Review

Friends and foes in synaptic transmission: the role of tomosyn in vesicle priming

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Priming is the process by which vesicles become available for fusion at nerve terminals and is modulated by numerous proteins and second messengers. One of the prominent members of this diverse family is tomosyn. Tomosyn has been identified as a syntaxin-binding protein; it inhibits vesicle priming, but its mode of action is not fully understood. The inhibitory activity of tomosyn depends on its N-terminal WD40-repeat domain and is regulated by the binding of its SNARE motif to syntaxin. Here, we describe new physiological information on the function of tomosyn and address possible interpretations of these results in the framework of the recently described crystal structure of the yeast tomosyn homolog Sro7. We also present possible molecular scenarios for vesicle priming and the involvement of tomosyn in these processes.

Vesicle priming as a key process in synaptic transmission

Synaptic transmission at the nerve terminal involves several steps that lead to the timely and controlled release of neurotransmitter. After the arrival and docking of a vesicle at the plasma membrane (PM), it undergoes a series of maturation steps that render it fusion competent. These steps are collectively known as the vesicle priming process and are crucial for vesicle fusion. Priming is coordinated by a series of protein–protein interactions that occur between cytosolic, vesicular and PM proteins [1–3]. A key step in vesicle priming is the formation of multiple heterotrimeric SNARE complexes between the PM SNARE proteins syntaxin1a and synaptosome-associated protein 25 kDa (SNAP-25) and the vesicular protein synaptobrevin (also known as VAMP2) [4–8]. Full assembly of the SNARE complex is thought to bring the vesicle into close apposition with the PM and can catalyze the fusion reaction [9]. The priming process is highly regulated by several accessory proteins such as Munc18, Munc13, tomosyn, rabphilin and complexin. Changes in the levels of some synaptic proteins alter specific steps in synaptic transmission and, as a consequence, affect synaptic plasticity processes [10–14]. All of these proteins interact with at least one member of the SNARE protein family. The purpose of this review is to discuss the priming process and the effect of tomosyn on vesicle priming and synaptic transmission.

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Tomosyn

Tomosyn was first discovered as a syntaxin1a-binding protein in a pulldown assay from rat cerebral cytosol and, accordingly, received its Japanese name: *tomo* ('friend' in Japanese) of *syn* (syntaxin) [15]. It is expressed in the brain and colocalizes with syntaxin in synapse-forming regions such as cerebellar molecular layers, but it is also found in nonsynaptic regions [15]. Tomosyn is partially colocalized with syntaxin1, synaptophysin and bassoon in cultured superior cervical ganglion (SCG) [16] and with synaptobrevin in *Caenorhabditis elegans* [17], indicating presynaptic localization. Tomosyn is known as a cytosolic protein but has also been found associated with synaptic vesicles [15,17,18] and other organelles such as insulin-containing granules [19,20], and it also localizes to the PM through its interaction with syntaxin [21,22]. Tomosyn has three distinct domains (Figure 1): a C-terminal region containing an R-SNARE, synaptobrevin-like coiled-coil domain; an N-terminal region enriched with WD40 repeats that are predicted to fold into a propeller-like structure, and a hypervariable linker domain that differs between several splice variants [23–25]. According to recent alignment with Sro7, the yeast tomosyn homolog, tomosyn might fold into a twin β-propeller-like structure and the hypervariable linker is predicted to be a part of the C-terminal propeller [26] (Figure 1).

Two mammalian tomosyn genes were identified, encoding tomosyn-1 and tomosyn-2. Tomosyn-1 is alternatively spliced into three distinct isoforms and tomosyn-2 [23] has four different splice variants [23,25]. The s-tomosyn and m-tomosyn isoforms are brain specific [25] and are enriched in synapse-specific domains, indicating a role in synaptic transmission, whereas b-tomosyn is ubiquitously expressed [25] and has been shown to have a role in regulated exocytosis in non-neuronal cell types [22]. The transcription of tomosyn-2 is steeply upregulated during development: its expression increases 30-fold between E10 and P12, following a pattern similar to syntaxin and Munc18. mRNA of tomosyn-1, by contrast, increases only by a factor of 2.7 during this period [23]. Although the antibodies used in immunohistochemical staining on brain slices cannot distinguish among the isoforms, one can speculate that, whereas tomosyn-2 is involved in specific restriction of vesicle fusion during synapse formation, tomosyn-1 has a more general role, acting as a regulator of axon elongation [23,27]. At the synapses, their expression also differs, indicating a similar function at different

