve terminals, and the virtual disappearance from nerve fibers in adult brain. The switch between exon 5 encoded fatty acylation domains, therefore, may be involved in selectively targeting SNAP-25 to axons and terminals. Alternatively, these sequences could provide subtle but distinct functions, possibly through interaction of the fatty acid moiety with the plasma membrane and thus contribute directly to mediating different vesicular fusion events required for neurite outgrowth and mature neurotransmitter release.

Additional support for the variety of roles played by SNAP-25 isoforms comes from studies where expression of SNAP-25 is perturbed. Blocking SNAP-25 expression with antisense oligonucleotides in cortical neurons and PC12 cells *in vitro*, and chicken retinal neurons *in vivo*, prevents neurite extension but not initial neurite outgrowth, as evidenced by a decrease in length but not number of neurites (50). Since PC12 cells, as neonatal cortical neurons, primarily express SNAP-25a mRNA (>90%) (48), this is consistent with the primary involvement of SNAP-25a in the fusion of PPVs during process elongation. In contrast, in synaptosomes of adult brain where SNAP-25b isoform is by far the major species, the specific carboxyl-terminal cleavage by BoNT/A and BoNT/E of SNAP-25 (51, 52) effectively blocks Ca-dependent glutamate release and thus clearly defines the function of this isoform in neurotransmission. However, BoNT/A also inhibits exocytosis and catecholamine release from adrenal chromaffin cells (53) and PC12 cells (54), which predominantly express SNAP-25a. This suggests the further possibility that the SNAP-25a isoform may also participate in docking and fusion of SGs, which, in contrast to glutaminergic vesicles, are not tightly clustered at an active zone and are triggered more slowly in response to Ca influx. The proposed dual function of SNAP-25, therefore, might be attributed to the part(s) played by the SNAP-25a isoform in physiological processes common to both axonal elongation and exocytosis from SGs and LDCVs, including peptide transmitters, whereas SNAP-25b may perform a more highly specialized role in fast release of classical neurotransmission.

These observations support the notion that alternative isoforms of membrane-associated proteins involved in membrane docking and fusion events play critical roles in providing added refinement required by neural cells for regulated vesicular trafficking. The specificity of such interactions, in fact, has been recently illustrated by the observation that neuron-specific isoforms of Syb interact with some, but not all, plasma membrane-associated members of the Sytx gene family (25). One intriguing possibility is that different isoforms of these proteins may contribute to directing vesicles to different classes of voltage-gated Ca ion channels on the plasma membrane (55) that would impose distinct physiological differences in signaling neurotransmitter release. In addition to these vesicle targeting proteins, isoform variants also have been described for other synaptic vesicle proteins, including synapsins (56), which function in tethering SVs to cytoskeletal actin cables, and synaptophysin (57) and several other members of the rab family of small GTP binding proteins whose precise functions in trafficking remain to be fully elucidated (58). Together with the membrane targeting proteins, these proteins may contribute further levels of diversity to the repertory for sorting, storage, and cycling of vesicles in a variety of neural cells. In light of these recent findings, the present challenge is to unravel how the expression of similar but distinct isoforms of presynaptic proteins changes during development and in response to different environmental stimuli. These may be the "details" that help to modulate the retooling of neurons during maturation and regeneration and, importantly, the synaptic plasticity that underlies learning and behavior.

We are grateful to Drs. Iain L. Campbell and Ellen J. Tisdale for critical reading and comments on this manuscript and Stefano Catsicas for many stimulating discussions. This research was supported in part by National Science Foundation Grant IBN9121121 and Public Health Service Grant MH48989 (M.C.W.).

