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A Fast Mode of Membrane Fusion Dependent on Tight SNARE Zippering

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SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins have a key role in membrane fusion. It is commonly assumed that pairing of SNARE proteins anchored in opposing membranes overcomes the repulsion energy between membranes, thereby catalyzing fusion. In this study, we have increased the distance between the coiled-coil SNARE motif and the transmembrane domain of the vesicular SNARE synaptobrevin-2 by insertion of a flexible linker to analyze how an increased intermembrane distance affects exocytosis. Synaptobrevin-2 lengthening did not change the frequency of exocytotic events measured at 1 μM free calcium but prevented the increase in the secretory activity triggered by higher calcium concentration. Exocytotic events monitored in adrenal chromaffin cells by means of carbon fiber amperometry were classified in two groups according to the rate and extent of fusion pore expansion. Lengthening the juxtamembrane region of synaptobrevin-2 severely reduced the occurrence of rapid single events, leaving slow ones unchanged. It also impaired the increase in the fast-fusion mode that normally follows elevation of intracellular Ca2+ levels. We conclude that mild stimuli trigger slow fusion events that do not rely on a short intermembrane distance. In contrast, a short intermembrane distance mediated by tight zippering of SNAREs is essential to a component of the secretory response elicited by robust stimuli and characterized by rapid dilation of the fusion pore.

Key words: exocytosis; membrane fusion; neurosecretion; SNARE; amperometry; synaptic vesicle release

Introduction

Membrane fusion is essential to a host of biological processes such as exocytosis, intracellular trafficking, fertilization, or viral entry. This is not a spontaneous process because biological membranes are highly stable and repulsion forces oppose their approach. Energy must be supplied to approach membranes, to promote curvature deformations generated during the approach-step-and-stalk formation (the initial junction formed by the rearrangement of lipids in the two cis-leaflets), to create and to enlarge the pore (Chernomordik et al., 2006; Lee and Schick, 2007).

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins are essential to intracellular membrane fusion events and may supply the force needed to surmount the free energy barriers (Jahn and Scheller, 2006). At the synapse and in neuroendocrine cells, the vesicle (v)-SNARE synaptobrevin-2 (Syb2) is inserted into the secretory vesicle membrane and can form a complex with plasma membrane-associated target-localized (t)-SNAREs (syntaxin-1 and SNAP-25). Assembly of the SNARE bundle proceeds from the membrane-distal to the membrane-proximal region of the proteins in a zipper-like manner (Hanson et al., 1997). In vitro assays of liposome fusion after reconstitution of recombinant proteins suggested that SNAREs constitute a minimal fusion machinery (Weber et al., 1998). Increasing the length of the juxtamembrane region of SNAREs reduced the efficacy of fusion, providing support to the “zipper model” according to which SNAREs catalyze fusion by reducing the intermembrane distance (McNew et al., 1999). However, liposome fusion is very slow unless SNARE-associated proteins are added (Martens et al., 2007; Shen et al., 2007). Therefore, it is not clear yet whether SNAREs catalyze fusion merely by reducing intermembrane distance. The energy released during SNARE assembly may also be used to stabilize the stalk or to dilate the pore. SNAREs could also be seen as scaffold proteins acting synergistically with other fusion proteins such as synaptotagmins (Sorensen et al., 2006; Zimmerberg et al., 2006; Martens et al., 2007).

