

Regulation of Exocytosis by Complexin

Ok-Ho Shin

Ok-Ho Shin, Department of Neuroscience and Cell Biology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, USA

Correspondence to: Ok-Ho Shin, Department of Neuroscience and Cell Biology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, USA

Email: okshin@utmb.edu

Telephone: +1-409-772-1188

Fax: +1-409-762-9382

Received: February 26, 2015

Revised: April 1, 2015

Accepted: April 6, 2015

Published online: June 6, 2015

ABSTRACT

Complexin (Cpx), which is expressed mainly in the nervous system, binds efficiently to the SNARE complex that is composed of either Syntaxin 1 or Syntaxin 3. Both Syntaxin 1 and Syntaxin 3 are involved in mediating synchronous neurotransmitter release in the nervous system. Cpx stabilizes the SNARE complex upon binding, and potentiates the efficacy of synchronous exocytotic process. Cpx is consisted of N-terminal, accessory α -helix, central α -helix, and C-terminal domains. Each domain of Cpx has a distinct function that is differentially involved in the regulation of priming, clamping, and activating in the exocytotic process. These functions of Cpx domains coordinately potentiate the efficacy of synchronous exocytotic process.

© 2015 ACT. All rights reserved.

Key words: Complexin; Exocytosis; SNARE Complex; Syntaxin; Transmitter release

Shin OH. Regulation of Exocytosis by Complexin. *Journal of Biochemistry and Molecular Biology Research* 2015; 1(2): 25-29 Available from: URL: <http://www.ghrnet.org/index.php/jbmr/article/view/1087>

Abbreviations

AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid;

Cpx: Complexin (also known as Synaphin);

GST: glutathione S-transferase;

IGF-1: insulin-like growth factor-1;

KD: knock-down;

KO: knock-out;

LTP: long-term potentiation;

SNAP-23 or -25: synaptosomal-associated protein 23 or 25;

SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor;

SNARE complex: a ternary complex composed of Syntaxin, Synaptobrevin, and either SNAP-23 or SNAP-25;

Syb2: Synaptobrevin 2 (also known as vesicle-associated membrane protein 2, VAMP2);

Syt1: Synaptotagmin 1;

Syt10: Synaptotagmin 10;

WT: wild-type.

INTRODUCTION

Ca²⁺-triggered exocytosis is an essential cellular process that mediates transmitter release to the extracellular space for intercellular communications particularly in neurons^[1-3]. The same or similar processes are used for intracellular trafficking to deliver membrane patches or proteins to the plasma membrane^[4,5]. Stimulation of neurons cause an increase in cytosolic Ca²⁺ concentrations and trigger fusions of synaptic vesicles into the plasma membrane to release transmitters to the extracellular space^[1-3]. Synaptotagmin 1 (Syt1), a vesicular Ca²⁺ binding C2 domain protein, regulates Ca²⁺-triggered neurotransmitter release by interacting with membranes and the SNARE complex in a Ca²⁺-dependent manner^[6,7]. Synaptobrevin 2 (Syb2; also known as vesicle-associated membrane protein 2, VAMP2), a vesicular SNARE protein, forms a ternary SNARE complex with Syntaxin and SNAP-25 that are located in the target plasma membrane^[8]. The interaction between Syt1 and the membrane is essential for the Ca²⁺-binding to the C2 domains of

Syt1 in physiological Ca^{2+} concentrations^[7,9,10]. Meanwhile, the Ca^{2+} -dependent affinities of interactions between Syt1 and SNARE complexes are correlated with the efficacies of the corresponding exocytosis^[6]. Complexin (Cpx; also known as Synaphin) binds to the assembled SNARE complex, stabilizes the SNARE complex upon binding, and potentiates the efficacy of Syt1-regulated neurotransmitter release^[11-14].

CPX IS EXPRESSED MAINLY IN THE NERVOUS SYSTEM

Cpx is initially identified as a cytosolic protein that interacts with the SNARE complex^[12,13]. Cpx binds to the assembled SNARE complex composed of Syntaxin 1, Syb2, and SNAP-25 with a high affinity^[11-13]. The structure of Cpx-bound SNARE complex revealed that Cpx interacts directly with all components of the SNARE complex^[11]. Consistent with this observation, Cpx does not bind to partially assembled SNARE complexes^[13,15]. Cpx family has four isoforms (Cpx1-4) that are expressed mainly in the nervous system such as the brain, spinal cord, and retina (Figure 1A)^[13,16]. However, trace amounts of Cpx1-3 isoforms are also expressed in the liver, pancreas, kidney, spleen, skeletal muscle, and lung^[13,16]. Cpx1 and Cpx2 are soluble cytosolic proteins^[12,13]. Meanwhile, the C-terminals of Cpx3 and Cpx4 are farnesylated, and this modification is required for the targeting of these Cpx isoforms to the presynaptic plasma membrane^[16].

