Neurochem Res (2008) 33:1915–1919
DOI 10.1007/s11064-008-9648-2

OVERVIEW

Searching for Molecular Players Differentially Involved in Neurotransmitter and Neuropeptide Release

Tao Xu · Pingyong Xu

Accepted: 29 February 2008 / Published online: 10 April 2008

Abstract Neurotransmitters and neuropeptides are stored in small clear vesicles (SCVs) and large dense core vesicles (LDCVs), respectively. Many differences in the properties of SCVs and LDCVs suggest that these two classes of secretory organelles may employ different sets of molecules in exocytosis. Relatively little is known, however, about factors that differentially participate in SCVs and LDCVs release. This article briefly overviews some key molecules that are possibly involved in the differential regulation of the trafficking, docking, priming and fusion of SCVs and LDCVs.

Keywords Neurotransmitter · Neuropeptide · Small clear vesicle · Large dense core vesicle · Exocytosis

Introduction

Neurons communicate with one another through the release of molecules from synaptic small clear vesicles (SCVs) and large dense core vesicles (LDCVs, also called dense core granules). Classical neurotransmitters are packaged in SCVs and undergo fast phasic release at active zones of the nerve terminal. In contrast, secretory proteins, specifically neuropeptides, are synthesized in the cell body and condensed in LDCVs. LDCVs can be released in the soma as well as in the nerve terminals away from active zones. Initial formation of immature LDCVs occurs at the *trans*-Golgi network (TGN). Once formed, immature LDCVs must be processed and remodeled to form mature LDCVs, which will then be transported to underneath the plasma membrane waiting to be released. SCVs, however, can be locally supplied in the synaptic terminal via endocytic pathways and be reloaded by vesicular transporters. Furthermore, SCVs and LDCVs differ in speeds and latencies for exocytosis upon Ca^{2+} stimulation.

Apparently, SCVs and LDCVs are distinct organelles responsible for different types of neurosecretion. Surprisingly, despite the differences, the exocytosis of SCVs and LDCVs share conserved features. For example, both SCVs and LDCVs are triggered to release by Ca^{2+} and Synaptotagmin I has been shown to be required for the Ca^{2+} sensing of both types of release [1]. Likewise, the same set of SNARE proteins are used by both SCVs and LDCVs [2, 3]. Furthermore, Munc18-1 protein, which is involved in the docking as well as priming of vesicles, is dispensable for both SCV and LDCV exocytosis [4]. Finally, both SCV and LDCV exocytosis are sensitive to various regulations, such as Ca^{2+}-dependent priming [5], PKC- and PKA-dependent facilitation [6]. Despite the ever-increasing list of proteins that are involved in the transportation, docking, priming and fusion of regulated exocytosis, relatively little is known about the factors that differentially participate in SCV and LDCV exocytosis. Is there any molecule that is differentially used by SCVs and LDCVs? How could the many differences in the release of SCVs and LDCVs be achieved molecularly?

Rab Proteins

Rab proteins comprise the largest family within the Ras superfamily of small GTPase. Rab proteins increase from

Special issue article in honor of Dr. Ji-Sheng Han.

T. Xu (✉) · P. Xu
National Laboratory of Biomacromolecules,
Institute of Biophysics, Chinese Academy of Sciences,
Beijing 100101, P.R. China
e-mail: xutao@ibp.ac.cn

T. Xu
College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P.R. China

11 members in yeast to 63 members in human, closely correlating the evolution with increasing endomembrane complexity. Individual Rab proteins are localized to distinct, characteristic organelles, suggesting that each regulates a particular trafficking step of a particular vesicle type. A wide variety of biological activities arise from the combinatorial interactions of a given Rab with a wide array of downstream effectors and their respective binding partners. Rab effectors have been shown to be involved in the initial tethering of vesicles to target membrane, in the movement along microtubule via motor proteins, and in actin-based mobility through interactions with class V myosins [7]. Hence, SCVs and LDCVs are likely to use different Rab proteins to coordinate translocation along different tracks.

