Journal Club

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Do SNARE Protein Isoforms Determine Fusion Pore Characteristics?

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Review of Chang et al.

Membrane fusion is a crucial element of many cellular processes, including the release of neurotransmitters from synaptic vesicles in neurons and the release of neuropeptides, neurotrophic factors, and hormones from dense core vesicles (DCVs) in brain and neuroendocrine cells. Vesicle secretion is mediated by the formation of the soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) complex. This SNARE complex comprises vesicle-associated membrane protein (VAMP), syntaxin, and synaptosomal-associated protein (SNAP). Different isoforms of these proteins are involved in membrane fusion at different sites, with the canonical SNARE complex of synaptic vesicles consisting of synaptobrevin 2, syntaxin 1, and SNAP25 (Kasai et al., 2012). Both synaptobrevin 2 and syntaxin 1 contain a membrane-spanning α -helix—the transmembrane domain—that spans the vesicle membrane and the plasma membrane, respectively. In addition, these proteins contain an α -helical SNARE motif, while SNAP25 contains two. These α -helices can form a tight bundle, which brings the vesicular and plasma membranes into close contact. Subsequently, the membranes fuse and cargo is released (Jahn

and Fasshauer, 2012; Rizo and Südhof, 2012).

One essential component of membrane fusion is the formation of the fusion pore, an intermediate, temporary structure that bridges the vesicular and plasma membranes. The composition of this pore is currently under debate. One hypothesis is that the fusion pore is composed purely of lipids of both membranes. According to this hypothesis, the SNARE complex accelerates pore formation by bringing the membranes in close proximity. Another model proposes a proteinaceous fusion pore, in which the transmembrane domains of the SNARE proteins are incorporated into the pore (Jackson and Chapman, 2008; Fang and Lindau, 2014). The latter hypothesis is supported by previous work by Han et al. (2004), who showed that replacing any of three specific amino acids of the syntaxin 1 transmembrane domain with the large amino acid tryptophan altered flux through the fusion pore. These amino acids are located at the same face of the α -helix, suggesting that this α -helical face lines the fusion pore. These data led the authors to propose a model in which the fusion pore consists of a circular arrangement of five to eight syntaxin 1 transmembrane domains (Han et al., 2004). This raises the question of whether the transmembrane domain of synaptobrevin 2 might also be involved in fusion pore formation.

A study recently published in *The Journal of Neuroscience* by Chang and

colleagues (2015) investigates the involvement of the synaptobrevin 2 transmembrane domain in the fusion pore. In this study, the authors used amperometry to measure the release of catecholamines from DCVs. Secretion of a single DCV results in a spike of current upon membrane fusion. However, just before this spike, small amounts of cargo are secreted through the fusion pore. This shows up in amperometric measurements as a pre-spike foot (PSF) (Jackson and Chapman, 2006). For their experiments, Chang et al. (2015) used chromaffin cells from synaptobrevin 2 and cellubrevin (VAMP2 and VAMP3, respectively) double knock-out (DKO) mice, in which DCV fusion is almost completely abolished. Similar to the study by Han et al. (2004), 21 mutants of synaptobrevin 2—each with a single amino acid replaced with tryptophan in the transmembrane domain (positions 96–116)—were individually expressed in the DKO cells. Expression of any of these constructs rescued DCV release frequency to wild-type levels (eight times higher than DKO). In addition, four of these mutants (L99W, V101W, C103W, and I105W) showed reduced PSF amplitude. Replacement of these amino acids with the small amino acid glycine resulted in an increased PSF. Therefore, the authors concluded that the residues of these four amino acids influence flux through the fusion pore in a size-dependent manner, with larger residues obstructing the fusion pore.

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To verify these findings, Chang et al. (2015) measured membrane capacitance and pore conductance. Membrane capacitance is a measure of the surface area of the plasma membrane. When a vesicle fuses, its membrane is incorporated into the plasma membrane, which increases the cell's membrane capacitance. The tryptophan substitutions that decreased PSF amplitude did not influence the changes in capacitance resulting from vesicle fusion, which means that vesicle size was not affected. However, the substitutions did decrease conductance of the fusion pore, which indicates that the physical properties of the pore were altered. This further supports the idea that these amino acids line the fusion pore and influence its characteristics. The authors also asked whether the charge of residues lining the fusion pore affect the flux through the pore. Indeed, they show that incorporation of negatively charged amino acid residues increased the PSF amplitude, whereas incorporation of positively charged amino acid residues decreased PSF amplitude and therefore the outflow of positively charged catecholamines.