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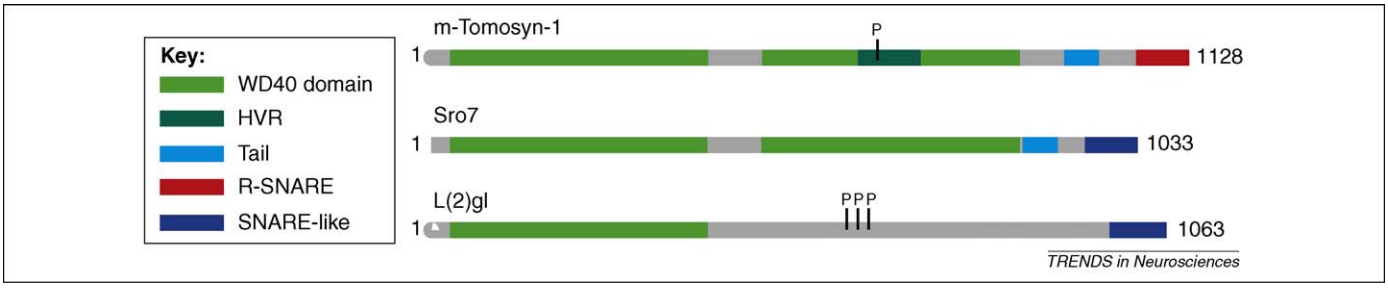

Figure 1. Domain structure of tomosyn and its homologs. The N terminus of all mammalian tomosyn isoforms is enriched with WD40 repeats that are predicted to fold into an N-terminal propeller-like structure and a C-terminal propeller-like structure (light green). The C terminus of all tomosyn isoforms contains an R-SNARE, which is a synaptobrevin-like coiled-coil domain (red). Mouse m-tomosyn is shown as a representative of all tomosyn isoforms. A SNARE-like domain exists in Sro7 and in L(2)gl (dark blue). A hypervariable domain (HVR; dark green) is part of the putative C-terminal propeller-like structure and varies in length between the different splice variants (only the m-tomosyn isoform is shown here). The PKA phosphorylation site in tomosyn and the three aPKC phosphorylation sites in L(2)gl are labeled with P. Sro7 has two β-propeller structures (green). The first and the last amino acids are labeled for each of the proteins.

synapses [23]. However, all of the information known to date relates to tomosyn-1, and the role of tomosyn-2 is not known.

Physiological effects of tomosyn

Tomosyn was indicated to displace Munc18 from syntaxin and form a novel four-protein complex consisting of syntaxin, SNAP-25 and the calcium sensor synaptotagmin [15]. Munc18 displacement was thought to release the inhibition imposed by Munc18 on syntaxin, and the new four-protein complex was hypothesized to be an intermediate complex before the formation of the SNARE core complex [15,28]. According to this scenario, tomosyn would be expected to have a catalytic role in neurotransmitter release. However, in this original study, tomosyn was already observed to inhibit exocytosis from PC12 cells [15] and recent work has shown that Munc18 does not inhibit exocytosis and is actually required for proper synaptic transmission [29–32]. In addition, synaptobrevin was not able to displace tomosyn from the SNARE core complex [24], indicating that if indeed the tomosyn–SNARE complex is formed under physiological conditions it might constitute a 'dead-end' complex that titrates out functional SNARE proteins [24,33]. Subsequent studies demonstrated that tomosyn causes a substantial reduction in exocytosis in several neurosecretory cells and in neurons [15,20–22,33–37]. Complementary support came from a series of genetic studies in *C. elegans* that demonstrated that in two different mutants in which tom-1, the tomosyn homolog, was disrupted neurotransmitter release was enhanced, giving rise to prolonged asynchronous, late release [38,39]. Mechanistically, it was shown that tomosyn inhibits the priming step in both neuroendocrine cells and neurons [16,17,37,39].

The effects of tomosyn on vesicle mobility were recently investigated using total internal reflection fluorescence microscopy [36]. Tomosyn overexpression was shown to change the vesicle dynamics near to the PM: it attenuated the immobilization of newly arriving vesicles, which caused an increase in vesicle mobility and a reduction in the time the vesicles remain near the PM. In addition, tomosyn reduced the release of already-immobile vesicles. These findings indicated that the inhibition of priming is mediated, in part, by attenuation of the immobilization of newly arriving vesicles and by a reduction in the release probabilities of docked vesicles [36].