Recent work has established an important role of Rab27 in secretory lysosome and LDCV exocytosis in melanocytes, cytotoxic T lymphocytes, and neuroendocrine cells. In melanocytes, Rab27a is localized to the pigment-containing melanosome granules, which is a type of secretory lysosome, and is essential for their transportation to the cell periphery. The Rab27a effector melanophilin links Rab27a-positive granules to the actin motor myosin-Va. Without myosin-Va-dependent capture of these organelles on actin filaments, melanosomes cannot be transferred to the cell periphery. In addition, mice and patients with mutations in Rab27a also lack the ability to secrete from the lytic granules of cytotoxic T lymphocytes [8, 9]. Rab27 has also been implicated in regulation of LDCV exocytosis in various endocrine and neuroendocrine cells [10, 11].

SCVs contain members of at least three families of Rab proteins: Rab3 (Rab3A, 3B, 3C, and 3D), Rab5, and Rab11 [12]. Of these, Rab3 proteins are the most abundant. Rab3A alone accounts for $\sim$25% of the total Rab GTP binding in brain. Rab3A has been shown to regulate neurotransmission by limiting the recruitment of SCVs into the RRP [13]. In a complete genetic analysis of Rab3 proteins, it has been shown the Rab3 is required for survival in mice and that the four Rab3 proteins are functionally redundant in this role [14]. Furthermore, Rab3 proteins are not in itself essential for SCV exocytosis but functions to modulate the basic release machinery. Unlike the role of Rab27 in the translocation of LDCVs, it is unclear at present whether Rab3 participate in the translocation of SCVs to underneath the terminal membrane. Rab3 mutants in *C. elegans* are viable but demonstrate less SCVs close to the active zone, suggesting a role of Rab3 in SCV translocation or tethering [15].

Rab Effectors

Probably the best-characterized Rab effectors are involved in the initial tethering of vesicles to target membranes. These include the exocyst complex (Sec4 effector in yeast exocytosis) [16, 17], the HOPS protein complex (Ypt7 effector in transport to the vacuole) [18, 19], p115 (Rab1 effector in Golgi transport) [20], and EEA1 (Rab5 effector in endocytosis) [21, 22]. It is thus perceptible that Rab27 and Rab3, together with their specific effectors, could function in the tethering/docking of LDCVs and SCVs to the plasma membrane. Analysis of Rab27a-deficient ashen mice has revealed involvement of Rab27a in the membrane tethering/docking step of the LDCVs in pancreatic β cells [23] and secretory lysosomes in cytotoxic T-lymphocytes [8, 9]. The synaptotagmin-like protein (Slp) family, which includes Slp1/JFC1, Slp2-a, Slp3-a, Slp4/granuphilin, Slp5 and rabphilin, has been identified as one group of Rab27 effectors. The members in Slp family contain an N-terminal Rab27-binding Slp homology domain (SHD) and C-terminal tandem C2 domains. Although the SHD of Slp4-a interacts with three distinct Rab species (Rab3, Rab8, and Rab27) in vitro, Slp4-a functions as a Rab27A effector during DCV exocytosis under physiological conditions. In Slp4/granuphilin knockout mice, a decrease in the number of plasma membrane-docked insulin-containing vesicles has been observed [24]. Despite a decrease in docking, however, Slp4/granuphilin-deficiency enhances insulin secretion. Biochemical analysis has shown that Slp4 interacts with Munc18-1 or Munc18-1 and Syntaxin-1 complex. Thus it has been proposed that Slp4 negatively regulates LDCV exocytosis by forcing the vesicle into a non-functional docked state. Unlike Slp4, Slp1, Slp2, Slp3, and Slp5 has been reported to specifically interact with Rab27 and do not interact with other Rabs, including Rab3. Slp2 is most abundantly expressed in the gastric surface mucous cells. Analysis of Slp2 mutant mice has revealed a reduced number of mucus granules, probably by reducing the docking of granules at the apical plasma membrane in the mucous cells [25]. Thus, it has been hypothesized that Rab27 and its effector Slp proteins may participate in putative docking machineries in different types of cells where they are endogenously expressed [26]. However, it is not clear whether all members of the Slp family participate in the docking, or whether Slp proteins only participate in a non-productive docking as suggested by the phenotype of Slp4-deficiency. Rabphilin has long been recognized as the effector of Rab3 as well as Rab27. Deletion of rabphilin produced no detectable effect in mice [27] and only a mild phenotype in *C. elegans* [28]. A recent study suggests that the binding of rabphilin to SNAP-25 accelerates the exocytosis of SVs only after the RRP has been exhausted [29]. Apparently, the exact functions of Slp proteins and how Rab27/Rab3 mediates the docking of vesicles to the plasma membrane remain to be further explored.