1. De Camilli, P. & Jahn, R. (1990) *Annu. Rev. Physiol.* **52**, 625–645.
2. Kelly, R. B. (1993) Review supplement to *Cell* **10**, *Neuron* **10**, 45–53.
3. Südhof, T. C. & Jahn, R. (1991) *Neuron* **6**, 665–677.
4. Trimble, W. S., Lineal, M. & Scheller, R. H. (1991) *Annu. Rev. Neurosci.* **14**, 93–122.
5. Huttner, W. B. (1993) *Nature (London)* **365**, 104–105.
6. Bennett, M. K. & Scheller, R. H. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2559–2563.
7. Lockerbie, R. O., Miller, V. E. & Pfenninger, K. H. (1991) *J. Cell Biol.* **112**, 1215–1227.
8. Thureson-Klein, Å. K. & Klein, R. L. (1990) *Int. Rev. Cytol.* **121**, 67–126.
9. Zhu, P. C., Thureson-Klein, Å. & Klein, R. L. (1986) *Neuroscience* **19**, 43–54.
10. Verhage, M., McMahon, H. T., Ghijsen, W. E. J. M., Boomsma, F., Scholten, G., Wiegant, V. M. & Nicholls, D. G. (1991) *Neuron* **6**, 517–524.
11. Smith, S. J. & Augustine, G. J. (1988) *Trends NeuroSci.* **11**, 458–464.
12. Augustine, G. J. & Neher, E. (1992) *Curr. Opin. Neurobiol.* **2**, 302–307.
13. Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E. (1993) *Nature (London)* **362**, 318–324.
14. Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H. & Rothman, J. E. (1993) *Cell* **75**, 409–418.
15. Matteoli, M., Haimann, C., Torri-Tarelli, F., Polak, J. M., Ceccarelli, B. & De Camilli, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7366–7370.
16. Brose, N., Petrenko, A. G., Südhof, T. C. & Jahn, R. (1992) *Science* **256**, 1021–1025.
17. DeBello, W. M., Betz, H. & Augustine, G. J. (1993) *Cell* **74**, 947–950.
18. Bennett, M. K., Calakos, N. & Scheller, R. H. (1992) *Science* **257**, 255–259.
19. Yoshida, A., Oho, C., Omori, A., Kuwahara, R., Ito, T. & Takahashi, M. (1992) *J. Biol. Chem.* **267**, 24925–24928.
20. Ushkaryov, Y. A., Petrenko, A. G., Geppert, M. & Südhof, T. C. (1992) *Science* **257**, 50–56.