The role of the endogenous mammalian tomosyn was investigated using silencing techniques and recently by investigating the phenotype of tomosyn-1 knockout mice. However, these studies yielded conflicting results: according to the notion that tomosyn inhibits priming and exocytosis, one would predict that deletion of tomosyn should enhance exocytosis. However, tomosyn knockdown in SCG neurons reduced synaptic transmission, similar to tomosyn overexpression in these cells [16]. Likewise, downregulation of tomosyn reduced insulin secretion from an insulin-secreting INS-1E cell line [19] but enhanced exocytosis in mouse β-cells [20]. In a recent study the phenotype of the first mouse tomosyn-1 mutant was described. In agreement with the overexpression experiments and the *C. elegans* tom-1 mutants, deletion of tomosyn-1 in these mice caused enhanced synaptic transmission and reduced paired pulse facilitation [40]. These data support the hypothesis that tomosyn has an inhibitory role and, therefore, deletion of tomosyn-1 enhances the release probability [37].

It has previously been suggested that tomosyn has a positive, in addition to the established negative, role in neurotransmitter release. This is supported by the finding that after intense stimulation tomosyn-overexpressing cells exhibited enhanced late secretion; this was evident as an enhanced sustained component during long flash stimulation in chromaffin cells [37,41] and as enhanced asynchronous release during high-frequency stimulation in SCG neurons [16]. It is possible that under high calcium conditions the inhibitory effect of tomosyn is reduced or that its permissive effects are enhanced. In adipocytes, b-tomosyn and Munc18c could interact simultaneously with syntaxin4; this complex has been suggested to prime syntaxin on the PM, providing further support for a positive role for tomosyn [22]. Therefore, although tomosyn is thought to play a negative part, there is some indication of a second, permissive role that still needs to be evaluated.

Structural domains of tomosyn and modes of action

Tomosyn is classified as an R-SNARE protein because the C terminus of all of its isoforms contains a coiled-coil domain, including 16 fully conserved hydrophobic amino acids and the conserved arginine in layer 0 of the central heptad repeat of the α-helix [24,28,33,42]. This domain shows very high homology to synaptobrevin and serves as the main high-affinity interaction site with syntaxin1a

[24,28,33]. The tomosyn coiled-coil domain also binds to SNAP-23 and syntaxin4 [22,28] and forms a stable core SNARE complex *in vitro* with SNAP-25 and syntaxin1a [24,33]. It has been suggested that full-length tomosyn binds to both SNAP-25 and syntaxin1a on the PM only in the presence of its SNARE domain [21].

The tomosyn–SNAP-25–syntaxin1a complex is very similar in its biophysical and structural properties to the SNARE complex containing synaptobrevin and it is formed at a similar rate [19,24,33]. Both complexes disassemble only with the addition of *N*-ethylmaleimide-sensitive factor (NSF) although the tomosyn complex is not resistant to SDS [24,33] and it is slightly weaker than the complex with synaptobrevin, as determined by atomic force microscopy [19]. The main difference between the structures of the tomosyn and neuronal SNARE complexes is found on the surface, where binding of complexin is not detected in the tomosyn complex [24].

These findings support the hypothesis that tomosyn, when highly abundant, inhibits vesicle fusion through the interaction of its R-SNARE domain with syntaxin. This interaction forms non-fusogenic SNARE complexes and prevents synaptobrevin from entering the SNARE complexes. However, a tomosyn mutant that lacks its SNARE domain [41] or a mutant that has a reduced affinity for syntaxin [34] still inhibit exocytosis, albeit to a lesser extent. These data indicate that the SNARE motif is not the sole mediator of the inhibitory activity of tomosyn. Nevertheless, its presence enhances the inhibitory effect of tomosyn after lysophosphatidic acid (LPA) activation (which enhances the interaction with syntaxin) [21]. Thus, this domain clearly has a role in tomosyn inhibition and can boost the activity of tomosyn under certain conditions. This interaction can be modulated by other tomosyn domains, given that the full-length tomosyn binds to syntaxin more efficiently than the SNARE domain alone [24,25]. Similarly, the ability of the C-terminal domain of Sro7 to bind to Sec9 (the yeast SNAP-25 homolog) is enhanced by the presence of the N-terminal WD40 region [26,43,44]. Taken together, this indicates that regions upstream of the SNARE motif of tomosyn are involved in syntaxin binding [24].

The WD40 motifs present in the N-terminal region of tomosyn (Figure 1) represent the most highly conserved region among tomosyn homologs [24]. WD40 repeats consist of a conserved core of ~40 amino acids that typically starts with the dipeptides GH (glycine-histidine) and ends with the dipeptides WD (tryptophan-aspartic acid). A stretch of 11–24 residues separates adjacent repeat motifs. Each WD repeat forms a single blade in a β-propeller structure and the number of repeats determines the number of blades in the protein (typically between five to seven repeats) [45,46]. The N-terminal region of tomosyn shares a high degree of similarity with other WD40-repeat proteins such as the *Drosophila melanogaster* protein [47], the mammalian Lgl homolog [48] and Sro7/Sro77 in yeast [44,49] (Figure 1). Lgl is a tumor-suppressor protein [50] that belongs to a family of cytoskeletal regulators associated with the direction of vesicle transport [51]. Sro7/77 proteins are involved in polarized secretion in yeast and deletion of Sro7/77 blocks exocytosis [44]. These data indicate a role in exocytosis and cell polarity for these homologs.