Munc13 Proteins

Vesicles need to undergo a priming step to prepare them for fusion in response to Ca^{2+} signal. The priming of vesicles into a fusion competent state is thought to involve, at least in part, the assembly of *trans* SNARE complexes [30, 31]. The precise mechanism by which priming is catalyzed remains to be revealed. Munc13 proteins constitute a family of 4 mammalian homologues of *C. elegans* Unc-13 and Drosophila Dunc-13 [32]. Genetic deletion of Munc13-1/u causes total arrest of synaptic transmission due to a complete loss of fusion-competent synaptic vesicles [33, 34]. Munc13 proteins contain C2 domain and a DAG binding C1 domain. The binding of DAG/phorbol-ester to C1 domain leads to enhanced priming activity of Munc13-1 [35, 36]. Despite an essential role Munc13 proteins in SCV priming, however, the requirement of Munc13 proteins for LDCV exocytosis, is controversial. Although exogenously expressed Munc13-1 strongly stimulates LDCV secretion in chromaffin cells [37] and pancreatic β cells [38], LDCV secretion remains relatively normal in chromaffin cells isolated from Munc13-1 knockouts [39]. Our recent study in pancreatic β cells suggests that Munc13-1 is required in accelerated priming of LDCVs, a function that involves DAG binding to the C1 domain [40]. However, the basal maintenance of fusion competent RRP does not seem to require Munc13-1. The involvement of Munc13 proteins in neuropeptide release from dense core vesicles has also been confirmed in the *unc-13* mutant *C. elegans* [6]. The differential requirement for Munc13 proteins in the release of SCVs and LDCVs suggests that these two types of vesicles probably use different mechanism in the priming or stabilization of fusion competent vesicles.

CAPS Proteins

Ca^{2+}-dependent activator protein for secretion (CAPS) was identified as an essential protein required for LDCV exocytosis [41]. CAPS is highly conserved in evolution: a single CAPS isoform (UNC-31) is found in *C. elegans*, whereas two closely related isoforms are expressed in vertebrates: CAPS1 and CAPS2. CAPS contains a conserved MH domain which is also found in members of the Munc13 family. CAPS appears to be required for LDCV but not SCV exocytosis [42, 43]. However, by deleting the CAPS1 gene, it has been demonstrated that CAPS1 is probably not involved in the Ca^{2+} -triggered LDCV exocytosis per se, but rather in the uptake or storage of monoamines into LDCVs [44]. It should be pointed out that this study has not addressed the redundancy of CAPS2, which is expressed at 8-fold higher levels than CAPS1 in adrenal glands. By contrast, recent experiments using *unc-31* mutant of *C. elegans* [6, 45] and our recent study clearly demonstrated a requirement of CAPS for LDCV exocytosis [46]. We observed a small but significant defect in SCV release in the *unc-31* mutant. This result is consistent with previous studies showing that ablation of CAPS/UNC-31 in *C. elegans* [43] and *Drosophila* [47] results in decreased synaptic transmission at the NMJ. Although these results suggest a role of CAPS/UNC-31 in SCV exocytosis, it is unclear whether CAPS/UNC-31 functions directly in SCV exocytosis or indirectly by regulating LDCV exocytosis [43]. By double knockout of both CAPS genes in mice, a recent study showed that CAPS1 and CAPS2 are essential components of the SCV priming machinery [48]. CAPS-deficient neurons contain no or very few fusion competent SCVs, which causes a selective impairment of fast phasic transmitter release. Thus unlike the previous thought that CAPS may be specific for LDCVs, it seems that CAPS proteins are both involved in the priming of SCVs and LDCVs although the exact requirement may be different under various cellular environment for different cell types.

