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Review

Plasma membrane targeting of exocytic SNARE proteins

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Abstract

SNARE proteins play a central role in the process of intracellular membrane fusion. Indeed, the interaction of SNAREs present on two opposing membranes is generally believed to provide the driving force to initiate membrane fusion. Eukaryotic cells express a large number of SNARE isoforms, and the function of individual SNAREs is required for specific intracellular fusion events. Exocytosis, the fusion of secretory vesicles with the plasma membrane, employs the proteins syntaxin and SNAP-25 as plasma membrane SNAREs. As a result, exocytosis is dependent upon the targeting of these proteins to the plasma membrane; however, the mechanisms that underlie trafficking of exocytic syntaxin and SNAP-25 proteins to the cell surface are poorly understood. The intracellular trafficking itinerary of these proteins is particularly intriguing as syntaxins are tail-anchored (or Type IV) membrane proteins, whereas SNAP-25 is anchored to membranes via a central palmitoylated domain—there is no common consensus for the trafficking of such proteins within the cell. In this review, we discuss the plasma membrane targeting of these essential exocytic SNARE proteins.
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Keywords: SNARE; Syntaxin; SNAP-25; Exocytosis; Membrane fusion; Munc18

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1. Introduction

Intracellular membrane fusion is essential for numerous cellular processes, including the maintenance of cellular architecture, cell division, and secretion. The fidelity of membrane fusion depends upon the coordinated actions of a wide range of cellular proteins, and the targeting of these proteins to specific cellular compartments. The fusion of intracellular vesicles with the plasma membrane occurs in a process called exocytosis. This membrane fusion pathway is essential for the targeting of newly synthesized proteins and lipids to the plasma membrane, and also regulates the

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release of various molecules from the cell. A more specialized form of exocytosis is 'regulated exocytosis'; in this pathway, membrane fusion requires a specific stimulus, in most cases an increase in intracellular calcium concentration. Regulated exocytosis underlies the controlled release of numerous physiologically important molecules, including neurotransmitters, catecholamines, and insulin.

Central to the process of membrane fusion are SNARE proteins, which are localized to various intracellular organelles and membranes [1]. Specific SNAREs present on two opposing membranes interact to form a highly stable 'SNARE complex'; the formation of this protein complex is tightly coupled to membrane fusion, and indeed SNAREs are sufficient to catalyze the fusion of lipid vesicles in vitro [2,3]. SNARE complex assembly involves the interaction of coiled-coil (helical) domains present in the individual SNARE proteins to form a parallel, twisted four-helix bundle [4–6]. Three of the helices are contributed by Q-SNAREs present on one membrane, with the other helix provided by an R-SNARE present on the opposing membrane. The classification of SNAREs as either 'Q' or 'R' derives from the presence of a highly conserved glutamine or arginine residue, respectively, which are located within the core of the helical bundle [7]. In the majority of intracellular membrane fusion pathways, the three helical domains contributed by Q-SNAREs are present in three distinct proteins [8]. However, in exocytic membrane fusion, one Q-SNARE helix is provided by a syntaxin protein and the other two helices are present in a single SNARE protein, SNAP-25 [5].

SNARE complex assembly is regulated at many levels. In neuronal and neuroendocrine cells the protein munc18-1 (also called nSec1) binds to syntaxin 1A and prevents its interaction with its SNARE partners [9,10]. The expression of munc18-1 is most abundant in neuronal/neuroendocrine cells, whereas munc18-2 and munc18-3 display a more widespread tissue distribution [11]. Munc18 proteins are likely to be required for the controlled assembly of SNARE complexes and are essential for membrane fusion. Other important SNARE regulatory molecules include SNAP and NSF, which catalyze SNARE complex disassembly following membrane fusion [2,12,13]. In addition to these proteins, a host of other factors have been shown to regulate SNARE proteins at many levels.