CPX BINDS EFFICIENTLY TO THE ASSEMBLED SNARE COMPLEXES THAT ARE COMPOSED OF EITHER SYNTAXIN 1 OR SYNTAXIN 3

Eukaryotic cells express at least four different Syntaxin isoforms (Syntaxin 1, 2, 3, and 4) that are involved in mediating various types of exocytotic processes^[18]. Systematic analyses of interactions between Cpx and SNARE complexes by employing both glutathione S-transferase (GST)-pull-downs and a yeast four-hybrid interaction assay showed that Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3^[17-20]. Cpx does not bind efficiently to the SNARE complexes that are composed of either Syntaxin 2 or Syntaxin 4^[18,20]. Consistent with these findings, the Syntaxin 1 residues that interact directly with Cpx are conserved in Syntaxin 3, but not in Syntaxin 2 and Syntaxin 4 (Figure 1B).

SNARE COMPLEXES THAT ARE INVOLVED IN MEDIATING SYNCHRONOUS RELEASE ARE PROBABLY ASSOCIATED WITH CPX

Stimulated neurons release neurotransmitters in two distinct modes, synchronous and asynchronous (Figure 2A). Synchronous release causes rapid actions, while asynchronous release triggers long-lasting actions of the target cells^[7,21]. Both modes of release are required to maintain the functions of biological systems. In addition, neurons are also capable of releasing neurotransmitters without evoked stimulations but still mainly in a Ca^{2+} -dependent manner, which is called spontaneous release (Figure 2A)^[7,21]. Four Cpx isoforms (Cpx1-4) are expressed mainly in the nervous system such as the brain, spinal cord, and retina, where neurotransmitters are released in a synchronous manner (Figure 2A)^[13,16]. In particular, membrane-bound Cpx3 and Cpx4 are expressed in retinal ribbon synapses

where strong synchronous neurotransmitter release is required^[17]. Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3^[17-20]. These Syntaxin isoforms are expressed at presynaptic nerve terminals where neurotransmitters are released in a synchronous manner^[6,17]. Neurons also express other Syntaxin isoforms such as Syntaxin 2 and Syntaxin 4, which are not involved in mediating synchronous neurotransmitter release^[22,23]. Therefore, the differential binding of Cpx to SNARE complexes is probably required for the regulation of subsequent interaction between the SNARE complex and responsible Ca^{2+} -sensors. Cpx-deficient neurons, in the presence of Syt1, display a significant reduction of the efficacy of Syt1-regulated synchronous release^[14]. However, these neurons still maintain the mode of synchronous release^[14]. Meanwhile, Syt1-deficient neurons, in the presence of Cpx, change the mode of release to asynchronous from synchronous release^[24]. These observations suggest that Syt1 is a key regulator for synchronous neurotransmitter release and that Cpx potentiates the Syt1-regulated synchronous release (Figure 2B).

CPX IS INVOLVED IN REGULATING VARIOUS TYPES OF EXOCYTOTIC PROCESSES

Cpx-deficient neurons consistently display a significant reduction of the efficacy of synchronous neurotransmitter release in many different release systems (Table 1). Cpx is also involved in regulating large dense-core vesicle exocytosis^[25-27] and rapid delivery of AMPA receptors to the plasma membrane^[3]. Cpx-deficient chromaffin cells show a significant decrease in both evoked norepinephrine release and the pool size of releasable vesicles^[25,26]. In olfactory neurons,