21. Hata, Y., Davletov, B., Petrenko, A. G., Jahn, R. & Südhof, T. C. (1993) *Neuron* **10**, 307–315.
22. O'Connor, V. M., Shamotienko, O., Grishin, E. & Betz, H. (1993) *FEBS Lett.* **326**, 255–260.
23. Horikawa, H. P. M., Saisu, H., Ishizuka, T., Sekine, Y., Tsugita, A., Odani, S. & Abe, T. (1993) *FEBS Lett.* **330**, 236–240.
24. Hata, Y., Slaughter, C. A. & Südhof, T. C. (1993) *Nature (London)* **366**, 347–351.
25. Calakos, N., Bennett, M. K., Peterson, K. E. & Scheller, R. H. (1994) *Science* **263**, 1146–1149.
26. Gundersen, C. B. & Umbach, J. A. (1992) *Neuron* **9**, 527–537.
27. Mastrogiacomo, A., Parsons, S. M., Zampigili, G. A., Jenden, D. J., Umbach, J. A. & Gundersen, C. B. (1994) *Science* **263**, 981–982.
28. Zinsmaier, K. E., Eberle, K. K., Buchner, E., Walter, N. & Benzer, S. (1994) *Science* **263**, 977–980.
29. Archer, B. T., Özçelik, T., Jahn, R., Francke, U. & Südhof, T. C. (1990) *J. Biol. Chem.* **265**, 13007–13015.
30. Baumert, M., Maycox, P. R., Navone, F., De Camilli, P. & Jahn, R. (1989) *EMBO J.* **8**, 379–384.
31. Trimble, W. S., Gray, T. S., Elferink, L. A., Wilson, M. C. & Scheller, R. H. (1990) *J. Neurosci.* **10**, 1380–1387.
32. McMahon, H. T., Ushkaryov, Y. A., Edelmann, L., Link, E., Binz, T., Niemann, H., Jahn, R. & Südhof, T. C. (1993) *Nature (London)* **364**, 346–349.
33. Perin, M. S., Fried, V. A., Mignery, G. A., Jahn, R. & Südhof, T. C. (1990) *Nature (London)* **345**, 260–263.
34. Geppert, M., Archer, B. T., III & Südhof, T. C. (1991) *J. Biol. Chem.* **266**, 13548–13552.
35. Bennett, M. K., García-Arrarás, J. E., Elferink, L. A., Peterson, K., Fleming, A. M., Hazuka, C. D. & Scheller, R. H. (1993) *Cell* **74**, 863–873.
36. Bark, I. C. (1993) *J. Mol. Biol.* **233**, 67–76.
37. Bark, I. C. & Wilson, M. C. (1993) *Gene* **139**, 291–292.
38. Walch-Solimena, C., Takei, K., Marek, K. L., Midyett, K., Südhof, T. C., De Camilli, P. & Jahn, R. (1993) *J. Neurosci.* **13**, 3895–3903.
39. Oyler, G. A., Higgins, G. A., Hart, R. A., Battenberg, E., Billingsley, M., Bloom, F. E. & Wilson, M. C. (1989) *J. Cell Biol.* **109**, 3039–3052.
40. Catsicas, S., Larhammar, D., Blomqvist, A., Sanna, P. P., Milner, R. J. & Wilson, M. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 785–789.
41. Risinger, C. & Larhammar, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 10598–10602.
42. Risinger, C., Blomqvist, A., Lundell, I., Lambertsson, A., Nässel, D., Pieribone, V. A., Brodin, L. & Larhammar, D. (1993) *J. Biol. Chem.* **268**, 24408–24414.
43. Schiavo, G., Santucci, A., DasGupta, B. R., Mehta, P. P., Jontes, J., Benfenati, F., Wilson, M. C. & Montecucco, C. (1993) *FEBS Lett.* **335**, 99–103.
44. Binz, T., Blasi, J., Yamasaki, S., Baumeister, A., Link, E., Südhof, T. C., Jahn, R. & Niemann, H. (1993) *J. Biol. Chem.* **269**, 1617–1620.
45. Carr, C. M. & Kim, P. S. (1993) *Cell* **73**, 823–832.
46. Hess, D. T., Slater, T. M., Wilson, M. C. & Skene, J. H. P. (1992) *J. Neurosci.* **12**, 4634–4641.
47. Hess, D. T., Patterson, S. I., Smith, D. S. & Skene, J. H. P. (1993) *Nature (London)* **366**, 562–565.
48. Bark, I. C., Mehta, P. P. & Wilson, M. C. (1993) *Soc. Neurosci. Abstr.* **19**, 1059.
49. Oyler, G. A., Polli, J. W., Wilson, M. C. & Billingsley, M. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5247–5251.
50. Osen-Sand, A., Catsicas, M., Staple, J. K., Jones, K. A., Ayala, G., Knowles, J., Grenningloh, G. & Catsicas, S. (1993) *Nature (London)* **364**, 445–448.
51. Blasi, J., Chapman, E. R., Link, E., Binz, T., Yamasaki, S., De Camilli, P., Südhof, T. C., Niemann, H. & Jahn, R. (1993) *Nature (London)* **365**, 160–163.
52. Shiavo, G., Rossetto, O., Catsicas, S., Polverino De Laureto, P., DasGupta, B. R., Benfenati, F. & Montecucco, C. (1993) *J. Biol. Chem.* **268**, 23784–23787.
53. Penner, R., Neher, E. & Dreyer, F. (1986) *Nature (London)* **324**, 76–78.
54. Lomneth, R., Martin, T. F. J. & DasGupta, B. R. (1991) *J. Neurochem.* **57**, 1413–1421.
55. Takahashi, T. & Momiyama, A. (1993) *Nature (London)* **366**, 156–158.
56. Südhof, T. C., Czernik, A. J., Kao, H.-T., Takei, K., Johnston, P. A., Horiuchi, A., Kanazir, S. D., Wagner, M. A., Perin, M. S., De Camilli, P. & Greengard, P. (1989) *Science* **245**, 1474–1480.
57. Fykse, E. M., Takei, K., Walch-Solimena, C., Geppert, M., Jahn, R., De Camilli, P. & Südhof, T. C. (1993) *J. Neurosci.* **13**, 4997–5007.
58. Simons, K. & Zerial, M. (1993) *Neuron* **11**, 789–799.