Chapter 5
Roles of Tomosyn in Neurotransmitter Release

Yasunori Yamamoto and Toshiaki Sakisaka

Abstract SNARE complexes and synaptotagmin mediate synaptic vesicle fusion with the plasma membrane of the active zone for the neurotransmitter release from presynaptic nerve terminals responding to neuronal signals. Many regulatory proteins for the SNARE complex formation have been identified. Among them, our originally identified protein, tomosyn, is likely to be a key molecule for the regulation of the SNARE complex-involved pre-fusion step and the Ca^{2+}-triggered synaptic vesicle fusion step. Tomosyn inhibits SNARE complex formation and thereby inhibits synaptic vesicle fusion by sequestering target SNAREs through its C-terminal VAMP-like domain in a Ca^{2+}-independent manner. The N-terminal WD40 repeats are the site for its binding to synaptotagmin-1, a Ca^{2+}-sensor protein, in a Ca^{2+}-dependent manner. The interaction negatively regulates the Ca^{2+}-dependent synaptic vesicle fusion mediated by synaptotagmin-1. Thus, tomosyn is a potent inhibitor, temporally and stepwisely regulating the synaptic vesicle fusion at the active zone, for the synchronized and fast neurotransmitter release.

Keywords Neurotransmitter release • Exocytosis • SNARE • Tomosyn • Synaptotagmin-1

5.1 Introduction

Tomosyn is a syntaxin-1-binding protein that we originally identified (Fujita et al. 1998). Tomosyn means tomo (friend in Japanese) of syntaxin-1. Tomosyn contains N-terminal WD40 repeats, a tail domain, and a C-terminal domain homologous to VAMP2. The C-terminal VAMP-like domain is responsible for binding to syntaxin-1 (Fujita et al. 1998) (Fig. 5.1a). Tomosyn belongs to the Lgl (lethal giant larvae) family that is conserved from yeast to human (Kagami et al. 1998; Hattendorf et al. 2007; Ashery et al. 2009) (Fig. 5.1b). The Lgl family is characterized by N-terminal fourteen WD40 repeats constituting two β-propeller structures (seven WD40 repeats constitute one β-propeller structure) (Hattendorf et al. 2007) and

Y. Yamamoto • T. Sakisaka (✉)
Division of Membrane Dynamics, Department of Physiology and Cell Biology,
Kobe University Graduate School of Medicine, Kobe 650-0017, Japan
e-mail: sakisaka@med.kobe-u.ac.jp

© Springer Japan 2015

S. Mochida (ed.), *Presynaptic Terminals*, DOI 10.1007/978-4-431-55166-9_5

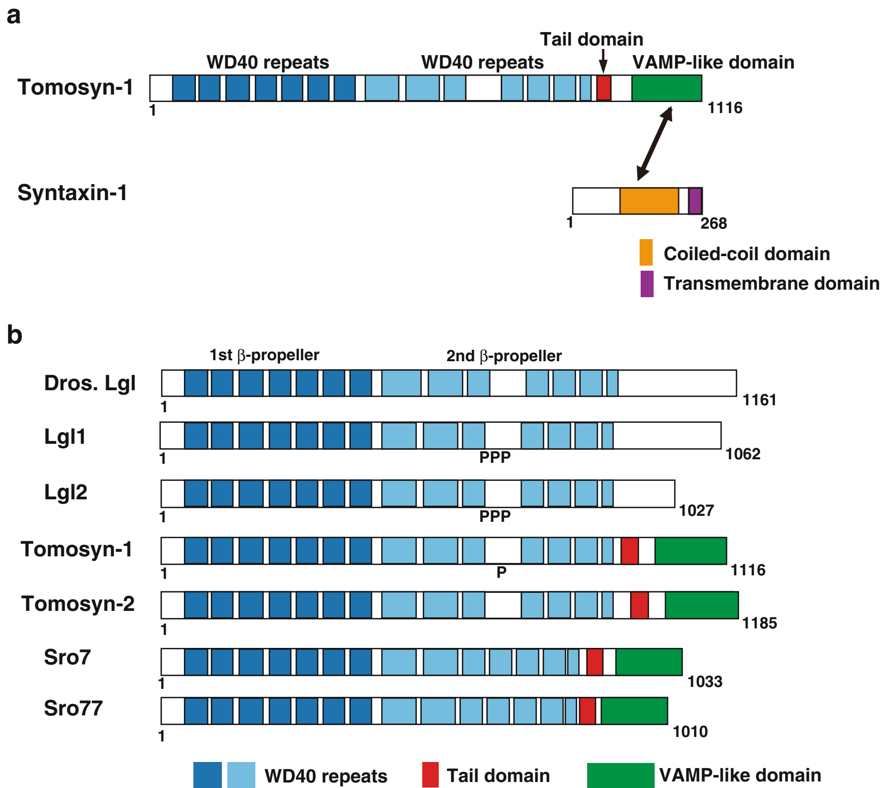

Fig. 5.1 Schematic representation of tomosyn. (**a**) Tomosyn is a syntaxin-1-binding protein. Tomosyn is composed of N-terminal WD40 repeats, a tail domain, and a C-terminal VAMP-like domain. Tomosyn binds to syntaxin-1 through the C-terminal VAMP-like domain. (**b**) Tomosyn belongs to the Lgl family. The Lgl family is characterized by the N-terminal WD40 repeats that constitute two β-propeller structures. Sro7 and Sro77 are yeast orthologues of tomosyn. Among the family members, only Lgl does not have the tail domain and the C-terminal VAMP-like domain. Phosphorylation sites are indicated with P