According to recent alignment with Sro7, tomosyn was predicted to have up to 14 WD40 repeats [26] and might similarly fold into a double β-propeller-like structure. This structure would then comprise both the N-terminal part of tomosyn (already predicted to fold into a propeller-like structure) and the hypervariable linker that might be part of the second, C-terminal β-propeller (Figure 1). The contribution of these domains, which constitute 90% of the protein, has not been studied extensively. Recently, disruption of the WD40 domain has been shown to reduce tomosyn inhibition, both in chromaffin cells expressing a mutant that lacks parts of N terminus of tomosyn [41] and in *C. elegans*, in which a tom-1 mutant lacking part of its WD40 region showed enhanced neurotransmitter release [38]. Interestingly, the minimal domain necessary for tomosyn inhibition includes both the WD40 domain and the hypervariable linker region [41], and this domain is sufficient to target tomosyn to the synapse [17]. Thus, if tomosyn is folded into two propellers, both are required for its inhibitory activity, given that disrupting either one of them abolishes its inhibitory effects [41].

How does this domain function? Despite the importance of the N-terminal WD40 repeats, the protein–protein interactions of the tomosyn N terminus remain elusive. Interestingly, in the *Drosophila* lethal(2)giant larvae [l(2)gl] protein, the region responsible for binding to non-muscle myosin II, also possesses a high degree of homology with the WD40-repeat region of tomosyn [47]. Moreover, the yeast tomosyn homologs Sro7/77 were shown to bind to yeast Myo1p and Myo2p (equivalent to non-muscle myosin II and myosin V in mammalian cells) [49]. Note that myosin II and myosin V have recently been suggested to be involved in a post-docking step in chromaffin cells and neurons [52–58]. It is, therefore, possible that the N-terminal domain of tomosyn interacts with cytoskeletal proteins and modulates exocytosis through these interactions; one possible scenario might be that the N-terminal domain of tomosyn inhibits exocytosis by interfering with the syntaxin–myosin interaction, which is important for exocytosis [56]. It was recently demonstrated that the tomosyn WD40 domain interacts directly with SNAP-25 and syntaxin, enhances the oligomerization of SNARE complexes and inhibits synaptic transmission [40]. This study indicated a dual inhibitory role for tomosyn: both its C-terminal coiled-coil domain and its N-terminal WD40 domain interact with syntaxin and SNAP-25 and inhibit the formation of productive SNARE complexes: the C-terminal coiled-coil domain by binding to syntaxin and SNAP-25, and the N-terminal WD40 domain by the formation of SNARE complex oligomers [40].

In Sro7, the N terminus of the WD40 domain contains a hydrophilic binding site for the C-terminal 'tail' of the protein [26]. It was suggested that Sro7 exists in two conformational states with different affinities for the C-terminal tail. The 'open' conformation has a lower affinity for the C-terminal 'tail' and enables binding of the SNARE motifs of Sec9, and this can regulate the availability of Sec9 to create SNARE complexes. The equilibrium between these states might be affected by other factors that

regulate Sro7 activity. Tomosyn seems to retain the regulatory tail, similarly to Sro7, and might possess an equivalent regulatory mechanism that controls syntaxin or SNAP-25 availability and vary according to presence of factors that control the C-terminal tail. Evidence for such intra-molecular regulation was recently reported [40].

Modulation of the interaction of tomosyn and function by second messengers

The interaction of tomosyn with syntaxin is modulated by several mechanisms. Nicotinic stimulation activates Rho-associated serine/threonine kinase (ROCK) and stimulates the formation of tomosyn–syntaxin complexes [27] at the PM [21]. Further activation of ROCK by LPA enhances the inhibitory effect of tomosyn, and this additional inhibition depends on the SNARE domain of tomosyn [21], indicating that the SNARE-domain–syntaxin interaction is important for tomosyn inhibition. RhoA is associated with large dense-core vesicles (LDCVs) and activation of G protein on the LDCVs inhibits the priming step [59,60]. Therefore, one possible scenario is that this inhibition is mediated through the syntaxin–tomosyn complex. Another level of regulation might stem from the activity of NSF. Inhibition of NSF activity enhances the tomosyn–syntaxin interaction on the PM [21] but causes displacement of b-tomosyn from the adipocyte PM [22]. Hence, coordinated activity of NSF and LPA can change the binding and unbinding kinetics of tomosyn with syntaxin–SNAP-25 complexes and control the activity of tomosyn [21,22]. The time course of ROCK activation and tomosyn–syntaxin complex formation is on the order of minutes [21]. Thus, it is reasonable to assume that this interaction does not affect the release of already-primed vesicles but becomes more relevant during intense neuronal activity when it modulates vesicle recruitment and the degree of vesicular pool refilling and can contribute to short-term plasticity.