Synaptotagmins

Synaptotagmins constitute a large family of at least 16 members and individual Synaptotagmin isoforms exhibit distinct Ca^{2+}-binding properties and subcellular localization. It is speculated that differential localization of synaptotagmins to different types of vesicles may form fusion machineries with various Ca^{2+}-sensitivities. Synaptotagmin I is best characterized as an abundant SCV-associated protein essential for rapid synchronous synaptic transmission. Synaptotagmin IX has been postulated to function as a major Ca^{2+} sensor for LDCV exocytosis in neuroendocrine cells. Synaptotagmin IX represents a close homologue to Synaptotagmin I based on sequence alignments. Nevertheless, the unique binding properties of Synaptotagmin IX with Ca^{2+}, phospholipid and SNARE complex suggest a substantially different biological roles from those of Synaptotagmin I. Because of the presence of multiple synaptotagmin genes [49] and the existence of multiple synaptotagmin isoforms on single vesicles [50], it remains a challenge to demonstrate whether and how synaptotagmin compositions confer the different Ca^{2+}-sensing properties of SCV and LDCV fusion. It is not clear whether multiple Synaptotagmin isoforms function independently on different types of vesicles or can function cooperatively on certain type of vesicle to confer distinct Ca^{2+}-sensing and kinetic properties. Our recent study [51] found that down-regulation of Synaptotagmin I and IX simultaneously resulted in a significant loss of Ca^{2+}-dependent LDCV exocytosis. By contrast, LDCV

exocytosis persisted in cells expressing either Synaptotagmin I or IX alone. Our result suggests that Synaptotagmin I and IX function redundantly in Ca^{2+}-sensing and fusion pore dilation of LDCVs in PC12 cells.

Protein Kinases

A large number of studies on many different cell types have implicated protein phosphorylation in the control of regulated exocytosis. PKA and PKC have been shown to enhance triggered exocytosis in essentially all cell types examined including neurons, neuroendocrine cells, endocrine cells, platelets, and so on. An array of exocytotic proteins has been identified as the substrates for PKA and PKC in vitro, and to a lesser extent in vivo. Because the apparent co-localization of SCVs and LDCVs in the same neuron, it is unclear whether the specificity of regulation is achieved by activation of different isoforms of protein kinases or by spatial-restricted activation of the same protein kinase. A recent study in *C. elegans* has shed light on how the specificity of regulation is achieved [6]. The PKC-1 protein of *C. elegans*, which is an ortholog of vertebrate PKCε and PKCη isoforms, has been shown to regulate exocytosis of neuropeptide-containing DCVs in *C. elegans* motor neurons. By contrast, SCV release occurred normally in *pkc-1* mutants. Similar neuropeptide secretion defects of the *pkc-1* mutants were found in mutants lacking *unc-31* or *unc-13*. This is the first study showing that different isoforms of PKC may differentially regulate SCV and LDCV exocytosis, which adds another layer of regulation of these two types of secretory organelles to fulfill their distinct physiological functions.

Prospects

The last decade has witnessed a remarkable explosion in our knowledge of many proteins that are required for regulated exocytosis. It is now acknowledged that both SCV and LDCV exocytose through mechanisms with many aspects in common and most likely employ the same basic protein components. Yet, SCVs and LDCVs are different organelles that are distinct in biogenesis, transport, docking, priming, fusion and regulation. Further studies are expected to reveal factors that contribute to the many differences in multiple steps along the cycling of these two famous types of secretory vesicles that play important roles in neural signaling.

Acknowledgments The laboratory of TX was supported by grants from the National Science Foundation of China (30630020, 30670504), the Major State Basic Research Program of P.R. China (2004CB720000, 2006CB911002).