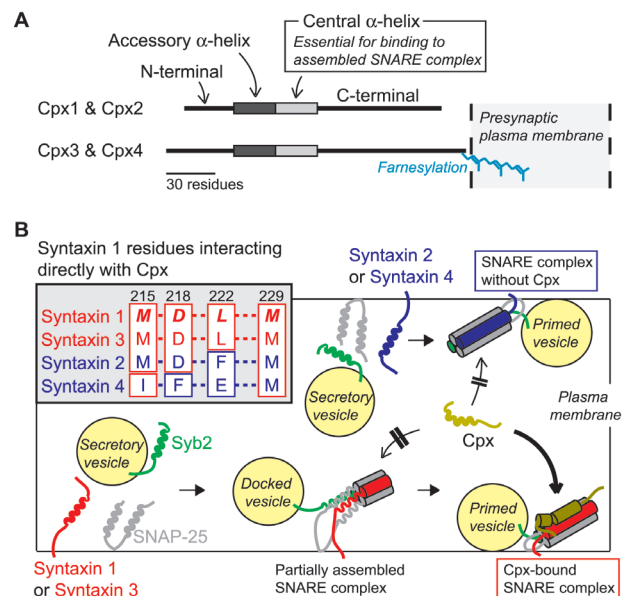


Figure 1 Cpx binds to the SNARE complex that mediates exocytosis. **A**. All Cpx isoforms (Cpx1-4) are consisted of N-terminal, accessory α -helix, central α -helix, and C-terminal domains. The central α -helix domain is essential for the Cpx binding to the assembled SNARE complex. Cpx1 and Cpx2 are soluble cytosolic proteins, while Cpx3 and Cpx4 are localized in the membrane because the C-terminals of Cpx3 and Cpx4 are farnesylated. **B**. Cpx binds efficiently to the assembled SNARE complexes composed of either Syntaxin 1 or Syntaxin 3. Consistent with this observation, the Syntaxin 1 residues (M215, D218, L222, and M229) that interact directly with Cpx are conserved in Syntaxin 3, but not in Syntaxin 2 and Syntaxin 4. The N-terminal Habc domains of Syntaxin are not shown. Cpx, Complexin; Syb2, Synaptobrevin 2.

Cpx-deficiency is associated with an impairment of Synaptotagmin 10 (Syt10)-regulated insulin-like growth factor-1 (IGF-1) release^[27]. These findings suggest that Cpx is involved in regulating various types of exocytotic processes in the nervous system. Trace amounts of Cpx1-3 isoforms are also expressed in many non-neuronal tissues such as the liver, pancreas, kidney, spleen, skeletal muscle, and lung^[13,16]. Therefore, Cpx is also likely involved in regulating various synchronous exocytotic processes occurring in these non-neuronal cells.

CPIX DOMAINS HAVE DISTINCT FUNCTIONS THAT COORDINATELY POTENTIATE THE EFFICACY OF SYNCHRONOUS RELEASE

Cpx-deficient cortical neurons and neuromuscular junctions show an increase in spontaneous release and a decrease in the pool size of releasable vesicles, in addition to a significant reduction of the efficacy of synchronous release (Table 1). These observations suggest that Cpx is involved in regulating at least three different steps of the exocytotic process: priming of vesicles, clamping of spontaneous vesicle fusions, and activation of Ca²⁺-triggered vesicle fusions (Figure 3A). However, Cpx-deficient hippocampal neurons do not show any increase in spontaneous release or decrease in the pool size of releasable vesicles^[14,28]. These differences among different neurotransmitter release systems are probably caused by the differential expression of other Ca²⁺-sensors or regulatory proteins. To resolve this discrepancy, isolated/purified vesicle fusion assays, such as *in vitro* flipped SNARE cell fusion assay and single vesicle-vesicle microscopy imaging, are developed and employed^[29-31]. The investigation of Cpx functions by employing these methods supports the multiple roles of Cpx in the regulation of priming, clamping, and activating during the Ca²⁺-triggered vesicle fusion process. Cpx is consisted of N-terminal, accessory α -helix, central α -helix, and C-terminal domains (Figure 3B). And each domain of Cpx has a distinct function that is differentially involved in the regulation of priming, clamping, and activating during Ca²⁺-triggered vesicle fusions. These distinct functions of Cpx domains coordinately contribute to potentiate the efficacy of synchronous release (Figure 3B)^[3,25,26,29-37].