A third tier of regulation involves phosphorylation of tomosyn. Tomosyn has been shown to be directly phosphorylated by protein kinase A (PKA) both *in vivo* and *in vitro* within the hypervariable linker region at Ser724 [16]. This phosphorylation reduces the interaction between tomosyn and syntaxin, possibly contributing to the effects of PKA on synaptic release in neurons. Although non-phosphorylated tomosyn inhibits release more strongly than a phosphomimetic mutant of tomosyn, wild-type tomosyn has an effect similar to that of the phosphomimetic mutant [16], shedding doubt on the functional importance of the regulation of tomosyn by PKA in the synapse (see also Ref. [40]). The Lgl protein is phosphorylated by atypical protein kinase C (aPKC) in a region that links the N- and C-terminal regions of the protein [61], similar to the PKA phosphorylation site in tomosyn [16]; this phosphorylation enables an intramolecular interaction between the two halves of the protein and inhibits its interaction with myosin II [61]. Such an auto-inhibitory intramolecular interaction might also occur in tomosyn, serving as an on/off switch. However, based only on structured-based alignment with Sro7 [26,62], the phosphorylation site is found in a loop within the second WD40 propeller and its phosphorylation might not have the ability to regulate an interaction between the two halves of the protein. Nevertheless, the tail domain of Sro7 has an auto-inhibitory effect on Sro7 binding to Sec9 [26] and, because the tail is conserved in tomosyn, similar auto-inhibitory regulation might occur also in tomosyn.

Mechanisms of docking and priming: interplay between Munc13, tomosyn and syntaxin

Recent findings have revealed that manipulations of M-unc13, tomosyn and syntaxin also considerably affect vesicle docking [14,36,39,63–65]. Deletion of unc13 or syntaxin causes a reduction in vesicle docking in *C. elegans* [64,66] and chromaffin cells [63]. Tomosyn knockdown also causes redistribution of synaptic vesicles at the *C. elegans* neuromuscular junction [39], whereas tomosyn overexpression enhances the turnover rate of vesicles near the PM in chromaffin cells [36]. How can we explain these data? Munc13 is suggested to stabilize the open form of syntaxin. In agreement, an unc13 docking defect is rescued by overexpression of an open form of syntaxin, indicating that an open syntaxin molecule is needed for vesicle docking and for vesicle priming [67] in some synapses. Furthermore, the morphological phenotype of unc13 mutant animals is also rescued by crossing with a tom-1 mutant [66]. However, functionally, these synapses are only partially rescued and only crossing unc13 and tom-1 mutants with an open syntaxin mutant improves animal mobility compared with the unc13 and open syntaxin mutant [17]. These findings place syntaxin in a central position in both vesicle docking and priming, and tomosyn and unc13 can act through syntaxin in an antagonistic manner. These data also indicate that docking and priming are molecularly interlinked and can be regarded as successive steps in the protein–protein interactions [68], leading to the formation of assembled SNARE complexes and fusion [14].

Mechanistically, if equilibrium between Munc13 and tomosyn exists [14], any perturbation of this homeostasis will change the availability of syntaxin. Thus, in the absence of unc13, tomosyn can prevent syntaxin from forming SNARE complexes. Accordingly, Munc13 might prevent tomosyn from forming complexes with SNARE proteins, or alternatively it can facilitate the disassembly of the non-fusogenic tomosyn–SNARE complexes. Moreover, the absence of tomosyn causes accumulation of unc13 at release sites [17]. Hence, changes in tomosyn expression levels might constitute a mechanism that modulates release probability and synaptic strength. A caveat to this assumption is that the level of tomosyn is only 3% of that of syntaxin [15] and under physiological conditions it seems unlikely that this low level could have such a profound effect. These findings can be reconciled if we assume that tomosyn is concentrated in the active zone and at release sites (for example in hot spots or syntaxin clusters; Figure 2) [69], similar to the accumulation of syntaxin and tomosyn in growth cones [27].