composed of Lgl, tomosyn, Sro7, and Sro77. Among the family members, only Lgl does not have a tail domain and a C-terminal domain homologous to VAMP2. Lgl, which was firstly identified as a tumor suppressor gene in *Drosophila*, has been shown to be involved in polarity formation and cell-cell adhesion in epithelial cells (Wirtz-Peitz and Knoblich 2006; Yamanaka and Ohno 2008). Sro7 and Sro77, yeast orthologues of tomosyn, have been shown to regulate exocytosis in yeast (Lehman et al. 1999). Lgl, tomosyn, and Sro7 directly interact with SNARE proteins (Fujita et al. 1998; Lehman et al. 1999; Müsch et al. 2002; Gangar et al. 2005), suggesting that the Lgl family plays roles in the SNARE-dependent vesicle trafficking. However, the common mode of action among the Lgl family members remains elucidated. Among the Lgl family members, the origins of tomosyn and Sro7 are evolutionally old (Kloepper et al. 2008). During the evolution from prokaryotes to metazoan, Lgl is thought to be generated by gene duplication of tomosyn (Kloepper et al. 2008).

Therefore, elucidating modes of action for tomosyn and Sro7 is important for understanding the function of the Lgl family. In this chapter we describe, based on our findings, roles of tomosyn in the synaptic vesicle fusion phase. In the vertebrate nervous system, tomosyn-1 is expressed dominantly in the whole brain, while tomosyn-2 is expressed in the restricted area of the brain (Groffen et al. 2005). From here, we refer to tomosyn-1 as tomosyn.

5.2 SNARE-Dependent Vesicle Fusion Machinery

Synaptic vesicles are transported to the active zone in the presynaptic plasma membrane where Ca^{2+} channels are located. Depolarization induces Ca^{2+} influx into the cytosol of nerve terminals through the Ca^{2+} channels, and this Ca^{2+} influx initiates the fusion of the vesicles with the plasma membrane, finally leading to exocytosis of neurotransmitters (Südhof 2004). Soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein (SNAP) receptors (SNAREs) are essential for the synaptic vesicle exocytosis (Sutton et al. 1998; Weber et al. 1998; Jahn and Scheller 2006; Rizo and Rosenmund 2008). Synaptic vesicles are endowed with vesicle-associated membrane protein 2 (VAMP2) as a vesicular SNARE (v-SNARE), whereas the presynaptic plasma membrane is endowed with syntaxin-1 and SNAP-25 as target SNAREs (t-SNAREs) (Fig. 5.2a). VAMP2 interacts with SNAP-25 and syntaxin-1 to form a stable SNARE complex (Trimble et al. 1988; Bennett et al. 1992; Söllner et al. 1993; Chen and Scheller 2001). The formation of the SNARE complex then brings synaptic vesicles and the plasma membrane into close apposition and provides the energy that drives the mixing of the two lipid bilayers (Weber et al. 1998; Chen and Scheller 2001; Jahn and Scheller 2006; Rizo and Rosenmund 2008). Although the previous study using the SNARE complex reconstituted on liposomes demonstrated that the SNARE complex was sufficient for membrane fusion, the fusion kinetics was very slow (Weber et al. 1998). These facts indicate in vivo existence of a special factor(s) to regulate SNARE assembly for fast membrane fusion characteristic of neurotransmitter release.

5.3 Tomosyn Regulates SNARE Complex Formation Through the C-Terminal VAMP-Like Domain

Tomosyn interacts with SNAP-25 and syntaxin-1 to form a stable tomosyn-SNARE complex through the C-terminal VAMP-like domain (Fujita et al. 1998) (Fig. 5.2b). The tomosyn-SNARE complex formation sequesters t-SNAREs on the presynaptic plasma membrane, leading to inhibition of the SNARE complex formation (Fujita et al. 1998; Hatsuzawa et al. 2003; Pobbati et al. 2004; Sakisaka et al. 2008;