Once initiated, the fusion process may proceed toward complete merging of the two membranes (full-collapse fusion). Fusion pore expansion may also arrest at some stage, followed by rapid reversal of the pore or conservation of vesicle shape during some time [a process known as “kiss-and-run” (KR) or “cavircapture”]. Full-collapse fusion and KR coexist in neurons and endocrine cells (Harata et al., 2006). These different modes of fusion are thought to condition vesicle reuse and transmitter efflux. In adrenal chromaffin cells, mild stimuli trigger the release of catecholamines by KR, whereas more robust stimuli evoke the discharge of the dense peptide core (Fulop et al., 2005) and increase the amount of catecholamines released per vesicle (Elhamdani et al., 2001). These changes in fusion pore dynamics are calcium dependent, but the underlying molecular mechanisms are not known.
In this study, we have analyzed the effect of a flexible linker inserted into the juxtamembrane region of Syb2 on exocytosis of secretory granules (SGs) of adrenal chromaffin cells to test the zipper model in living cells and to investigate the function of SNAREs in fusion pore dynamics. Cat-echolamine release from single SGs was monitored using carbon fiber amperometry, a highly sensitive technique that can resolve the dynamics of the fusion pore from its opening to its final dilation on membrane merging. We found that Syb2 lengthening has little effect on exocytosis triggered by moderate calcium elevation but impairs calcium-dependent increase in fusion probability and severely reduces the proportion of exocytotic events characterized by fast dilation of the fusion pore.

Materials and Methods

Materials. Plasmids encoding rat Syb2 and Syb2 (in pDNA3.1-mycHis vector; Invitrogen) were kindly provided by M. Seagar (Faculté de Médecine Secteur Nord, Inserm UMR 641, Marseille, France) and the one encoding bovine green fluorescent protein (GFP)-Syb2 [in the pEGFP (plasmid enhanced GFP) vector (Clontech)] by T. Galli (Institut Jacques Monod, CNRS UMR 7592, Paris, France). The plasmid pBN13-BoNT/B (botulinum neurotoxin type B) light chain (LC) encoding the light chain of BoNT/B was a gift from T. Binz (Institut für Biochemie, Medizinische Hochschule Hannover, Hannover, Germany). Collagenase A (used for dissociation of chromaffin cells) was from Roche Diagnostics. Carbon fiber electrodes were from ALA Scientific Intruments. Cell culture reagents were obtained from PAA Laboratories. DNA purifications were done using kits from Macherey-Nagel. Chemicals were from Sigma.

Plasmid constructs. A BamHI site was added to the sequence of Syb2R using the QuickChange mutagenesis kit (Stratagene) according to manufacturer’s instructions and the following primers: SybBamHI-f (forward), 5'-GGATCCGAGGTTTTTCCACC. The insertion generated the mutations K94G and M95S. The plasmid was then digested by BamHI and dephosphorylated and ligated to two prehybridized single-stranded phosphorylated oligonucleotides: GGS-BamHI-f, 5'-GGATCCGAGGTTTTTCCACC and BamHI-reverse, 5’-CAGGGAGGGTGCGCGACAGGATCCCGGGTTCCCGG. The double-stranded oligonucleotide codes for the 9 aa repeat sequence GGSGGSGGS. When this oligonucleotide is inserted into the parent vector in the correct orientation, the BamHI site is maintained. This annealed double-stranded oligonucleotide was ligated into the parent vector digested by BamHI. The plasmid pCDNA-Syb2R-i11-Myc-His encoding by expressing toxin-insensitive Syb2 proteins (Quetglas et al., 2002). These modified Syb2 proteins (Syb2R) carry point muta-
membrane-spanning domain (Fig. 1). Modified Syb2R proteins (Syb2R-i11) carrying a flexible linker of 11 residues control the concentration of intracellular Ca\textsuperscript{2+} (left panels) or permeabilized with digitonin in the presence of 1 (middle panels) or 100 (right panels) μM free Ca\textsuperscript{2+}. The data suggest that fusion probability depends on the completeness of SNAREs zippering at high but not at low [Ca\textsuperscript{2+}], i.e., in Syb2R-i11-expressing cells (Fig. 2). To bypass Ca\textsuperscript{2+} release was not observed in Syb2R-i11-transfected cells (Fig. 2). However, this extra component of release may be because of the fact that catecholamine collection by the electrode, especially during spike trail, is better for sharp spikes than for broad ones. Spike widening was confirmed by measuring shape parameters on single events. Compared with controls, HW/I\textsubscript{max} was increased by ~60%, whereas I\textsubscript{max}/Q was reduced in Syb2R-i11 cells (Table 1). Spike widening was also observed in cells stimulated by high K\textsuperscript{+} depolarization and at 1 μM [Ca\textsuperscript{2+}], but the effect was less pronounced under these conditions (Table 1). To illustrate these changes, the “mean spike” obtained by averaging all fusion events monitored under a given condition is shown in Figure 3 E,F. These results suggest that linker insertion slows the dilation of the fusion pore (increased rise time and reduced I\textsubscript{max}) and reduced the diameter of the pore (slowed decay).