The possible modes of action of tomosyn

Tomosyn can be envisioned as functioning in two steps: the tomosyn SNARE motif could serve as a spatial signaling marker recruiting tomosyn to specific sites on the PM that are enriched with syntaxin, and this interaction itself could inhibit SNARE-complex formation and priming

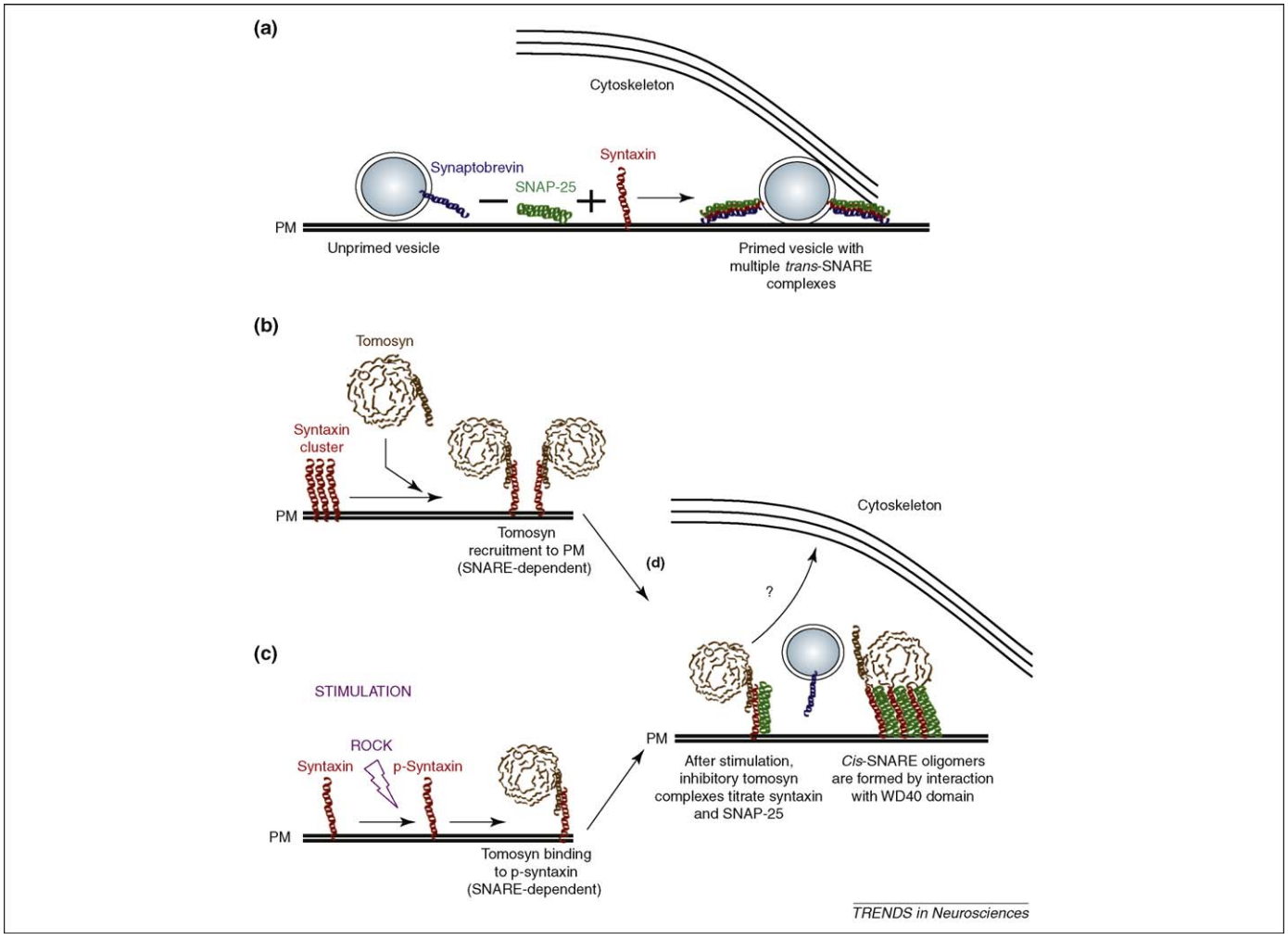

Figure 2. A putative model for the inhibitory activity of tomosyn under resting conditions and after stimulation. **(a)** Under resting conditions, a docked, unprimed vesicle forms *trans*-SNARE complexes via interaction of its membrane-bound synaptobrevin with PM-bound syntaxin and SNAP-25 to form multiple SNARE complexes. This process renders the vesicle fusion competent (primed) and it can then fuse upon elevation of $[Ca^{2+}]_i$. **(b)** Tomosyn is recruited through its SNARE motif to areas on the PM enriched with syntaxin molecules and inhibits SNARE-complex formation. (**c**) After intense stimulation, ROCK phosphorylates syntaxin at Ser14 and this increases the interaction between tomosyn and phosphorylated syntaxin, and more tomosyn molecules are recruited to the membrane through binding to phosphorylated syntaxins. (**d**) Dual inhibition by tomosyn. In addition to the syntaxin–tomosyn interaction, the WD domain enhances oligomerization of *cis*-SNARE complexes and further reduces vesicle priming. The WD domain might also interact with the cytoskeleton (marked on the figure with a '?'). Thus, under tomosyn overexpression or upon specific stimulation, tomosyn can impose greater inhibition by forming complexes with SNAP-25 and syntaxin or by enhancing oligomerization of *cis*-SNARE complexes, limiting the amount of uncomplexed PM-bound SNAREs and, therefore, reducing the formation of *trans*-SNARE complexes. For clarity, only the SNARE domain of syntaxin is shown.