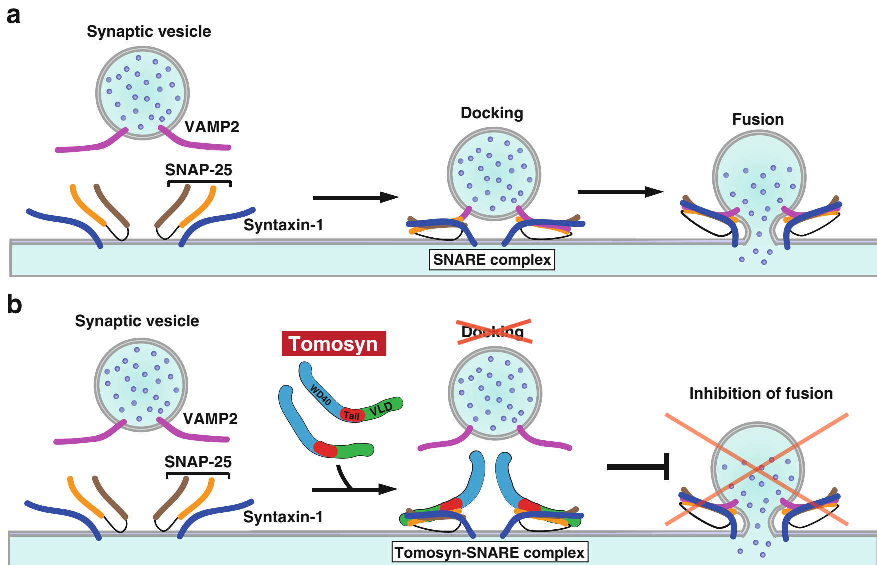

Fig. 5.2 Regulation of the SNARE complex formation by tomosyn. (**a**) Synaptic vesicle fusion driven by SNARE complexes. Syntaxin-1, SNAP-25, and VAMP2 form a ternary SNARE complex, allowing the synaptic vesicle to dock at the presynaptic membrane. Subsequently, the SNARE complexes catalyze membrane fusion in a zipper-like fashion. (**b**) Inhibition of the SNARE complex formation by tomosyn. Tomosyn binds to syntaxin-1 and SNAP-25 through the C-terminal VAMP-like domain (VLD), thereby forming a stable tomosyn-SNARE complex. The tomosyn-SNARE complex formation inhibits the SNARE complex formation by sequestering the t-SNAREs, leading to inhibition of membrane fusion

Yamamoto et al. 2009; Ashery et al. 2009). Consistent with the inhibitory activity on the SNARE complex formation, the genetic ablation of tomosyn in mice and *C. elegans* leads to enhancement of neurotransmitter release (Sakisaka et al. 2008; Gracheva et al. 2006; McEwen et al. 2006), and the overexpression in superior cervical ganglion (SCG) neurons inhibits neurotransmitter release induced by an action potential (Baba et al. 2005). Accumulating evidence suggests that tomosyn controls exocytotic efficacy of synaptic vesicles. Paired-pulse facilitation at mossy fiber synapses of hippocampi is decreased in the tomosyn-deficient mice (Sakisaka et al. 2008). In response to repetitive presynaptic action potentials, the tomosyn-overexpressing neurons show severe synaptic depression, in contrast to remarkable synaptic facilitation in control neurons (Baba et al. 2005). However, it is unclear how tomosyn controls the Ca^{2+}-dependent exocytosis, since the C-terminal VAMP-like domain sequesters the t-SNAREs in a Ca^{2+}-independent manner. We showed that tomosyn is directly phosphorylated by protein kinase A (PKA), which in turn reduces its interaction with syntaxin-1 and enhances the formation of the SNARE complex (Baba et al. 2005). In addition, Rho-associated serine/threonine kinase (ROCK) activated by Rho small G protein phosphorylates syntaxin-1, which in turn increases the affinity of syntaxin-1 for tomosyn and forms a stable complex with

tomosyn, resulting in inhibition of the formation of the SNARE complex during neurite extension (Sakisaka et al. 2004). Thus, the inhibitory activity of the C-terminal VAMP-like domain is regulated via the well-known signal transduction pathways.

5.4 Tomosyn Regulates SNARE Complex Formation Through the N-Terminal WD40 Repeats

The N-terminal WD40 repeats of tomosyn are also responsible for potent inhibition of neurotransmitter release. It has been demonstrated that catecholamine secretion is potently inhibited in chromaffin cells by overexpressing the N-terminal WD40 repeats (Yizhar et al. 2007). Intriguingly, the inhibitory activity of the N-terminal WD40 repeats in the chromaffin cells depends on Ca^{2+} concentration (Yizhar et al. 2004, 2007). We have also demonstrated that acetylcholine release from SCG neurons in long-term culture is potently inhibited by microinjecting the tomosyn fragment encompassing the N-terminal WD40 repeats (Sakisaka et al. 2008). Similarly to the inhibitory activity of the N-terminal WD40 repeats in the chromaffin cells, the inhibitory activity of tomosyn in the SCG neurons is influenced by Ca^{2+} concentration (Baba et al. 2005). In *C. elegans*, tomosyn associates with synaptic vesicles through the N-terminal WD40 repeats (McEwen et al. 2006), raising the possibility that the N-terminal WD40 repeats may negatively regulate the function of synaptic vesicle in a Ca^{2+}-dependent manner. While we have shown that tomosyn oligomerizes the SNARE complex through the N-terminal WD40 repeats (Sakisaka et al. 2008), this does not account for the Ca^{2+}-dependent inhibitory activity of the N-terminal WD40 repeats since the oligomerization takes place in a Ca^{2+}-independent manner. Therefore, the N-terminal WD40 repeats are expected to functionally interact with a Ca^{2+}-responsive protein(s) involved in the regulation of the synaptic vesicle fusion.