This review focuses on the mechanisms and pathways used to traffic syntaxin and SNAP-25 to the plasma membrane, where these proteins exert their essential function in exocytic membrane fusion.

1.1. Syntaxin

Members of the syntaxin family of SNARE proteins are found on numerous intracellular organelles, and their function is required for a wide range of intracellular membrane fusion pathways [14]. There are 15 mammalian syntaxin genes, and four of the expressed proteins (syntaxins 1–4) are localized to the plasma membrane and function in exocytic pathways [14]. Additionally, syntaxins 1–4 can be expressed as alternatively spliced isoforms. These plasma membrane syntaxins are differentially expressed in different cell types, and can also display distinct localizations within the plasma membrane of polarized cells. Syntaxins belong to a family of proteins that are 'tail-anchored' (also called Type IV membrane proteins); such proteins have an NH_2 terminal cytoplasmic domain that is membrane-bound by virtue of a single C-terminal hydrophobic domain and have no ectodomain [15]. Tail-anchored proteins (unlike classical Type II membrane proteins) are inserted posttranslationally into membranes [15]. Depending on the length of the hydrophobic domain and the nature of the surrounding amino acid residues, tail-anchored proteins insert into either the ER membrane or the mitochondrial outer membrane. The mechanism of insertion of tail-anchored proteins into the ER membrane can occur by a number of mechanisms that differ in ATP dependence and receptors [15]. However, the exact requirements for syntaxin insertion into the ER membrane have not been analyzed. The C-terminal membrane anchor of syntaxins 1–4 is preceded by a membrane proximal ~60-amino-acid coiled-coil region that participates in SNARE complex assembly. The function of specific syntaxin proteins is required for defined exocytosis pathways. For example, syntaxin 1 (which is expressed almost exclusively in neuronal and neuroendocrine cells) functions in exocytosis pathways such as presynaptic neurotransmitter release [16], whereas syntaxin 4, which has a more ubiquitous tissue distribution, functions in pathways such as the exocytosis of vesicles containing the facilitative glucose transporter, Glut4, in adipocytes [17].

1.1.1. The transmembrane domains (TMDs) of exocytic syntaxins confer plasma membrane localization

Analysis of the membrane targeting of chimeric syntaxin molecules has demonstrated that the TMDs are sufficient to confer membrane binding. In Madin Darby canine kidney (MDCK) cells, the TMDs of syntaxin 3 and syntaxin 4 target the reporter molecule GFP to the plasma membrane [18], and the TMD of syntaxin 3 also localized GFP to the plasma membrane in 3T3-L1 adipocytes [19]. The trafficking of the syntaxin 3 TMD to the plasma membrane in adipocytes was dependent upon its length; reducing the 25-amino-acid TMD to a 17-amino-acid sequence caused its accumulation in the Golgi [19]. The length of the TMD of the yeast syntaxin homologue Sso1p also plays a prominent role in the plasma membrane targeting of this exocytic SNARE protein [20]. In addition, it appears that distinct syntaxin TMDs may contain specific targeting information: syntaxin 3 TMD targeted GFP to the apical plasma membrane in MDCK cells, whereas the syntaxin 4 TMD resulted in a predominantly basolateral distribution of GFP [18].

The plasma membrane appears to be the 'default' destination for a number of syntaxin proteins in the absence of cytoplasmic domain internalization signals. In PC12 cells (a

rat adrenal chromaffin cell line), chimeras consisting of the cytoplasmic domain of syntaxin 1A fused to the TMD of the endosomal/TGN syntaxin 6, 7 and 8 isoforms accumulated at the plasma membrane [21]. In contrast, when the cytoplasmic domains of syntaxin 6, 7 or 8 were fused to the TMD of syntaxin 1A, the proteins were correctly localised to endosomal/TGN membranes in rat liver Clone 9 cells [21], suggesting that cytoplasmic sequences are required to direct syntaxin isoforms from the plasma membrane to their correct intracellular location.