References

1. Voets T, Moser T, Lund PE et al (2001) Intracellular calcium dependence of large dense-core vesicle exocytosis in the absence of synaptotagmin I. Proc Natl Acad Sci USA 98(20):11680–11685
2. Jena BP (2005) Cell secretion and membrane fusion. Domest Anim Endocrinol 29(1):145–165
3. Sorensen JB (2005) SNARE complexes prepare for membrane fusion. Trends Neurosci 28(9):453–455
4. Toonen RF (2003) Role of Munc18-1 in synaptic vesicle and large dense-core vesicle secretion. Biochem Soc Trans 31(Pt 4):848–850
5. Kits KS, Mansvelder HD (2000) Regulation of exocytosis in neuroendocrine cells: spatial organization of channels and vesicles, stimulus-secretion coupling, calcium buffers and modulation. Brain Res Brain Res Rev 33(1):78–94
6. Sieburth D, Madison JM, Kaplan JM (2007) PKC-1 regulates secretion of neuropeptides. Nat Neurosci 10(1):49–57
7. Grosshans BL, Ortiz D, Novick P (2006) Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci USA 103(32):11821–11827
8. Stinchcombe JC, Barral DC, Mules EH et al (2001) Rab27a is required for regulated secretion in cytotoxic T lymphocytes. J Cell Biol 152(4):825–834
9. Haddad EK, Wu X, Hammer JA 3rd et al (2001) Defective granule exocytosis in Rab27a-deficient lymphocytes from Ashen mice. J Cell Biol 152(4):835–842
10. Tsuboi T, Fukuda M (2006) Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. J Cell Sci 119(Pt 11):2196–2203
11. Tolmachova T, Anders R, Stinchcombe J et al (2004) A general role for Rab27a in secretory cells. Mol Biol Cell 15(1):332–344
12. Fischer von Mollard G, Stahl B, Li C et al (1994) Rab proteins in regulated exocytosis. Trends Biochem Sci 19(4):164–168
13. Geppert M, Bolshakov VY, Siegelbaum SA et al (1994) The role of Rab3A in neurotransmitter release. Nature 369(6480):493–497
14. Schluter OM, Basu J, Sudhof TC et al (2006) Rab3 superprimes synaptic vesicles for release: implications for short-term synaptic plasticity. J Neurosci 26(4):1239–1246
15. Nonet ML, Staunton JE, Kilgard MP et al (1997) *Caenorhabditis elegans* rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. J Neurosci 17(21):8061–8073
16. Itzen A, Rak A, Goody RS (2007) Sec2 is a highly efficient exchange factor for the Rab protein Sec4. J Mol Biol 365(5):1359–1367
17. Novick P, Brennwald P, Walworth NC et al (1993) The cycle of SEC4 function in vesicular transport. Ciba Found Symp 176:218–228; discussion 29–32
18. Stroupe C, Collins KM, Fratti RA et al (2006) Purification of active HOPS complex reveals its affinities for phosphoinositides and the SNARE Vam7p. Embo J 25(8):1579–1589
19. Bugnicourt A, Froissard M, Sereti K et al (2004) Antagonistic roles of ESCRT and Vps class C/HOPS complexes in the recycling of yeast membrane proteins. Mol Biol Cell 15(9):4203–4214
20. Allan BB, Moyer BD, Balch WE (2000) Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. Science 289(5478):444–448
21. Christoforidis S, McBride HM, Burgoyne RD et al (1999) The Rab5 effector EEA1 is a core component of endosome docking. Nature 397(6720):621–625
22. Simonsen A, Lippe R, Christoforidis S et al (1998) EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. Nature 394(6692):494–498