Indeed, we have demonstrated that tomosyn directly binds to synaptotagmin-1, a synaptic vesicle protein with two C_2 domains that both bind to Ca^{2+}, through the N-terminal WD40 repeats in a Ca^{2+}-dependent manner (Yamamoto et al. 2010a). Synaptotagmin-1 underlies Ca^{2+} responsiveness in the neurotransmitter release (Geppert et al. 1994; Sutton et al. 1995; Shao et al. 1998; Fukuda et al. 1999; Augustine 2001; Fernandez et al. 2001; Chapman 2008). Upon Ca^{2+} binding, synaptotagmin-1 induces positive curvature of the presynaptic membrane by inserting the hydrophobic loops in the C_2 domains into the presynaptic membrane, thereby catalyzing fast synaptic vesicle fusion in cooperation with the SNARE complex (Martens et al. 2007; Stein et al. 2007; Xue et al. 2008; Hui et al. 2009) (Fig. 5.3a). Importantly, the Ca^{2+}-dependent binding between tomosyn and synaptotagmin-1 impairs the synaptotagmin-1 catalysis (Yamamoto et al. 2010a) (Fig. 5.3b), indicating that tomosyn negatively regulates the synaptotagmin-1-mediated step of Ca^{2+}-dependent neurotransmitter release through the N-terminal WD40 repeats. Furthermore, the Ca^{2+}-dependent binding enhances the activity of the

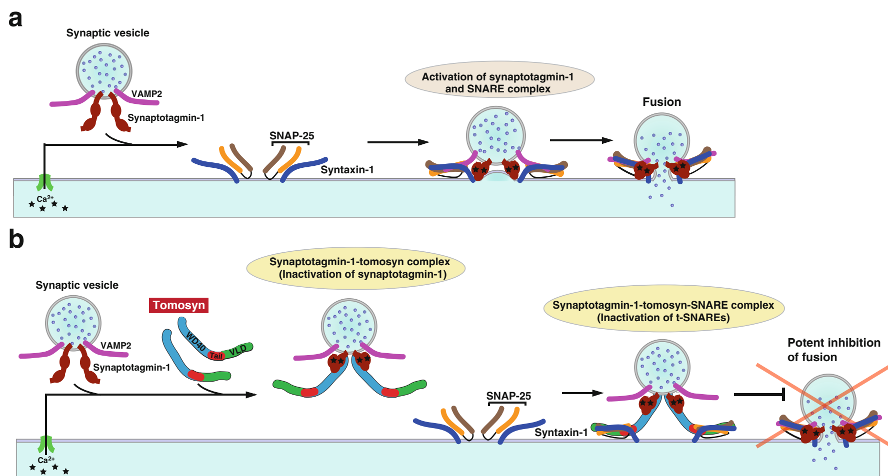

Fig. 5.3 Regulation of the Ca^{2+}-dependent synaptic vesicle fusion by tomosyn. (**a**) Synaptotagmin-1 is a Ca^{2+} sensor that cooperates with the SNARE complex toward membrane fusion. Upon Ca^{2+} influx, Ca^{2+} binds and activates synaptotagmin-1. Activated synaptotagmin-1 bends the presynaptic membrane, thereby facilitating the SNARE complex-driven membrane fusion. (**b**) Tomosyn acts as an alternative Ca^{2+} sensor that negatively regulates the synaptic vesicle fusion. Upon Ca^{2+} influx, tomosyn binds and inactivates Ca^{2+}-bound synaptotagmin-1 through the N-terminal WD40 repeats. The synaptotagmin-1 binding enhances sequestration of t-SNAREs by the C-terminal VAMP-like domain (VLD) of tomosyn. Eventually, membrane fusion is potently inhibited in a Ca^{2+}-dependent manner

C-terminal VAMP-like domain of tomosyn to sequester t-SNAREs (Yamamoto et al. 2010a) (Fig. 5.3b). These findings raise an attractive possibility that the interplay between tomosyn and synaptotagmin-1 underlies the inhibitory control of Ca^{2+}-dependent neurotransmitter release. In response to a rise in Ca^{2+} concentration, synaptotagmin-1 on the synaptic vesicle catches tomosyn and inactivates its own catalysis for membrane fusion. Simultaneously, the synaptotagmin-1-tomosyn complex enhances sequestering of t-SNAREs on the presynaptic membrane and blocks the SNARE assembly. Eventually, the Ca^{2+}-dependent synaptotagmin-1-tomosyn-SNARE complex formation will ensure inactivation of the fusion machineries on both the donor and target membranes and thereby inhibit priming of the synaptic vesicles. Synaptotagmin-1, cooperating with tomosyn on synaptic vesicles, would act as an alternative Ca^{2+} sensor to negatively control exocytotic efficacy of the synaptic vesicles, sensing Ca^{2+} concentration change near Ca^{2+} channel clusters.