1.1.2. Role of munc18 proteins in intracellular trafficking of syntaxin

Several studies have found that when syntaxin 1A (a neuronal-specific syntaxin isoform) is heterologously expressed in nonneuronal or neurosecretion-incompetent cells, the protein is retained intracellularly in the ER/Golgi [21–24]. Domain swapping experiments have indicated that this intracellular accumulation is dependent upon the cytoplasmic domain of syntaxin 1A [21]. Interestingly, co-expression of munc18-1 (the neuronal isoform of munc18) with syntaxin 1A relieves the intracellular block and leads to plasma membrane targeting [22]. At early time points following co-transfection, syntaxin 1A and munc18-1 co-localize in the Golgi region of the cell, suggesting that the two proteins traffic as a complex to the cell surface [22]. The precise role of munc18-1 in the membrane targeting of newly synthesized syntaxin 1A is not clear, but intracellular accumulation of syntaxin 1A in nonneuronal cells in the absence of munc18-1 may be a result of its interaction with SNARE proteins in the early secretory pathway [22]. Another possibility is that the plasma membrane targeting of syntaxin 1A requires its interaction with cholesterol-rich lipid rafts [23,24]. Munc18-1 has been suggested to facilitate the interaction of syntaxin 1A with such domains [23].

Syntaxin 1A exists in at least two distinct conformations: in an ‘open’ conformation syntaxin 1A is able to form functional SNARE complexes, whereas munc18-1 holds syntaxin 1A in a SNARE complex-incompatible ‘closed’ conformation [25]. Thus, munc18-1 may facilitate the correct intracellular trafficking of syntaxin 1A by stabilizing it in a protected conformation that prevents its participation in unfavourable SNARE complexes [22]. The dependence of syntaxin 1A on munc18-1 is further highlighted by studies showing that munc18-1 null mutants have a significant reduction in cellular syntaxin 1A levels [26].

In contrast to the intracellular accumulation of syntaxin 1A, syntaxin 3 and the yeast syntaxin homologue, Sso2p, were localized correctly to the plasma membrane when expressed in these same cells [22,27]. Does this imply that the dependence on munc18 for plasma membrane targeting is specific for syntaxin 1A? Although this may be the case, it is also possible that all mammalian plasma membrane syntaxins require munc18 proteins for correct intracellular trafficking; the nonneuronal cell types employed in these studies may express abundant levels of other munc18 isoforms which facilitate the correct targeting of nonneuronal syntaxin isoforms, such as syntaxin 3. Indeed, a study in