Adding linkers to Syb2 prevents rapid dilation of the fusion pore

The effect of linker insertion in Syb2 on the mean parameters of amperometric spikes may result either from a general modification of spike shape or from a more specific effect on a subset of events. To address this issue, we compared the distribution of different spike parameters measured at 1 or 100 μM [Ca\textsuperscript{2+}] in control cells, rise time, decay time, HW, and s\textsubscript{max} display a broad distribution, but log-transformed data are almost normally dis-
ttributed. However, the frequency plots are bimodal and better fitted with two normal density functions than with a single one, suggesting the existence of two categories of events (Fig. 4). Furthermore, rise time and decay time are correlated with $s_{\text{max}}$ and more loosely with $I_{\text{max}}$, but not with the guantal size (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). The data suggest that a population of rapid and large spikes coexists with a population of broader and smaller spikes. Raising the free calcium concentration from 1 to 100 μM increased the proportion of large and rapid spikes (Figs. 3, 4, supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

To determine whether the bimodal distribution of spike shape parameters observed in Syb2R-expressing cells resulted from the overexpression of Syb2, amperometric spikes from nontransfected cells were also analyzed. The distribution of spike rise velocity and rise time values was also consistent with the existence of two categories of fusion events (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). Furthermore, no significant difference in any of the mean spike parameters was observed between Syb2R-expressing cells and nontransfected ones (data not shown).

In contrast, in Syb2R-i11-expressing cells, the proportion of rapid spikes was reduced, compared with control cells, or even suppressed (Fig. 4). A population of rapid events can still be seen in frequency histograms of $s_{\text{max}}$. However, it is hardly detected in histograms of rise time or decay time values (Fig. 4, supplemental Fig. 2, available at www.jneurosci.org as supplemental material). In contrast to what was observed in control cells, elevating [Ca$^{2+}$], from 1 to 100 μM did not increase the proportion of large and rapid events in Syb2R-i11-expressing cells (Fig. 4A–F, supplemental Fig. 2, available at www.jneurosci.org as supplemental material). We conclude that Syb2 lengthening impairs one mode of fusion characterized by rapid dilation of the fusion pore.

The foot signal is reduced in Syb2R-i11-expressing cells

Amperometric spikes are preceded in ~30% of the cases by a "foot" signal (also called a pre-spike signal) that reflects the diffusion of transmitters through a narrow, slowly expanding fusion pore (Albillos et al., 1997). The onset of the spike itself corresponds to the sudden enlargement of the fusion pore. At 1 μM [Ca$^{2+}$], similar foot signals were observed in control cells (maximal amplitude = 3.8 ± 4.5 pA, n = 104) and in Syb2R-i11-expressing cells (3.9 ± 3.5 pA, n = 122). Raising [Ca$^{2+}$], to 100 μM increased the maximal amplitude of the foot current in Syb2R-transfected cells (7.5 ± 6.9 pA, n = 164) but not in Syb2R-i11-expressing cells (4.5 ± 4.5 pA, n = 118; p < 0.0001 on pooled spike values, p < 0.05 on mean values obtained in the different cells). The integral of the foot signal (data not shown) was also reduced in Syb2R-i11-expressing cells compared with Syb2R cells at 100 μM [Ca$^{2+}$], In contrast, the duration of the foot signal was not changed.