(Figure 2b). Then, the WD40 domain with the hypervariable linker region, either through oligomerization of *cis*-SNARE complexes or via interaction with myosin and/or cytoskeleton, could further control the inhibition of tomosyn at the PM (Figure 2d). Similarly, Hattendorf *et al.* [26] suggest that Sro7 interaction with the cytoskeleton is coupled with displacement of the C-terminal tail from its N-terminal binding site. In this partially open conformation, Sro7 could both recruit Sec9 to the fusion sites at the membrane and inhibit binding to other SNAREs [26].

Tomosyn can affect both vesicle priming and turnover if we assume that it regulates the transition of morphologically docked vesicles into a functionally docked state (Figure 3): vesicles undergo morphological docking or tethering through interactions with subcortical cytoskeleton or other proteins such as the exocyst complex [70] or Munc18 [31] (Figure 3, step 1). At this stage, their mobility near the PM is still high. Once a vesicle has formed a single *trans*-SNARE complex, which represents the first step in the priming process, its mobility decreases (Figure 3, step 2). Additional SNARE complexes are formed once the vesicle is immobilized, progressing vesicle priming without affecting its mobility [36] (Figure 3, step 3). Tomosyn could interfere with steps 2 or 3, both representing the formation of SNARE complexes [36]. Interfering with the formation of the first SNARE complex (Figure 3, step 2) would attenuate vesicle immobilization and increase the turnover rate of newly arriving vesicles. Interfering with the formation of subsequent SNARE complexes (Figure 3, step 3) would decrease the release probability of already immobile, resident vesicles [36]. Hence, under tomosyn overexpression, vesicles are bound by fewer SNARE complexes and are, therefore, less fusion competent.

Tomosyn possesses multiple functional domains that can regulate its activity. The interaction with syntaxin through its SNARE domain is dynamically regulated. We can speculate that, because the surface of the tomosyn–syntaxin–SNAP-25 complex differs from that of the