5.5 The Tail Domain Regulates the Activity of Tomosyn

We have demonstrated that the tail domain of tomosyn acts as a regulatory domain for the C-terminal VAMP-like domain (Yamamoto et al. 2009, 2010b). The tail domain can directly bind to either the C-terminal VAMP-like domain or the

N-terminal WD40 repeats (Yamamoto et al. 2009). The binding of the tail domain to the C-terminal VAMP-like domain represses the activity of the C-terminal VAMP-like domain to inhibit the SNARE complex formation, which is restored by the binding of the tail domain to the N-terminal WD40 repeats (Yamamoto et al. 2009). Therefore, tomosyn will be in a state of equilibrium between two conformational states upon the tail domain binding. In one conformational state (state I), the tail domain binds to the N-terminal WD40 repeats, leading to exposure of the C-terminal VAMP-like domain. The exposed C-terminal VAMP-like domain efficiently forms the tomosyn-SNARE complex, resulting in the potent inhibition of the SNARE complex formation. In the other conformational state (state II), the tail domain masks the C-terminal VAMP-like domain and thereby blocks the tomosyn-SNARE complex formation (Yamamoto et al. 2009) or enables VAMP2 to displace tomosyn from the tomosyn-SNARE complex (Yamamoto et al. 2010b), resulting in loss of the inhibition of the SNARE complex formation. In support of the idea of equilibrium, full-length tomosyn moderately inhibited the SNARE-driven membrane fusion relative to the tomosyn fragment encompassing only the C-terminal VAMP-like domain in vitro (Yamamoto et al. 2010b), suggesting that full-length tomosyn exists in both states. Given that full-length tomosyn inhibited the SNARE-driven membrane fusion more than the tomosyn fragment encompassing both the tail domain and the C-terminal VAMP-like domain did in vitro (Yamamoto et al. 2010b), the state I may be a dominant state. What drives the conformational change from state I to state II? We previously reported that PKA phosphorylates tomosyn, resulting in reducing the binding of the C-terminal VAMP-like domain to syntaxin-1 (Baba et al. 2005). Therefore, PKA might be a possible regulator to drive the conformational change. The physiological meaning of the structural regulation of tomosyn upon the tail binding still remains elusive. We have generated tomosyn-deficient mice, characterized them electrophysiologically, and revealed that the tomosyn-deficient mice lacked short-term potentiation (Sakisaka et al. 2008). Therefore, the structural regulation of tomosyn may be important for the short-term memory. Further studies will be needed for understanding the regulation of the conformational change of tomosyn.

5.6 Tomosyn Regulates the Readily Releasable Pool Size

Evidence is accumulating that tomosyn regulates the readily releasable pool (RRP) size in response to repetitive presynaptic activity. Tomosyn-deficient mice show reduced paired-pulse facilitation in hippocampi (Sakisaka et al. 2008), suggesting that, without tomosyn, the first action potential depletes synaptic vesicles in the RRP. Tomosyn-overexpressing presynaptic SCG neurons evoke smaller excitatory postsynaptic potentials (EPSPs) but cannot respond to following repetitive action potentials, thus inducing severe synaptic depression (Baba et al. 2005). In addition,

synchronization for repeated transmitter release was lost under tomosyn loss-function by point mutations in the N-terminal WD40 repeats (Baba et al. 2005). The SCG neuron in culture forms synapses with many varicosities wrapping the cell soma (Baba et al. 2005; Ma et al. 2009); therefore, the RRP size is relatively large, 84–180 synaptic vesicles (Ma et al. 2009; unpublished data), calculated from the depletion of the RRP with a train of high-frequency action potentials. The averaged EPSP amplitude is ≈20 mV (Baba et al. 2005; Ma et al. 2009), suggesting that the number of releasable vesicles in response to an action potential is well controlled. From the averaged EPSP integral, we estimate that neurotransmitters are released from ≈50 synaptic vesicles in the GFP-overexpressing or non-transfected SCG neurons (Baba et al. 2005; Ma et al. 2009). However, only 14 synaptic vesicles in tomosyn-overexpressing neurons (unpublished data) and 12–20 synaptic vesicles in the tomosyn mutant–overexpressing neurons (Baba et al. 2005) can be exocytosed in response to an action potential. Therefore, tomosyn is a key molecule to determine the size of the RRP.