Discussion

Two recent studies have addressed the effect of inserting a hydrophilic linker between the complex-forming SNARE motif of Syb2 and its transmembrane domain on exocytosis. Deák et al. (2006) found that lengthened Syb2 carrying a 12-aa-long linker could hardly rescue evoked neurotransmitter release in neurons from Syb2 KO mice but fully rescued spontaneous release (miniatures). Kesavan et al. (2007) found that linker insertion in Syb2 severely reduced the magnitude of the exocytotic burst in chromaffin cells (synchronous release) without changing the calcium dependence of the response and slowed fusion pore expansion.

Our data complement these findings. We found that (1) linker insertion in Syb2 does not reduce the occurrence of exocytotic events at low calcium, but prevents the increase in fusion probability normally observed at high calcium; and (2) fusion events can be categorized in two classes according to fusion pore dynamics. Linker insertion specifically abrogates "fast" fusion events, whereas "slow" fusion events remain unaffected. The effect on fusion pore dynamics is also more robust at high calcium because, schematically, the pore is slow at low calcium and fast at high calcium. Together, the data suggest that synchronous release and fast fusion are intimately linked because they are both inhibited by linker insertion. Moreover, both operate at high calcium levels and may rely on a calcium sensor with low affinity for.
impair the exocytotic process. The lack of effect of Syb2 length-/H11006-Decay time (ms) 55.7
stimuli that generate high [Ca2+/H9262+].
KR dominates at low [Ca2+/H9262+] and also, more loosely, with spike amplitude. Hence, a popu-
lation of amperometric spikes probably correspond to two modes of fusion has been obtained in adrenal chromaffin 
tissues. Two modes of exocytosis have been described previously. During full fusion, fusion pore dilation proceeds until completion of membrane merging, whereas it is restricted in KR/cavicapture (Harata et al., 2006). Evidence for both modes of fusion has been obtained in adrenal chromaffin cells and lactotrophs (Elhamdani et al., 2001, 2006; Taraska et al., 2003; Stenovec et al., 2004; Fulop et al., 2005; Vardjan et al., 2007). KR dominates at low [Ca2+/H9262+]i, whereas full fusion increases with stimuli that generate high [Ca2+/H9262+]. (Fulop et al., 2005; Elhamdani et al., 2006; Vardjan et al., 2007). Slow and rapid fusion events may thus correspond to KR/cavicapture and full fusion, respectively.

### Two modes of membrane fusion

Our categorization of fusion events is based on the finding that several parameters that describe amperometric spike shape (rise time, decay time, spike rise velocity) have a bimodal distribution in control cells. These parameters are correlated with each other and also, more loosely, with spike amplitude. Hence, a population of large and rapid spikes coexists with a second group of smaller and slower fusion events. The proportion of rapid events increases with calcium concentration. These two groups of fusion events might correspond to different-sized vesicles. However, rise and decay times are not correlated with quantal size. Furthermore, the size of dense core granules rather closely follows a Gaussian distribution (Plattner et al., 1997). Therefore, these two populations of amperometric spikes probably correspond to two modes of fusion with distinct rates of fusion pore dilation and not to two groups of vesicles. Two modes of exocytosis have been described previously. During full fusion, fusion pore dilation proceeds until completion of membrane merging, whereas it is restricted in KR/cavicapture (Harata et al., 2006). Evidence for both modes of fusion has been obtained in adrenal chromaffin cells and lactotrophs (Elhamdani et al., 2001, 2006; Taraska et al., 2003; Stenovec et al., 2004; Fulop et al., 2005; Vardjan et al., 2007). KR dominates at low [Ca2+/H9262+]i, whereas full fusion increases with stimuli that generate high [Ca2+/H9262+]. (Fulop et al., 2005; Elhamdani et al., 2006; Vardjan et al., 2007). Slow and rapid fusion events may thus correspond to KR/cavicapture and full fusion, respectively.