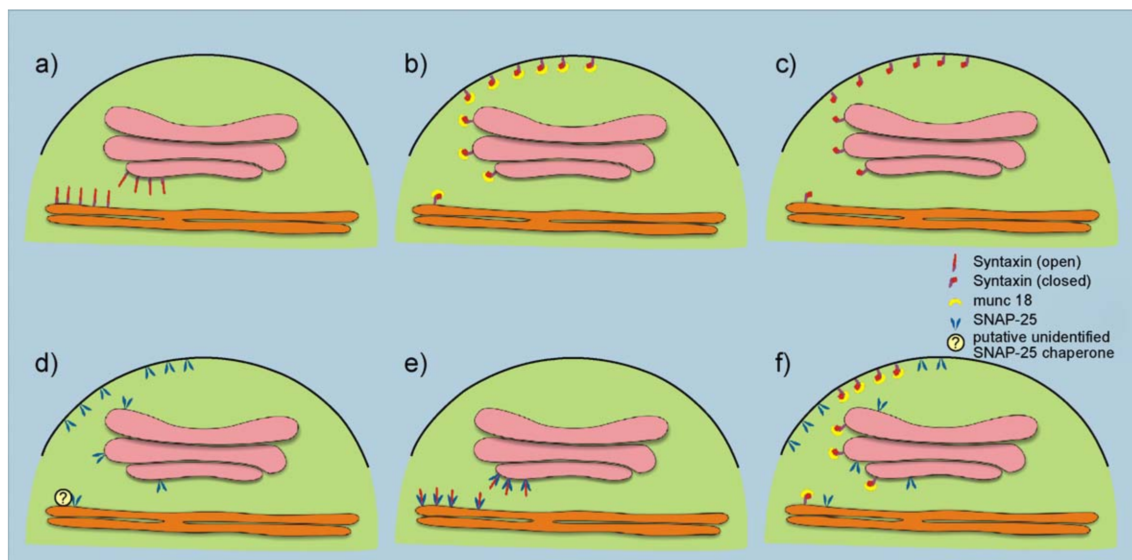

Fig. 1. Intracellular trafficking of exocytic syntaxin and SNAP-25 proteins. The plasma membrane targeting of syntaxin is proposed to be dependent upon its conformational state. In an open conformation, syntaxin is retained in the ER/Golgi region of the cell, possibly due to its interaction with other SNARE proteins present within these compartments (a); munc18 may stabilize the closed conformation of syntaxin isoforms such as syntaxin 1A, facilitating plasma membrane targeting (b); syntaxin isoforms that favour a closed conformation (such as the yeast protein, Ssop) may not require munc18 proteins for efficient trafficking to the plasma membrane (c). Plasma membrane targeting of SNAP-25 is also influenced by other factors. When expressed alone, SNAP-25 can target to the plasma membrane (d), however, the trafficking of SNAP-25 may depend upon other, as yet unidentified, factors; when co-expressed with syntaxin 1A, SNAP-25 accumulates intracellularly, probably as a result of its association with ER/Golgi-localized syntaxin 1A (e); co-expression of munc18, syntaxin and SNAP-25 may facilitate the plasma membrane trafficking of syntaxin and SNAP-25. It is proposed that the munc18-induced trafficking of syntaxin 1A to the plasma membrane would allow SNAP-25 transit to the cell surface by preventing its accumulation in the ER/Golgi (f).

human salivary gland HSY cells found that GFP-tagged syntaxin 4 required co-expression of munc18-3 for plasma membrane delivery [28], implying that munc18 proteins function as trafficking chaperones for syntaxin isoforms other than syntaxin 1A. Further work is clearly required in this area, and RNAi approaches may be successful in selectively reducing the levels of specific munc18 isoforms expressed in cells; this would allow a more comprehensive analysis of the munc18-dependence of plasma membrane trafficking of specific syntaxin isoforms in cells where they are endogenously expressed.

At this point, it is important to reiterate that the vast majority of studies that have implicated munc18-1 in the plasma membrane targeting of syntaxin 1A have been performed on nonneuronal cell lines. Undoubtedly, cells that do not express endogenous munc18-1 are a useful model in which to study the role of this protein in plasma membrane targeting of syntaxin 1A. However, there is evidence from (perhaps) more physiologically relevant systems that syntaxin 1A can reach the plasma membrane independently of munc18. In *C. elegans*, a constitutively open mutant of syntaxin which does not interact with UNC-18 (*C. elegans* homologue of munc-18) could fully rescue syntaxin null mutants [29]. In addition, syntaxin distribution was identical in wild-type and unc-18 null mutant *C. elegans*, although syntaxin levels were reduced by 50% in the mutants [30]. Similarly, syntaxin was suggested to be correctly targeted in munc18-1-deficient neurons, although no direct quantification of syntaxin plasma membrane levels was feasible in this study [31]. These results suggest either that syntaxin 1A trafficking has different requirements in neuronal and nonneuronal cells, or alternatively that neuronal-specific factors other than munc18 can regulate the intracellular trafficking of syntaxin 1A.

The potential role of munc18 proteins in syntaxin trafficking in yeast appears easier to decipher than for their mammalian counterparts. The yeast munc18 homologue, Sec1p, has a very low affinity (if any at all) for the yeast exocytic syntaxin homologue, Ssop, but binds specifically to assembled ternary SNARE complexes [32]. This suggests that Ssop must reach the plasma membrane independently of a munc18 protein, and this is consistent with the observed plasma membrane targeting of Sso2p in mammalian cells [27].