The size of the RRP in the central nervous system is extremely small (1–2 % of the total number of vesicles) (Schikorski and Stevens 2001; Sakaba et al. 2002; Rizzoli and Betz 2004). The vast majority of the synaptic vesicles are reserved at the presynaptic nerve terminals despite Ca^{2+} influx (Südhof 2000; Rizzoli and Betz 2005), and a subset of them is accordingly mobilized to prevent depletion of the RRP (Südhof 2000; Harata et al. 2001; Rizzoli and Betz 2005), resulting in maintaining of the RRP size. As mentioned above, tomosyn is the molecule that inactivates synaptotagmin-1 in response to Ca^{2+} influx and thereby perturbs the SNARE machinery activation (Yamamoto et al. 2010a) (Figs. 5.2b and 5.3b). Interestingly, the presynaptic interplay between tomosyn and synaptotagmin-1 controls the EPSP shape in the falling phase, enabling the neurons to respond to high-frequency action potentials (unpublished data). This result raises the possibility that the RRP size, i.e., exocytotic efficacy of release-ready synaptic vesicles, might be determined by the interaction between tomosyn and synaptotagmin-1 during arrivals of consecutive neuronal signals to the presynaptic terminal. The Ca^{2+}-dependent synaptotagmin-1-tomosyn-SNARE complex formation as depicted in Fig. 5.3b may ensure inactivation of the fusion machineries on both the donor and target membranes under high Ca^{2+} concentration accumulating with repetitive Ca^{2+} influxes to reserve the synaptic vesicles, leading to maintaining of the RRP size. By laser photolysis of caged calcium in a rat calyx of Held synapse, a rise in Ca^{2+} concentration to 1–2 μM readily evoked release (Bollmann et al. 2000; Felmy et al. 2003). Brief local Ca^{2+} rise to 10–25 μM is sufficient to achieve the amount and the kinetics of the physiological transmitter release (Schneggenburger and Neher 2005). An increase to >30 μM depleted the RRP in <0.5 ms (Bollmann et al. 2000). In addition, 40 μM is the peak concentration for synaptic vesicles at the release site during an action potential (half-width approximately 0.4 ms; Meinrenken et al. 2002). Therefore, in our scenario, the synaptotagmin-1-tomosyn-SNARE complex will be maximally formed in the high-range concentration (10–50 μM) of local Ca^{2+} rise that depletes the RRP (Meinrenken et al. 2002), in order to negatively control exocytotic efficacy of synaptic vesicle in a late phase

of transmitter release preventing the RRP depletion. Severe synaptic depression induced by repetitive action potentials in tomosyn-overexpressing neurons (Baba et al. 2005) supports that the synaptotagmin-1-tomosyn-SNARE complex strongly retains low efficacy of synaptic vesicles for exocytosis in the accumulated Ca^{2+} rise. By contrast, our in vitro Ca^{2+} titration analysis shows that the synaptotagmin-1-tomosyn-SNARE complex formation is not saturated at 10–50 μM and proceeds more as the Ca^{2+} concentration increases (unpublished data). However, this result does not debate on the physiological relevance of the synaptotagmin-1-tomosyn-SNARE complex formation. The in vitro biochemical reactions do not reconstitute the local Ca^{2+} rise as seen in neurons. It has been reported that a specific membrane lipid, PI(4,5)P2, increases Ca^{2+} affinity of synaptotagmin-1 (Radhakrishnan et al. 2009). Therefore, to address biochemically the precise dependency on Ca^{2+} concentration for the synaptotagmin-1-tomosyn-SNARE complex formation, it will be required to develop more physiologically relevant assay reflecting the local Ca^{2+} rise and the membranous environment.

5.7 Conclusions and Perspectives

The crystal structure of the N-terminal WD40 repeats of Sro7, the yeast orthologue of tomosyn, has been solved (Hattendorf et al. 2007). Based on the solved structure, Sro7 is suggested to bind to Sec9, a yeast counterpart of SNAP-25, through the N-terminal WD40 repeats and thereby inhibits the SNARE complex formation. While Sec9 binds to Sro7 through both the N-terminal region and the SNARE motifs, the N-terminal region of Sec9 is not conserved in mammalian SNAP-25 (Hattendorf et al. 2007). As far as we examined, the N-terminal WD40 repeats of tomosyn had no inhibitory activity on the SNARE complex formation. Therefore, the inhibitory activity of the N-terminal WD40 repeats of Sro7 on the SNARE complex formation may not be evolutionally conserved. On the other hand, the binding of tomosyn to synaptotagmin-1 is in good agreement with the association of tomosyn with the synaptic vesicles in *C. elegans* (McEwen et al. 2006), raising a possibility that the activity of the N-terminal WD40 repeats to inhibit the synaptotagmin-1 function might be evolutionally conserved between nematodes and mammals. The N-terminal WD40 repeats also have the activity to oligomerize the SNARE complex (Sakisaka et al. 2008). However, it remains to be elucidated whether the inhibitory activity on the synaptotagmin-1 function and the oligomerization activity on the SNARE complex are mutually exclusive or compatible. Tomosyn adopts two conformational states upon reciprocal intramolecular bindings of the tail domain (Yamamoto et al. 2009). In one conformational state where the tail domain binds to the N-terminal WD40 repeats, tomosyn potently inhibits the SNARE complex formation through the C-terminal VAMP-like domain. In the other conformational state where the tail domain binds to the C-terminal VAMP-like domain, the inhibitory activity of the C-terminal VAMP-like domain is decreased. The binding of synaptotagmin-1 to the N-terminal WD40 repeats

enhances the tomosyn-SNARE complex formation through the C-terminal VAMP-like domain (Yamamoto et al. 2010a). Therefore, synaptotagmin-1 binding may stabilize the former conformational state of tomosyn, leading to enhancement of the tomosyn-SNARE complex formation. Future structural studies of full-length tomosyn, the synaptotagmin-1-tomosyn complex, and the synaptotagmin-1-tomosyn-SNARE complex will be required to address these concerns.