### Intermembrane distance and membrane fusion

Fusion of giant unilamellar vesicles is steeply dependent on intermembrane distance (Heuvingh et al., 2004). Accordingly, theoretical models conclude that the free energy for the stalk intermediate increases with intermembrane distance (Chizmadzhev et al., 1995). Assuming an extended conformation of our 11-aa-long linker, the resulting augmentation of intermembrane distance (4.7 nm) is expected to increase the energy barrier for fusion by ~100 kBT (kBT, Boltzmann’s constant; T, absolute temperature) (Lee and Schick, 2007). Such a large penalty should impair the exocytotic process. The lack of effect of Syb2 lengthening on the occurrence of fusion events at low calcium is therefore intriguing and suggests that SNAREs do not catalyze fusion merely by pulling membranes together. One possibility, however, would be that fusion is powered at low calcium by partially assembled SNAREs. The slow rate of fusion would result from the rather large intermembrane distance; the observed insensitivity of the process to linker insertion would be caused by the small relative increment in the intermembrane distance resulting from linker addition to partially assembled Syb2. Further zipperping of the SNARE bundle could even compensate for this increment. Consistently, previous studies have indicated that SNARE complexes may exist in a partially zippered state in which they are still able to catalyze exocytosis (Xu et al., 1998, 1999; Sorensen et al., 2006). Implicit in this scheme is the fact that the energy released by SNARE assembly (Li et al., 2007) is not sufficient to overcome repulsion forces between apposing membranes.

High calcium levels increase the release probability, the temporal coupling between stimulus and response, and the kinetics of single fusion events. Linker insertion in Syb2 severely inhibits all of these effects that probably reflect the action of a calcium-dependent factor. A good candidate is synaptotagmin. At calyx-of-Held synapses, asynchronous release of neurotransmitters dominates at [Ca2+/H9262+]< 1 μM. Above this value, synchronous release operates and is dependent on synaptotagmin-2 (Sun et al., 2007). Synaptotagmins may thus contribute additional energy to assist SNAREpin zipperping, leading to further reduction of the intermembrane distance and to increased fusion probability. Such a mechanism would be obviously blocked by linker insertion in Syb2.

### Force transmission to the membrane

The intermembrane-distance paradigm may account for the observed data, but other possibilities should be considered. The membrane-proximal region of SNAREs is supposed to transmit force to the membrane-spanning domain. Interestingly, it interacts with phospholipids (Quetglas et al., 2002) and is even immersed in the bilayer (Chen et al., 2004). Recent molecular dynamics simulations have extended these observations by showing that the C-terminal end of the SNARE motif is also embedded in the membrane (M. Baaden, personal communication). On zipperping of the complex, the membrane-proximal region of SNAREs could thus bend or destabilize the membrane. This may account for the severe reduction of membrane fusion that results from mutations or deletion of the C-terminal hydrophobic layers of SNAREs (Sorensen et al., 2006; Siddiqui et al., 2007). Insertion of a hydrophilic linker in Syb2 could perturb the insertion of the membrane-proximal region into the phospholipid bilayer and thus force-transmission to the membrane (Baaden, personal communication). This could be particularly important at high calcium when additional factors would add strain on SNAREs.

### Fusion pore expansion

Linker insertion in Syb2 abrogates the fast-fusion mode and also reduces the initial expansion of the pore because the amplitude of...
of fusion pore dilation (Chizmadzhev et al., 1995, 2000; Amatore et al., 2000). Membrane tension may result both from the interaction of synaptotagmins with SNAREs and phospholipids at the neck of the fusing vesicle (Zimmerberg et al., 2006) and from swelling of the granule matrix. Influx of Na⁺ through the fusion pore displaces matrix-bound Ca²⁺ and catecholamines, and leads to matrix swelling and increased vesicular membrane tension (Amatore et al., 2000; Gong et al., 2007). Energy would be stored in the membrane until the tension exceeds the cohesion of the fusion machinery and would then drive pore enlargement.