23. Kasai K, Ohara-Imaizumi M, Takahashi N et al (2005) Rab27a mediates the tight docking of insulin granules onto the plasma membrane during glucose stimulation. J Clin Invest 115(2): 388–396
24. Fukuda M, Kanno E (2005) Analysis of the role of Rab27 effector Slp4-a/Granuphilin-a in dense-core vesicle exocytosis. Methods Enzymol 403:445–457
25. Saegusa C, Tanaka T, Tani S et al (2006) Decreased basal mucus secretion by Slp2-a-deficient gastric surface mucous cells. Genes Cells 11(6):623–631
26. Fukuda M (2006) Rab27 and its effectors in secretory granule exocytosis: a novel docking machinery composed of a Rab27.effector complex. Biochem Soc Trans 34(Pt 5):691–695
27. Schluter OM, Schnell E, Verhage M et al (1999) Rabphilin knock-out mice reveal that rabphilin is not required for rab3 function in regulating neurotransmitter release. J Neurosci 19(14):5834–5846
28. Staunton J, Ganetzky B, Nonet ML (2001) Rabphilin potentiates soluble N-ethylmaleimide sensitive factor attachment protein receptor function independently of rab3. J Neurosci 21(23): 9255–9264
29. Deak F, Shin OH, Tang J et al (2006) Rabphilin regulates SNARE-dependent re-priming of synaptic vesicles for fusion. Embo J 25(12):2856–2866
30. Xu T, Binz T, Niemann H et al (1998) Multiple kinetic components of exocytosis distinguished by neurotoxin sensitivity. Nat Neurosci 1(3):192–200
31. Xu T, Rammner B, Margittai M et al (1999) Inhibition of SNARE complex assembly differentially affects kinetic components of exocytosis. Cell 99(7):713–722
32. Koch H, Hofmann K, Brose N (2000) Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. Biochem J 349(Pt 1):247–253
33. Augustin I, Rosenmund C, Sudhof TC et al (1999) Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. Nature 400(6743):457–461
34. Richmond JE, Davis WS, Jorgensen EM (1999) UNC-13 is required for synaptic vesicle fusion in *C. elegans*. Nat Neurosci 2(11):959–964
35. Rhee JS, Betz A, Pyott S et al (2002) Beta phorbol ester- and diacylglycerol-induced augmentation of transmitter release is mediated by Munc13s and not by PKCs. Cell 108(1):121–133
36. Betz A, Ashery U, Rickmann M et al (1998) Munc13-1 is a presynaptic phorbol ester receptor that enhances neurotransmitter release. Neuron 21(1):123–136
37. Ashery U, Varoqueaux F, Voets T et al (2000) Munc13–1 acts as a priming factor for large dense-core vesicles in bovine chromaffin cells. Embo J 19(14):3586–3596
38. Sheu L, Pasyk EA, Ji J et al (2003) Regulation of insulin exocytosis by Munc13-1. J Biol Chem 278(30):27556–27563
39. Stevens DR, Wu ZX, Matti U et al (2005) Identification of the minimal protein domain required for priming activity of Munc13-1. Curr Biol 15(24):2243–2248
40. Kang L, He Z, Xu P et al (2006) Munc13-1 is required for the sustained release of insulin from pancreatic beta cells. Cell Metab 3(6):463–468
41. Walent JH, Porter BW, Martin TF (1992) A novel 145 kd brain cytosolic protein reconstitutes Ca(2+)-regulated secretion in permeable neuroendocrine cells. Cell 70(5):765–775
42. Berwin B, Floor E, Martin TF (1998) CAPS (mammalian UNC-31) protein localizes to membranes involved in dense-core vesicle exocytosis. Neuron 21(1):137–145
43. Cai T, Fukushige T, Notkins AL et al (2004) Insulinoma-associated protein IA-2, a vesicle transmembrane protein, genetically interacts with UNC-31/CAPS and affects neurosecretion in *Caenorhabditis elegans*. J Neurosci 24(12):3115–3124
44. Speidel D, Bruederle CE, Enk C et al (2005) CAPS1 regulates catecholamine loading of large dense-core vesicles. Neuron 46(1):75–88
45. Speese S, Petrie M, Schuske K et al (2007) UNC-31 (CAPS) is required for dense-core vesicle but not synaptic vesicle exocytosis in *Caenorhabditis elegans*. J Neurosci 27(23):6150–6162
46. Zhou KM, Dong YM, Ge Q et al (2007) PKA activation bypasses the requirement for UNC-31 in the docking of dense core vesicles from *C. elegans* neurons. Neuron 56(4):657–669
47. Renden R, Berwin B, Davis W et al (2001) Drosophila CAPS is an essential gene that regulates dense-core vesicle release and synaptic vesicle fusion. Neuron 31(3):421–437
48. Jockusch WJ, Speidel D, Sigler A et al (2007) CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell 131(4):796–808
49. Sugita S, Shin OH, Han W et al (2002) Synaptotagmins form a hierarchy of exocytotic Ca(2+) sensors with distinct Ca(2+) affinities. Embo J 21(3):270–280
50. Lynch KL, Martin TF (2007) Synaptotagmins I and IX function redundantly in regulated exocytosis but not endocytosis in PC12 cells. J Cell Sci 120(Pt 4):617–627
51. Zhu D, Zhou W, Liang T et al (2007) Synaptotagmin I and IX function redundantly in controlling fusion pore of large dense core vesicles. Biochem Biophys Res Commun 361(4):922–927