References

Ashery U, Bielopolski N, Barak B, Yizhar O (2009) Friends and foes in synaptic transmission: the role of tomosyn in vesicle priming. Trends Neurosci 32:275–282

Augustine GJ (2001) How does calcium trigger neurotransmitter release? Curr Opin Neurobiol 11:320–326

Baba T, Sakisaka T, Mochida S, Takai Y (2005) PKA-catalyzed phosphorylation of tomosyn and its implication in Ca^{2+}-dependent exocytosis of neurotransmitter. J Cell Biol 170:1113–1125

Bennett MK, Calakos N, Scheller RH (1992) Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 257:255–259

Bollmann JH, Sakmann B, Borst JG (2000) Calcium sensitivity of glutamate release in a calyx-type terminal. Science 289:953–957

Chapman ER (2008) How does synaptotagmin trigger neurotransmitter release? Annu Rev Biochem 77:615–641

Chen YA, Scheller RH (2001) SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol 2:98–106

Felmy F, Neher E, Schneggenburger R (2003) The timing of phasic transmitter release is Ca^{2+}-dependent and lacks a direct influence of presynaptic membrane potential. Proc Natl Acad Sci U S A 100:15200–15205

Fernandez I, Araç D, Ubach J, Gerber SH, Shin O, Gao Y, Anderson RG, Südhof TC, Rizo J (2001) Three-dimensional structure of the synaptotagmin 1 C_2B-domain: synaptotagmin 1 as a phospholipid binding machine. Neuron 32:1057–1069

Fujita Y, Shirataki H, Sakisaka T, Asakura T, Ohya T, Kotani H, Yokoyama S, Nishioka H, Matsuura Y, Mizoguchi A, Scheller RH, Takai Y (1998) Tomosyn: a syntaxin-1-binding protein that forms a novel complex in the neurotransmitter release process. Neuron 20:905–915

Fukuda M, Kanno E, Mikoshiba K (1999) Conserved N-terminal cysteine motif is essential for homo- and heterodimer formation of synaptotagmins III, V, VI, and X. J Biol Chem 274:31421–31427

Gangar A, Rossi G, Andreeva A, Hales R, Brennwald P (2005) Structurally conserved interaction of Lgl family with SNAREs is critical to their cellular function. Curr Biol 15:1136–1142

Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Südhof TC (1994) Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. Cell 79:717–727

Gracheva EO, Burdina AO, Holgado AM, Berthelot-Grosjean M, Ackley BD, Hadwiger G, Nonet ML, Weimer RM, Richmond JE (2006) Tomosyn negatively regulates CAPS-dependent peptide release at *Caenorhabditis elegans* synapses. PLoS Biol 4:e261

Groffen AJ, Jacobsen L, Schut D, Verhage M (2005) Two distinct genes drive expression of seven tomosyn isoforms in the mammalian brain, sharing a conserved structure with a unique variable domain. J Neurochem 92:554–568

Harata N, Pyle JL, Aravanis AM, Mozhayeva M, Kavalali ET, Tsien RW (2001) Limited numbers of recycling vesicles in small CNS nerve terminals: implications for neural signaling and vesicular cycling. Trends Neurosci 24:637–643