We propose that slow fusion is powered by partially zippered SNAREs. Activation of synaptotagmins at high calcium may promote fast fusion by adding strain on SNAREs or by strengthening ring assemblies at the neck of the fusing vesicle. Both mechanisms could be affected by linker insertion in Syb2. Reduced SNARE-to-membrane force transmission and weakened fusion machinery could account for its effects on the foot signal and on the main spike, respectively.

References

Albillos A, Dernick G, Horstmann H, Almers W, Alvarez de Toledo G, Lindau M (1997) The exocytotic event in chromaffin cells revealed by patch amperometry. Nature 389:509–512.

Amatore C, Bouret Y, Travis ER, Wightman RM (2000) Interplay between membrane dynamics, diffusion and swelling pressure governs individual vesicular exocytotic events during release of adrenaline by chromaffin cells. Biochimie 82:481–496.

Chen Y, Xu Y, Zhang F, Shin YK (2004) Constitutive versus regulated SNARE assembly: a structural basis. EMBO J 23:681–689.

Chernomordik LV, Zimmerberg J, Kozlov MM (2006) Membranes of the world unite! J Cell Biol 175:201–207.

Chizmadzhev YA, Cohen FS, Scherbakov A, Zimmerberg J (1998) Membrane mechanics can account for fusion pore dilation in stages. Biophys J 79:2489–2500.

Chizmadzhev YA, Kuzmin PI, Kumenko DA, Zimmerberg J, Cohen FS (2000) Dynamics of fusion pores connecting membranes of different tensions. Biophys J 78:2241–2256.

Deak F, Shin OH, Karalati ET, Sudhof TC (2006) Structural determinants of synaptobrevin 2 function in synaptic vesicle fusion. J Neurosci 26:6668–6676.

Desnos C, Schonn JS, Huet S, Tran VS, El-Amraoui A, Raposo G, Farget I, Chapius C, Ménasché G, de Saint Basile G, Petit C, Cribier
S, Henry JP, Darchen F (2003) Rab27A and its effector MyRIP link secretory granules to F-actin and control their motion towards release sites. J Cell Biol 163:819–830.

Elhamdani A, Palfrey HC, Artalejo CR (2001) Quantal size is dependent on stimulation frequency and calcium entry in calf chromaffin cells. Neuron 31:819–830.

Elhamdani A, Azizi F, Artalejo CR (2006) Double patch clamp reveals that transient fusion (kiss-and-run) is a major mechanism of secretion in calf adrenal chromaffin cells: high calcium shifts the mechanism from kiss-and-run to complete fusion. J Neurosci 26:3030–3036.

Fulop T, Radabaugh S, Smith C (2005) Activity-dependent differential transmitter release in mouse adrenal chromaffin cells. J Neurosci 25:7324–7332.

Gong LW, de Toledo GA, Lindau M (2007) Exocytotic catecholamine release is not associated with cation flux through channels in the vesicle membrane but Na+ influx through the fusion pore. Nat Cell Biol 9:915–922.

Grabner CP, Fox AP (2006) Stimulus-dependent alterations in quantal neurotransmitter release. J Neurophysiol 96:3082–3087.

Hanson PI, Roth R, Morisaki H, Jahn R, Heuser JE (1997) Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell 90:523–535.

Harata NC, Aravanis AM, Tsien RW (2006) Kiss-and-run and full-collapse fusion as modes of exo-endocytosis in neurosecretion. J Neurochem 97:1546–1570.

Heuvingh J, Pincet F, Criber S (2004) Hemifusion and fusion of giant vesicles induced by reduction of inter-membrane distance. Eur Phys J E Soft Matter 14:269–276.