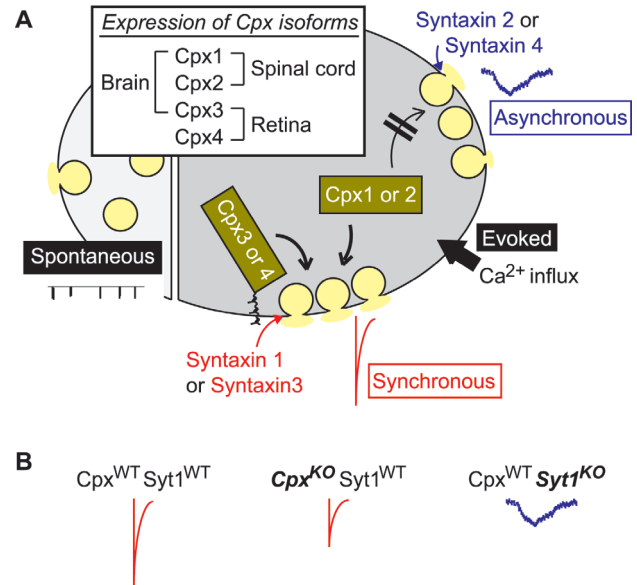


Figure 2 Regulation of different modes neurotransmitter release by Cpx. A. Evoked neurons release neurotransmitters in either synchronous or asynchronous mode. Syntaxin 1 and Syntaxin 3 are the major Syntaxin isoforms expressed at presynaptic nerve terminals and they are involved in mediating synchronous neurotransmitter release. Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3. Therefore, Cpx is probably involved in regulating the interactions between SNARE complexes and Ca²⁺-sensors for exocytosis. Neurons also spontaneously release neurotransmitters without evoked stimulations, but still mainly in a Ca²⁺-dependent manner. B. Syt1-deficient (Cpx^{WT}Syt1^{KO}) neurons change the mode of release from synchronous to asynchronous. Meanwhile, Cpx-deficient (Cpx^{KO}Syt1^{WT}) neurons do not change the mode of release, even though the efficacy of synchronous release is significantly reduced. Cpx, Complexin; KO, knock-out; Syt1, Synaptotagmin 1; WT, wild-type.

Table 1 Effects of Cpx-deficiency on various exocytotic processes.

| Species | Cells/Tissues | Phenotypes | References | |
|--|-----------------|--------------------------------|---|---|
| Cpx ⁺ 1,2,3-KO [†] | Mouse | Hippocampal & striatal neurons | Synchronous release ▼ Spontaneous release ▼ | [28] |
| | | Hippocampal neurons | Synchronous release ▼ | [14] |
| Cpx1,2-KO | Mouse | Cortical neurons | Synchronous release ▼ Spontaneous release ▲ Vesicle priming ▼ | [34,35] |
| | | | Cortical neurons | Synchronous release ▼ Spontaneous release ▲ Vesicle priming ▼ mRNA levels for Cpx3&4 ▲ |
| Cpx1,2-KD [§] | Rat | CA1 region of hippocampus | AMPA [†] receptor exocytosis during LTP ^{††} ▼ | [4] |
| Cpx1,2-KD | Mouse | Olfactory neuron | Syt10 ^{††} -regulated IGF-1 ^{§§} exocytosis ▼ | [27] |
| Cpx2-KO | Mouse | Chromaffin cells | Synchronous release ▼ Pool size of releasable vesicles ▼ | [25,26] |
| Cpx3,4-KO | Mouse | Retinal ribbon synapses | Synchronous release ▼ Evoked release ▼ | [17] |
| Cpx-KO | D. melanogaster | Neuromuscular junction | Asynchronous release ▲ Spontaneous release ▲ Growth of synapses ▲ | [39-41] |
| Cpx-KO | C. elegans | Neuromuscular junction | Evoked release ▼ Rate of tonic release ▲ Docked synaptic vesicles ▼ | [42,43] |

[†] Complexin, [‡] knock-out, [§] knock-down, [¶] α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, ^{††} long-term potentiation, ^{†††} Synaptotagmin 10, ^{§§} insulin-like growth factor-1, ▲ increase, ▼ decrease.