1.1.3. Role of the conformational state of syntaxin proteins in subcellular trafficking

The munc18 dependence of plasma membrane targeting of specific syntaxin proteins may depend upon the extent to which they exist in closed or open conformation. Whereas Sso1p appears to exist largely in a closed conformation [33], recent work has shown that monomeric syntaxin 1A favours an open conformation [34]. The closed conformation of syntaxin should prevent its interaction with SNAREs present in the secretory pathway. Thus, plasma membrane targeting of syntaxin proteins is probably most efficient when the protein is in a closed conformation, the dependence on munc18 being related to the favoured conformational state of specific syntaxin proteins (Fig. 1). This model is consistent with studies of unc-18 (*C. elegans*) and munc18 (mouse) knock-out organisms, syntaxin expression being markedly reduced in both systems [26,30]. This reduction in syntaxin expression may indicate that trafficking of syntaxin is less efficient in munc18/unc-18 deficient organisms. Nevertheless, syntaxin does reach the plasma membrane in these deficient cells, suggesting that syntaxin can traffic independently (although also inefficiently) of munc18, and there may be specific neuronal factors that can chaperone syntaxin 1A plasma membrane trafficking in the absence of munc18.

1.2. SNAP-25

Members of the SNAP-25 protein family contribute two of the four alpha helices that compose exocytic SNARE complexes. Alpha helical SNARE motifs are present at the N- and C-termini of SNAP-25 proteins and are separated by a central cysteine-rich membrane targeting/binding domain. The first identified SNAP-25 proteins were termed SNAP-25A and SNAP-25B; these isoforms are highly homologous, with only nine different amino acids [35,36]. SNAP-25A/B exhibit a restricted expression pattern, being most abundant in neuronal and neuroendocrine cells, and these proteins have a specialized function in fast regulated exocytosis pathways, such as synaptic vesicle exocytosis [37–39]. Another member of the SNAP-25 protein family is SNAP-23; this protein, which shares ~60% identity with SNAP-25, has a ubiquitous tissue distribution [40,41]. SNAP-23 has been implicated in regulated exocytosis in nonneuronal cell types such as adipocytes and mast cells [42,43], and may also have a general function in constitutive exocytosis [44]. SNAP-29 is another protein related to SNAP-25; however, as this protein is localized in many intracellular membranes and there is no evidence that it functions as a regular exocytic SNARE protein, it will not be discussed further [45–47]. SNAP-25 does not contain a TMD and membrane targeting of this protein has been shown to be dependent upon palmitoylation. Furthermore, syntaxin has been suggested to be required for efficient trafficking of SNAP-25 to the plasma membrane.

1.2.1. Palmitoylation of SNAP-25

Although members of the SNAP-25 protein family do not contain TMDs, they are bound tightly and efficiently to cell membranes. Association of SNAP-25 and SNAP-23 with membranes is dependent upon a central cysteine-rich domain; this domain is palmitoylated in vivo and mutants lacking these cysteine residues are cytosolic [48–52]. The cysteine-rich domains of SNAP-25A and SNAP-25B each contain four cysteine residues, although the position of one of these cysteines is different in the two isoforms [35,36], whereas SNAP-23 contains five cysteines in the cysteine-

rich domain [40,41]. Palmitoylation of SNAP-25 proteins is highly sensitive to even single cysteine substitutions, and mutation of any of the four cysteine residues within the cysteine-rich domain of SNAP-25 causes a dramatic decrease in palmitoylation: single cysteine mutations in the cysteine-rich domain of SNAP-25 reduced the level of protein palmitoylation by 58–91% in COS-7 cells depending on the specific cysteine that was mutated [53]. The minimal domain of SNAP-25 required for efficient palmitoylation is a 36-amino-acid sequence containing the cysteine-rich domain and the 28 amino acids that follow the cysteines [49]. This C-terminal 28-amino-acid domain contains a conserved QPARV motif that is essential for SNAP-25 palmitoylation [49]. Palmitoylation of SNAP-25 clearly plays an essential role in targeting to the plasma membrane [48–52]. However, palmitoylation has also been suggested to be required for the dynamic association of SNAP-25 with other SNARE proteins [52], and for its enrichment at specific domains of the plasma membrane [54].