Jahn R, Scheller RH (2006) SNAREs—engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–643.

Kesavan J, Borisovska M, Bruns D (2007) v-SNARE actions during Ca(2+)/H(1001) triggered exocytosis. Cell 131:351–363.

Lee JY, Schick M (2007) Dependence of the energies of fusion on the inter-membrane separation: optimal and constrained. J Chem Phys 127:075102.

Li F, Pincet F, Perez E, Eng WS, Melia TJ, Rothman JE, Tareste D (2007) Energetics and dynamics of SNAREpin folding across lipid bilayers. Nat Struct Mol Biol 14:890–896.

Martens S, Koslov MM, McMahon HT (2007) How synaptotagmin promotes membrane fusion. Science 316:1205–1208.

McNew JA, Weber T, Engelman DM, Sollner TH, Rothman JE (1999) The length of the flexible SNAREpin juxtamembrane region is a critical determinant of SNARE-dependent fusion. Mol Cell 4:415–421.

Plattner H, Artalejo AR, Neher E (1997) Ultrastructural organization of bovine chromaffin cell cortex-analysis by cryofixation and morphometry of aspects pertinent to exocytosis. J Cell Biol 139:1709–1717.

Quetglas S, Iborra C, Sasakiwa N, De Haro L, Kumakura K, Sato K, Leveque C, Seagar M (2002) Calmodulin and lipid binding to synaptobrevin regulates calcium-dependent exocytosis. EMBO J 21:3970–3979.

Schonn JS, Desnos C, Henry JP, Darchen F (2003) Transmitter uptake and release in PC12 cells overexpressing plasma membrane monoamine transporters. J Neurochem 84:669–677.

Shen J, Tareste DC, Paumet F, Rothman JE, Melia TJ (2007) Selective activation of cognate SNAREpins by Sec1/Munc18 proteins. Cell 128:183–195.

Siddiqui TJ, Vites O, Stein A, Heintzmann R, Jahn R, Fasshauer D (2007) Determinants of synaptobrevin regulation in membranes. Mol Biol Cell 18:2037–2046.

Sørensen JB, Wiederhold K, Müller EM, Milesevic I, Nagy G, de Groot BL, Grubmüller H, Fasshauer D (2006) Sequential N- to C-terminal SNARE complex assembly drives priming and fusion of secretory vesicles. EMBO J 25:955–966.

Sun J, Pang ZP, Qin D, Fahim AT, Adachi R, Sudhof TC (2007) A dual-Ca2+-sensor model for neurotransmitter release in a central synapse. Nature 450:676–682.

Taraska JW, Perrais D, Ohara-Imaiuzumi M, Nagamatsu S, Almers W (2003) Secretory granules are recaptured largely intact after stimulated exocytosis in cultured endocrine cells. Proc Natl Acad Sci U S A 100:2070–2075.

Vardjan N, Stenovec M, Jorgacevski J, Kreft M, Zorec R (2007) Subnanometer fusion pores in spontaneous exocytosis of peptidergic vesicles. J Neurosci 27:4737–4746.

Weber T, Zemelman BV, McNew JA, Westermann B, Gmachl M, Parlati F, Sollner TH, Rothman JE (1998) SNAREpins: minimal machinery for membrane fusion. Cell 92:759–772.

Xu T, Binz T, Niemann H, Neher E (1998) Multiple kinetic components of exocytosis distinguished by neurotoxin sensitivity. Nat Neurosci 1:192–200.

Xu T, Rammner B, Margittai M, Artalejo AR, Neher E, Jahn R (1999) Inhibition of SNARE complex assembly differentially affects kinetic components of exocytosis. Cell 99:713–722.

Zimmerberg J, Akimov SA, Frolov V (2006) Synaptotagmin: fusogenic role for calcium sensor? Nat Struct Mol Biol 13:301–303.