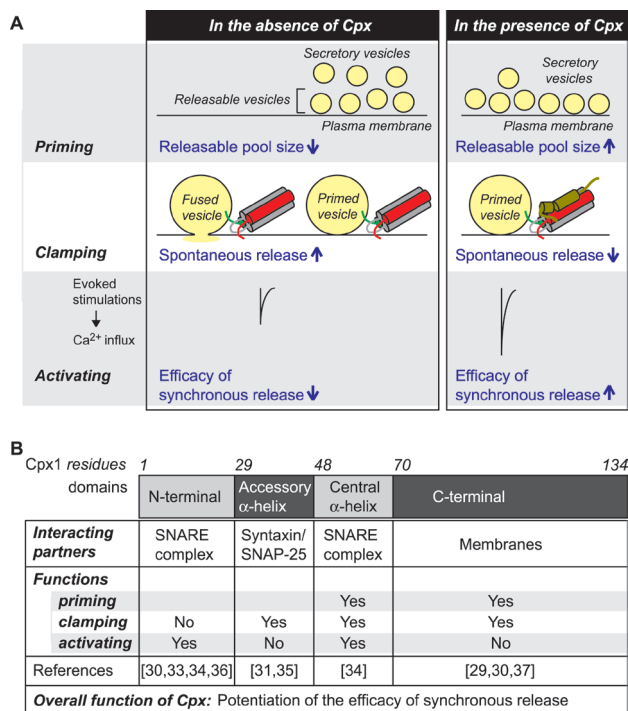


Figure 3 Cpx domains have distinct functions that coordinately potentiate the efficacy of synchronous neurotransmitter release. A. Cpx-deficient cortical neurons and neuromuscular junctions show: (i) a decrease in the pool size of releasable vesicles; (ii) an increase of spontaneous release; and (iii) a reduction of the efficacy of synchronous release. These observations, which are also supported by an *in vitro* single vesicle-vesicle microscopy imaging technique, indicate that Cpx is involved in the regulation of priming, clamping, and activating in the Ca^{2+} -triggered vesicle fusion process. B. Cpx isoforms (Cpx1-4) are consisted of N-terminal, accessory α -helix, central α -helix, and C-terminal domains. Each domain of Cpx has a distinct function that coordinately potentiates the efficacy of synchronous release. Cpx, Complexin; Cpx1, Complexin 1.

CONCLUSION

Cpx binds efficiently to the assembled SNARE complex that is composed of either Syntaxin 1 or Syntaxin 3, stabilizes the SNARE complex upon binding, and potentiates synchronous neurotransmitter release^[11,14,18,20]. In stimulated neurons, Syt1 interacts with membranes and the Cpx-bound SNARE complex in a Ca^{2+} -dependent manner to mediate synchronous neurotransmitter release^[6]. The efficacies of Syt1-regulated synchronous release are correlated with the strengths of interactions between Syt1 and SNARE complexes^[6]. Therefore, it is reasonable to assume that Cpx most likely facilitates the interaction between synchronous Ca^{2+} -sensors and SNARE complexes, at a minimum, by selecting, tagging, and stabilizing the assembled SNARE complex to potentiate the efficacy of synchronous release.

CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

REFERENCES

- Duncan CJ. Role of calcium in triggering the release of transmitters at the neuromuscular junction. *Cell Calcium* 1983; 4: 171-193.
- Südhof TC. Neurotransmitter release. *Handb Exp Pharmacol* 2008; 184: 1-21.
- Südhof TC. Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron* 2013; 80: 675-690. PMID: PMC 3866025.