Hatsuzawa K, Lang T, Fasshauer D, Bruns D, Jahn R (2003) The R-SNARE motif of tomosyn forms SNARE core complexes with syntaxin 1 and SNAP-25 and down-regulates exocytosis. J Biol Chem 278:31159–31166
Hattendorf DA, Andreeva A, Gangar A, Brennwald PJ, Weis WI (2007) Structure of the yeast polarity protein Sro7 reveals a SNARE regulatory mechanism. Nature 446:567–571
Hui E, Johnson CP, Yao J, Dunning FM, Chapman ER (2009) Synaptotagmin-mediated bending of the target membrane is a critical step in Ca(2^+)-regulated fusion. Cell 138:709–721
Jahn R, Scheller RH (2006) SNAREs–engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–643
Kagami M, Toh-e A, Matsui Y (1998) Sro7p, a *Saccharomyces cerevisiae* counterpart of the tumor suppressor l(2)gl protein, is related to myosins in function. Genetics 149:1717–1727
Kloepper TH, Kienle CN, Fasshauer D (2008) SNAREing the basis of multicellularity: consequences of protein family expansion during evolution. Mol Biol Evol 25:2055–2068
Lehman K, Rossi G, Adamo JE, Brennwald P (1999) Yeast homologues of tomosyn and lethal giant larvae function in exocytosis and are associated with the plasma membrane SNARE, Sec9. J Cell Biol 146:125–140
Ma H, Cai Q, Lu W, Sheng ZH, Mochida S (2009) KIF5B motor adaptor syntabulin maintains synaptic transmission in sympathetic neurons. J Neurosci 29:13019–13029
Martens S, Kozlov MM, McMahon HT (2007) How synaptotagmin promotes membrane fusion. Science 316:1205–1208
McEwen JM, Madison JM, Dybbs M, Kaplan JM (2006) Antagonistic regulation of synaptic vesicle priming by Tomosyn and UNC-13. Neuron 51:303–315
Meinrenken CJ, Borst JG, Sakmann B (2002) Calcium secretion coupling at calyx of held governed by nonuniform channel-vesicle topography. J Neurosci 22:1648–1667
Müsch A, Cohen D, Yeaman C, Nelson WJ, Rodriguez-Boulan E, Brennwald PJ (2002) Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. Mol Biol Cell 13:158–168
Pobbati AV, Razeto A, Boddener M, Becker S, Fasshauer D (2004) Structural basis for the inhibitory role of tomosyn in exocytosis. J Biol Chem 279:47192–47200
Radhakrishnan A, Stein A, Jahn R, Fasshauer D (2009) The Ca^{2+} affinity of synaptotagmin 1 is markedly increased by a specific interaction of its C2B domain with phosphatidylinositol 4,5-bisphosphate. J Biol Chem 284:25749–25760
Rizo J, Rosenmund C (2008) Synaptic vesicle fusion. Nat Struct Mol Biol 15:665–674
Rizzoli SO, Betz WJ (2004) The structural organization of the readily releasable pool of synaptic vesicles. Science 303:2037–2039
Rizzoli SO, Betz WJ (2005) Synaptic vesicle pools. Nat Rev Neurosci 6:57–69
Sakaba T, Schneggenburger R, Neher E (2002) Estimation of quantal parameters at the calyx of Held synapse. Neurosci Res 44:343–356
Sakisaka T, Baba T, Tanaka S, Izumi G, Yasumi M, Takai Y (2004) Regulation of SNAREs by tomosyn and ROCK: implication in extension and retraction of neurites. J Cell Biol 166:17–25
Sakisaka T, Yamamoto Y, Mochida S, Nakamura M, Nishikawa K, Ishizaki H, Okamoto-Tanaka-M, Miyoshi J, Fujiyoshi Y, Manabe T, Takai Y (2008) Dual inhibition of SNARE complex formation by tomosyn ensures controlled neurotransmitter release. J Cell Biol 183:323–337
Schikorski T, Stevens CF (2001) Morphological correlates of functionally defined synaptic vesicle populations. Nat Neurosci 4:391–395
Schneggenburger R, Neher E (2005) Presynaptic calcium and control of vesicle fusion. Curr Opin Neurobiol 15:266–274
Shao X, Fernandez I, Südhof TC, Rizo J (1998) Solution structures of the Ca^{2+}-free and Ca^{2+}-bound C_2A domain of synaptotagmin I: does Ca^{2+} induce a conformational change? Biochemistry 37:16106–16115
Söllner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE (1993) A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75:409–418

Stein A, Radhakrishnan A, Riedel D, Fasshauer D, Jahn R (2007) Synaptotagmin activates membrane fusion through a Ca^{2+}-dependent trans interaction with phospholipids. Nat Struct Mol Biol 14:904–911

Südhof TC (2000) The synaptic vesicle cycle revisited. Neuron 28:317–320

Südhof TC (2004) The synaptic vesicle cycle. Annu Rev Neurosci 27:509–547

Sutton RB, Davletov BA, Berghuis AM, Südhof TC, Sprang SR (1995) Structure of the first C_2 domain of synaptotagmin I: a novel Ca^{2+}/phospholipid-binding fold. Cell 80:929–938

Sutton RB, Fasshauer D, Jahn R, Brunger AT (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. Nature 395:347–353

Trimble WS, Cowan DM, Scheller RH (1988) VAMP-1: a synaptic vesicle-associated integral membrane protein. Proc Natl Acad Sci U S A 85:4538–4542

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

Wirtz-Peitz F, Knoblich JA (2006) Lethal giant larvae take on a life of their own. Trends Cell Biol 16:234–241

Xue M, Ma C, Craig TK, Rosenmund C, Rizo J (2008) The Janus-faced nature of the C_2B domain is fundamental for synaptotagmin-1 function. Nat Struct Mol Biol 15:1160–1168

Yamamoto Y, Mochida S, Kurooka T, Sakisaka T (2009) Reciprocal intramolecular interactions of tomosyn control its inhibitory activity on SNARE complex formation. J Biol Chem 284:12480–12490

Yamamoto Y, Mochida S, Miyazaki N, Kawai K, Fujikura K, Kurooka T, Iwasaki K, Sakisaka T (2010a) Tomosyn inhibits synaptotagmin-1-mediated step of Ca^{2+}-dependent neurotransmitter release through its N-terminal WD40 repeats. J Biol Chem 285:40943–40955

Yamamoto Y, Fujikura K, Sakaue M, Okimura K, Kobayashi Y, Nakamura T, Sakisaka T (2010b) The tail domain of tomosyn controls membrane fusion through tomosyn displacement by VAMP2. Biochem Biophys Res Commun 399:24–30

Yamanaka T, Ohno S (2008) Role of Lgl/Dlg/Scribble in the regulation of epithelial junction, polarity and growth. Front Biosci 13:6693–6707

Yizhar O, Matti U, Melamed R, Hagalili Y, Bruns D, Rettig J, Ashery U (2004) Tomosyn inhibits priming of large dense-core vesicles in a calcium-dependent manner. Proc Natl Acad Sci U S A 101:2578–2583

Yizhar O, Lipstein N, Gladycheva SE, Matti U, Ernst SA, Rettig J, Stuenkel EL, Ashery U (2007) Multiple functional domains are involved in tomosyn regulation of exocytosis. J Neurochem 103:604–616