The data presented by Chang and colleagues (2015) indicate that besides the transmembrane domain of syntaxin 1, the transmembrane domain of synaptobrevin 2 also lines the fusion pore. Interestingly, the four residues in the synaptobrevin 2 transmembrane domain that alter the PSF are located two-by-two on opposite faces of the α -helix, and therefore cannot line the fusion pore simultaneously. This is unlike the findings for syntaxin 1, where the residues that alter fusion pore flux are on one face of the α -helix. However, synaptobrevin 2 is known to dimerize (Fdez et al., 2010). Therefore, the authors propose a model in which three to four asymmetric dimers of synaptobrevin 2 line the fusion pore (Chang et al., 2015; their Fig. 5B). These would line up with six to eight syntaxin 1 monomers as proposed by Han et al. (2004) in a gap junction-like manner to form a protenaceous fusion pore (Chang et al., 2015; their Fig. 5C).

It is worth noting that although knocking out both synaptobrevin 2 (VAMP2) and cellubrevin (VAMP3) abolishes DCV release, knocking out synaptobrevin 2 alone reduces, but does not abolish, DCV release, and knocking out cellubrevin alone does not significantly influence DCV release. Moreover, cellubrevin is up-regulated in synaptobrevin 2 KO cells (Borisovska et al., 2005). This suggests that cellubrevin does not play a significant role in DCV release in the presence of syn-

aptobrevin 2, but it can partially take over its function when synaptobrevin 2 is absent. Interestingly, while the SNARE domains of synaptobrevin 2 and cellubrevin differ by only a single amino acid, the transmembrane domain of cellubrevin is only 44% homologous to the transmembrane domain of synaptobrevin 2. Of the four amino acids that Chang et al. (2015) found to influence fusion pore flux, three differ between synaptobrevin 2 and cellubrevin. At positions 101 and 103, cellubrevin expresses a serine and leucine, respectively. Compared to the residues present in synaptobrevin 2 (valine at 101 and cysteine at 103), the polarity of these residues is altered (from nonpolar to polar at residue 101 and from polar to nonpolar at residue 103). Residue polarity has been linked to ion permeation through the pore of an ion channel (Juntadech et al., 2014). Therefore, the altered residue polarity might also directly influence the flux of charged cargo through a fusion pore formed by cellubrevin. In addition, cellubrevin contains a glycine at position 99. The authors report that making this specific substitution in synaptobrevin 2 (L99G) appeared to increase PSF amplitude, although the difference did not reach statistical significance (Chang et al., 2015). Given these differences in amino acid residues at pore-lining sites between synaptobrevin 2 and cellubrevin, we suggest that fusion pores formed by these proteins will have different characteristics, and that pores formed by cellubrevin will have a higher PSF amplitude than pores formed by synaptobrevin 2. Consistent with this, Borisovska et al. (2005) showed that PSF amplitude was increased in a synaptobrevin 2 KO. This phenotype was rescued by synaptobrevin 2 overexpression, but not by overexpression of cellubrevin (Borisovska et al., 2005).

Because the composition of transmembrane domains differs across VAMP isoforms, fusion pore characteristics may depend on the particular isoform present on different types of vesicles. Furthermore, there are various isoforms of syntaxin, which also show variability in their transmembrane domains. Importantly, the three residues that alter fusion pore flux upon tryptophan substitution (I269, G276, I283) (Han et al., 2004) are different in multiple syntaxin isoforms. The composition of the SNARE complexes involved in membrane fusion differs between vesicles and cell types. For example, syntaxin 6 or syntaxin 16 together with VAMP4 regulate early endosome to *trans*-Golgi network trafficking (Shitara et al.,

2013), cellubrevin regulates vesicle exocytosis in astrocytes (Li et al., 2015), and synaptobrevin 1 mediates synaptic vesicle release in a subpopulation of hippocampal neurons (Zimmermann et al., 2014). The isoform composition of the SNARE complex as a whole may thus determine the characteristics of fusion pores formed by these proteins and therefore partly regulate temporal dynamics of release events and the amount of cargo that is secreted from a vesicle. At synapses, characteristics of the fusion pore could therefore influence kinetics of synaptic events, which can in turn influence synaptic integration (Magee, 2000). Also, low levels of release through a fusion pore have been hypothesized to act as an inhibitory signal, desensitizing postsynaptic receptors (Jackson and Chapman, 2008). One type of fusion in which the fusion pore plays a fundamental role is kiss-and-run. Here, the fusion pore closes after release of cargo and the vesicle retracts from the membrane, unlike full-collapse fusion, where the fusion pore dilates and the vesicle membrane is incorporated into the plasma membrane. Whether synaptic vesicles engage in kiss-and-run release is still under debate, but it has been well established for DCV secretion (Alabi and Tsien, 2013), for which kiss-and-run is used almost exclusively (Chiang et al., 2014). The composition of the SNARE complexes involved in different types of fusion may therefore be important in determining the release of cargo through the fusion pore from both dense core and synaptic vesicles.