- Ahmad M, Polepalli JS, Goswama D, Yang X, Kaeser-Woo YJ, Südhof TC, and Malenka RC. Presynaptic complexin controls AMPA receptor exocytosis during LTP. *Neuron* 2012; 73: 260-267. PMID: PMC 3269030.
- Chiergatti E, and Meldolesi J. Regulated exocytosis: new organelles for nonsecretory purposes. *Nat Rev Mol Cell Biol* 2005; 6: 181-187.
- Pang ZP, Shin OH, Meyer AC, Rosenmund C, and Südhof TC. A gain-of-function mutation in synaptotagmin-1 reveals a critical role of Ca^{2+} -dependent soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex binding in synaptic exocytosis. *J Neurosci* 2006; 26: 12556-12565.
- Shin OH. Exocytosis and synaptic vesicle function. *Compr Physiol* 2014; 4: 149-175.
- Südhof TC, and Rothman JE. Membrane fusion: Grapping with SNARE and SM proteins. *Science* 2009; 323: 474-477. PMID: PMC 3736821.
- Shin OH, Rizo J, and Südhof TC. Synaptotagmin function in dense core vesicle exocytosis studied in cracked PC12 cells. *Nat Neurosci* 2002; 5: 649-656.
- Zhang X, Rizo J, and Südhof TC. Mechanism of phospholipid binding by the C2A-domain of synaptotagmin I. *Biochemistry* 1998; 37: 12395-12403.
- Chen X, Tomchick DR, Kovrigin E, Arac D, Machius M, Südhof TC, and Rizo, J. Three-dimensional structure of the complexin/SNARE complex. *Neuron* 2002; 33: 397-409.
- Ishzuka T, Saisu H, Odani S, and Abe T. Synaphin: a protein associated with the docking/fusion complex in presynaptic terminal. *Biochem Biophys Res Commun* 1995; 213: 1107-1114.
- McMahon HT, Missier M, Li C, Südhof TC. Complexins: cytosolic proteins that regulate SNAP receptor function. *Cell* 1995; 83: 111-119.
- Reim K, Mansour M, Varoqueaux F, McMahon HT, Südhof TC, Brose N, Rosenmund C. Complexins regulate a late step in Ca^{2+} -dependent neurotransmitter release. *Cell* 2001; 104: 71-81.
- Liu J, Guo T, Wei Y, Liu M, Sui SF. Complexin is able to bind to SNARE core complexes in different assembled states with distinct affinity. *Biochem Biophys Res Commun* 2006; 347: 413-419.
- Reim K, Wegmeyer H, Brandstätter JH, Xue M, Resenmund C, Dresbach T, Hoffmann K, and Brose N. Structurally and functionally unique complexins at retina ribbon synapses. *J Cell Biol* 2005; 169: 669-680. PMID: PMC 2171701.
- Reim K, Regus-Leidig H, Ammermüller J, El-Kordi A, Radyushkin K, Ehrenreich H, Brandstätter JH, Brose N. Aberrant function and structure of retinal ribbon synapses in the absence of complexin 3 and complexin 4. *J Cell Sci* 2009; 122: 1352-1362.
- Shih AM, and Shin OH. Interactions among the SNARE proteins and complexin analyzed by a yeast four-hybrid assay. *Anal Biochem* 2011; 416:107-111.
- Tang J, Maximov A, Shin OH, Dai H, Rizo J, Südhof TC. A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. *Cell* 2006; 126: 1175-1187.
- Pabst S, Hazzard JW, Antonin W, Südhof TC, Jahn R, Rizo J, and Fasshauer D. Selective interaction of complexin with the neuronal SNARE complex. Differentiation of the binding regions. *J Biol Chem* 2000; 275: 19808-19818.
- Kaeser PS, and Regehr WG. Molecular mechanisms for synchronous, asynchronous, and spontaneous neurotransmitter release. *Annu Rev Physiol* 2014; 76: 333-367.
- Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, Rammner B, Fräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R. Molecular anatomy of a trafficking organelle. *Cell* 2006; 127: 831-846.
- Kennedy MJ, Davison IG, Robinson CG, and Ehlers MD. Syntaxin-4 defines a domain for activity-dependent exocytosis in den-