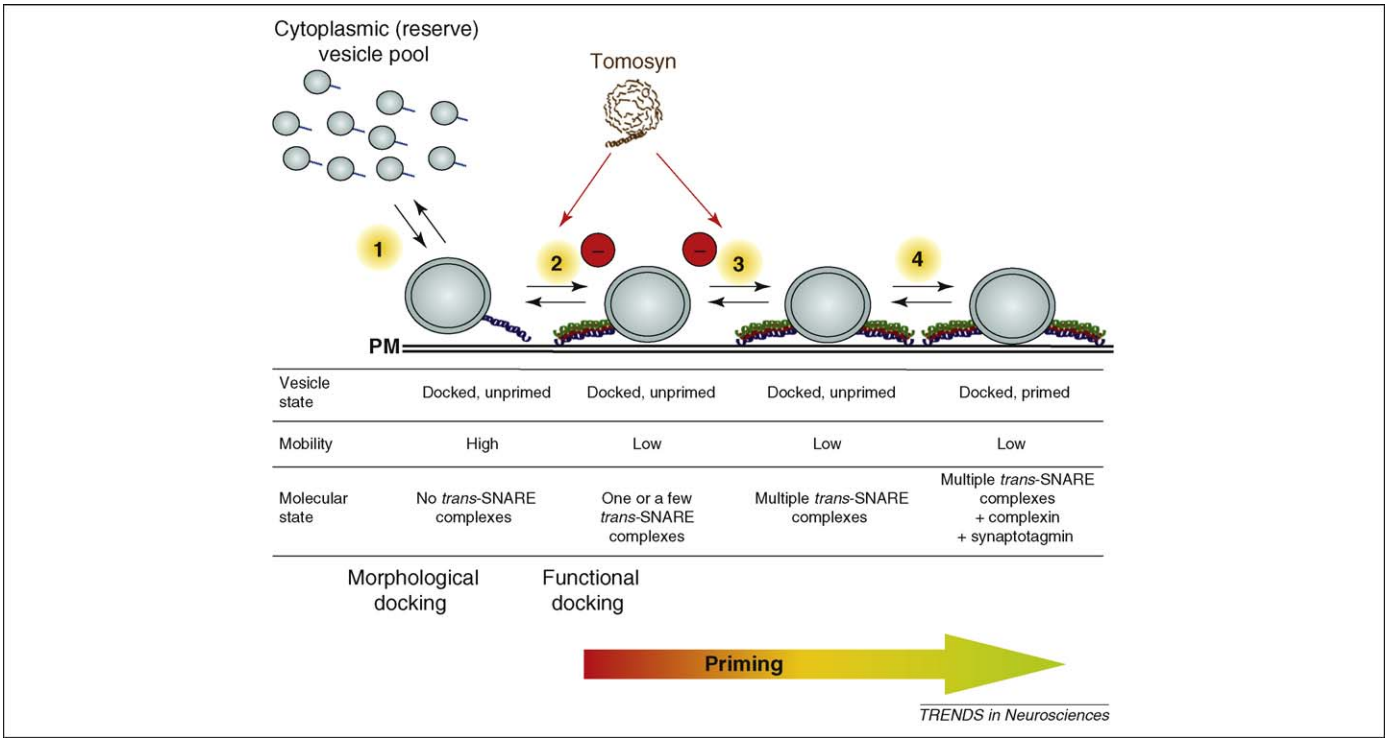

Figure 3. Schematic representation of vesicle docking and priming, and the effects of tomosyn on vesicle mobility and turnover. Vesicles undergo morphological docking or tethering through interactions with the subcortical cytoskeleton or with other proteins, such as the exocyst complex or Munc18 (step 1). After the formation of the first *trans*-SNARE complex, the mobility of vesicle decreases (step 2). This represents the first step in the priming process and the formation of a functional docked vesicle. Additional SNARE complexes are formed once the vesicle is immobilized, without affecting its mobility (step 3). Upon binding of accessory proteins such as complexin and/or synaptotagmin, priming is complete and the vesicle becomes fusion competent (step 4). Note that the priming process consists of several steps until the vesicle becomes fully fusion competent. Steps 2 and 3 involve an identical molecular process, the formation of *trans*-SNARE complexes, although step 2 involves a decline in vesicle mobility and step 3 does not. Tomosyn can interfere with the formation of SNARE complexes in steps 2 or 3. Interfering with step 2 will increase the turnover rate of newly arriving vesicles and inhibit their immobilization. Interfering with step 3 will reduce the fusion competence of the vesicles and decrease its release probability. Blue helix, synaptobrevin; green–red–blue helixes, SNARE complex.

synaptobrevin–syntaxin–SNAP-25 SNARE complex, it can interact with other proteins involved in exocytosis [24], providing another tier of regulation. The entire N-terminal WD40 domain and linker form an integral functional domain that is essential for the inhibitory activity of tomosyn [41] and might fold into a twin β-propeller structure similar to Sro7 [26]. This domain could interact with cytoskeletal elements [47,49] or with as yet unidentified synaptic proteins (e.g. exocyst protein Exo84 has been shown to interact with Sro7/77 [70]) and might also regulate the syntaxin–tomosyn interaction [23,24,26,33,41] by serving as an intramolecular switch. In addition, the WD40 repeats can control SNARE-complex oligomerization and provide another level of regulation of synaptic transmission. The ability of tomosyn to enhance the formation of SNARE oligomers as part of its inhibitory activity indicates that these oligomers should be non-productive, probably *cis*-SNARE, complexes. Hence, both its SNARE motif interaction with syntaxin and its WD40-repeat-induced SNARE oligomerization act to reduce the availability or the formation of *trans*-SNARE complexes that are essential for fusion. We are still lacking essential information as to whether other proteins, beside the SNARE proteins, interact with tomosyn and how they affect its activity. Finally, we need to gain a better understanding of the exact molecular mechanisms by which tomosyn operates and its involvement in various synaptic plasticity processes and whether tomosyn-1 and tomosyn-2 act similarly. Nevertheless, it is clear that tomosyn functions at a central stage of the vesicle maturation process in concert with Munc13 and syntaxin and can fine-tune synaptic strength via various types of mechanisms.

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