Currently, little is known about the structure of the fusion pore, and its exact function remains to be determined. However, the data shown by Chang et al. (2015) support the view of the fusion pore as an arrangement of proteins and may provide further insight into the functional role of the fusion pore and of different SNARE protein isoforms in regulating fusion events.

References

- Alabi AA, Tsien RW (2013) Perspectives on kiss-and-run: role in exocytosis, endocytosis, and neurotransmission. *Annu Rev Physiol* 75: 393–422. [CrossRef Medline](#)
- Borisovska M, Zhao Y, Tsytsyura Y, Glyvuk N, Takamori S, Matti U, Rettig J, Südhof T, Bruns D (2005) v-SNAREs control exocytosis of vesicles from priming to fusion. *EMBO J* 24:2114–2126. [CrossRef Medline](#)
- Chang CW, Hui E, Bai J, Bruns D, Chapman ER, Jackson MB (2015) A structural role for the synaptobrevin 2 transmembrane domain in dense-core vesicle fusion pores. *J Neurosci* 35: 5772–5780. [CrossRef Medline](#)

- Chiang HC, Shin W, Zhao WD, Hamid E, Sheng J, Baydyuk M, Wen PJ, Jin A, Momboisse F, Wu LG (2014) Post-fusion structural changes and their roles in exocytosis and endocytosis of dense-core vesicles. *Nat Commun* 5:3356. [CrossRef Medline](#)
- Fang Q, Lindau M (2014) How could SNARE proteins open a fusion pore? *Physiology* 29:278–285. [CrossRef Medline](#)
- Fdez E, Martínez-Salvador M, Beard M, Woodman P, Hilfiker S (2010) Transmembrane-domain determinants for SNARE-mediated membrane fusion. *J Cell Sci* 123:2473–2480. [CrossRef Medline](#)
- Han X, Wang CT, Bai J, Chapman ER, Jackson MB (2004) Transmembrane segments of syntaxin line the fusion pore of Ca(2+)-triggered exocytosis. *Science* 304:289–292. [CrossRef Medline](#)
- Jackson MB, Chapman ER (2006) Fusion pores and fusion machines in Ca²⁺-triggered exocytosis. *Annu Rev Biophys Biomol Struct* 35:135–160. [CrossRef Medline](#)
- Jackson MB, Chapman ER (2008) The fusion pores of Ca(2+)-triggered exocytosis. *Nat Struct Mol Biol* 15:684–689. [CrossRef Medline](#)
- Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. *Nature* 490:201–207. [CrossRef Medline](#)
- Juntadech T, Kanintrunkul Y, Kanchanawarin C, Katzenmeier G, Angsuthanasombat C (2014) Importance of polarity of the $\alpha 4$ – $\alpha 5$ loop residue—Asn166 in the pore-forming domain of the *Bacillus thuringiensis* Cry4Ba toxin: implications for ion permeation and pore opening. *Biochim Biophys Acta* 1838:319–327. [CrossRef Medline](#)
- Kasai H, Takahashi N, Tokumaru H (2012) Distinct initial SNARE configurations underlying the diversity of exocytosis. *Physiol Rev* 92:1915–1964. [CrossRef Medline](#)
- Li D, Hérault K, Zylbersztejn K, Lauterbach MA, Guillon M, Oheim M, Ropert N (2015) Astrocyte VAMP3 vesicles undergo Ca(2+)-independent cycling and modulate glutamate transporter trafficking. *J Physiol* 593:2807–2832. [CrossRef Medline](#)
- Magee JC (2000) Dendritic integration of excitatory synaptic input. *Nat Rev Neurosci* 1:181–190. [CrossRef Medline](#)
- Rizo J, Südhof TC (2012) The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices—guilty as charged? *Annu rev cell dev biol* 28:279–308. [CrossRef Medline](#)
- Shitara A, Shibui T, Okayama M, Arakawa T, Mizoguchi I, Shakakura Y, Takuma T (2013) VAMP4 is required to maintain the ribbon structure of the Golgi apparatus. *Mol Cell Biochem* 380:11–21. [CrossRef Medline](#)
- Zimmermann J, Trimbuch T, Rosenmund C (2014) Synaptobrevin 1 mediates vesicle priming and evoked release in a subpopulation of hippocampal neurons. *J Neurophysiol* 112:1559–1565. [CrossRef Medline](#)