- drite spines. *Cell* 2010; 141: 524-535. PMID: PMC 2874581.
24. Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, and Südhof TC. Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. *Cell* 1994; 79: 717-727.
 25. Cai H, Reim K, Varoqueaux F, Tapechum S, Hill K, Sorenson JB, Brose N, and Chow RH. Complexin II plays a positive role in Ca^{2+} -triggered exocytosis by facilitating vesicle priming. *Proc Natl Acad Sci USA* 2008; 105: 19538-19543. PMID: PMC 2614796.
 26. Dhara M, Yarzagaray A, Schwart Y, Dutta S, Grabner C, Moghadam PK, Bost A, Schirra C, Rettig J, Reim K, Brose N, Mohrmann R, and Bruns D. Complexin synchronizes primed vesicle exocytosis and regulates fusion pore dynamics. *J Cell Biol* 2014; 204: 1123-1140. PMID: PMC 3971750.
 27. Cao P, Yang X, and Südhof TC. Complexin activates exocytosis of distinct secretory vesicles controlled by different synaptotagmins. *J Neurosci* 2013; 33: 1714-1727. PMID: PMC 3711587.
 28. Xue M, Stradomska A, Chen H, Brose N, Zhang W, Rosenmund C, and Reim K. Complexins facilitate neurotransmitter release at excitatory and inhibitory synapses in mammalian central nervous system. *Proc Natl Acad Sci USA* 2008; 105: 7875-7880. PMID: PMC 2409395.
 29. Diao J, Cipriano DJ, Zhao M, Zhang Y, Shah S, Padolina MS, Pfuetzner RA, Brunger AT. Complexin-I enhances the on-rate of vesicle docking via simultaneous SNARE and membrane interactions. *J Am Chem Soc* 2013; 135: 15274-15277. PMID: PMC 3854000.
 30. Lai Y, Diao J, Cipriano DJ, Zhang Y, Pfuetzner RA, Padolina MS, Brunger AT. Complexin inhibits spontaneous release and synchronizes Ca^{2+} -triggered synaptic vesicle fusion by distinct mechanisms. *Elife* 2014; 3: e03756. PMID: PMC 4130161.
 31. Giraudo CG, Garcia-Diaz A, Eng WS, Yamamoto A, Melia TJ, Rothman JE. Distinct domains of complexins bind SNARE complexes and clamp fusion *in vitro*. *J Biol Chem* 2008; 283: 21211-21219. PMID: PMC 2475712.
 32. Giraudo CG, Eng WS, Melia TJ, Rothman JE. A clamping mechanism involved in SNARE-dependent exocytosis. *Science* 2006; 313: 676-680.
 33. Xue M, Craig TK, Xu J, Chao HT, Rizo J, and Rosenmund C. Binding of the complexin N terminus to the SNARE complex potentiates synaptic vesicle fusogenicity. *Nat Struct Mol Biol* 2010; 17: 568-575. PMID: PMC 3172005.
 34. Maximov A, Tang J, Yang X, Pang ZP, and Südhof TC. Complexin controls the force transfer from SNARE complexes to membranes in fusion. *Science* 2009; 323: 516-521. PMID: PMC 3235366.
 35. Yang X, Kaeser-Woo YJ, Pang ZP, and Südhof TC. Complexin clamps asynchronous release by blocking a secondary Ca^{2+} -sensor via its accessory α helix. *Neuron* 2010; 68: 907-920. PMID: PMC 3050570.
 36. Xue M, Reim K, Chen X, Chao HT, Deng H, Rizo J, Brose N, and Rosenmund C. Distinct domains of complexin I differentially regulate neurotransmitter release. *Nat Struct Mol Biol* 2007; 14: 949-958.
 37. Kaeser-Woo YJ, Yang X, and Südhof TC. C-terminal complexin sequence is selectively required for clamping and priming but not for Ca^{2+} triggering of synaptic exocytosis. *J Neurosci* 2012; 32: 2877-2885. PMID: PMC 3742123.
 38. Yang X, Cao P, and Südhof TC. Deconstructing complexin function in activating and clamping Ca^{2+} -triggered exocytosis by comparing knockout and knockdown phenotypes. *Proc Natl Acad Sci USA* 2013; 110: 20777-20782. PMID: PMC 3870694.
 39. Buhl LK, Jorguera RA, Akbergenova Y, Huntwork-Rodriguez S, Volfson D, and Littleton JT. Differential regulation of evoked and spontaneous neurotransmitter release by C-terminal modifications of complexin. *Mol Cell Neurosci* 2013; 52: 161-172. PMID: PMC 3540146.
 40. Huntworks S, and Littleton JT. A complexin fusion clamp regulates spontaneous neurotransmitter release and synaptic growth. *Nat Neurosci* 2007; 10: 1235-1237.
 41. Jorguera RA, Huntwork-Rodriguez S, Akbergenova Y, Cho RW, and Littleton JT. Complexin controls spontaneous and evoked neurotransmitter release by regulating the timing and properties of synaptotagmin activity. *J Neurosci* 2012; 32: 18234-18245. PMID: PMC 3530744.
 42. Hobson RJ, Liu Q, Watanabe S, and Jorgensen EM. Complexin maintains vesicles in the primed state in *C. elegans*. *Curr Biol* 2011; 21: 106-113. PMID: PMC 3048763.
 43. Martin JA, Hu Z, Fenz KM, Fernandez J, and Dittman JS. Complexin has opposite effects on two modes of synaptic vesicle fusion. *Curr Biol* 2011; 21: 97-105. PMID: PMC 3026084.
- Peer reviewer:** Zui Pan, PhD, Associate Professor, Davis Heart and Lung Research Institute, Comprehensive Cancer Center, The Ohio State University Wexner Medical Center, 460 West 12th Ave. BRT. Rm398, Columbus, OH 43210, USA.