Interestingly, the half-life of SNAP-25 palmitoylation was shown to be shorter than the half-life of the protein, suggesting that palmitoylation of SNAP-25 may be dynamic [53]. This intriguing observation may present a mechanism to regulate either the membrane association of SNAP-25 or its interaction with specific membrane subdomains.

1.2.2. Mechanism and location of SNAP-25 palmitoylation

SNAP-25 can be palmitoylated in vitro in the absence of a palmitoylacyl transferase (PAT) enzyme; this spontaneous palmitoylation of SNAP-25 is markedly enhanced in the presence of syntaxin 1 [55]. This suggests that palmitoylation of SNAP-25 in vivo may also occur by a noncatalytic mechanism. However, if this were the case, then it is more difficult to explain the effects of single cysteine substitutions on SNAP-25 global palmitoylation, and this may indicate that palmitoylation of SNAP-25 in vivo requires a specific palmitoyl acyl transferase (PAT). Although there are no common consensus sequences identified for protein S-palmitoylation, the position of the cysteines and other amino acid residues in the 36 amino acid cysteine-rich domain may form a specific site required for palmitoylation of SNAP-25.

PAT activity has been detected at various locations within the cell, including the plasma membrane, Golgi and ER [56–58]. The cellular site of SNAP-25 palmitoylation is unknown; however, palmitoylation is dependent upon a functional secretory pathway, suggesting that SNAP-25 must enter this pathway to get palmitoylated [59]. Similarly, palmitoylation of H-ras also requires transport through the secretory pathway [60,61]. However, whereas disruption of the secretory pathway by Brefeldin A (BFA, a drug that blocks transport through the Golgi) treatment abolished SNAP-25 palmitoylation, this drug did not prevent the palmitoylation of H-ras [61]. These results imply that the cellular location of SNAP-25 palmitoylation is distinct from that of H-ras, and emphasizes that there are several PAT activities within the cell having specific substrate preferences. In yeast, Ras2p is palmitoylated by a PAT located in the ER [58,62] and this may be similar for the mammalian ras isoforms.

Inhibition of SNAP-25 palmitoylation by BFA therefore implies that SNAP-25 is palmitoylated in a post-ER compartment, presumably either the Golgi or plasma membrane. Alternatively, the effects of BFA on SNAP-25 palmitoylation may be indirect: SNAP-25 palmitoylation may require another protein that traffics through the secretory pathway [59]. Identification of the subcellular location of SNAP-25 palmitoylation could be further probed by analyzing SNAP-25 palmitoylation at 19 °C, a temperature that blocks protein exit from the TGN; lack of SNAP-25 palmitoylation at this low temperature would imply that the protein is palmitoylated at the cell surface. The plasma membrane is enriched in PAT activity against other palmitoylated proteins, such as G protein alpha subunits [63]. Furthermore, this PAT activity was enriched in cholesterol-rich microdomains of the plasma membrane called 'lipid rafts' [64]. Interestingly, SNAP-25 and SNAP-23 associate with lipid raft domains in a variety of cell types [65–69], suggesting the possibility that this association may be important for palmitoylation of SNAP-25 by a specific raft-associated PAT. In this regard, it would be interesting to examine whether disruption of lipid rafts (through cholesterol depletion) affects SNAP-25 palmitoylation. It would also be interesting to determine the role played by lipid rafts in the surface delivery of SNAP-25 and SNAP-23.

1.2.3. Role of syntaxin in SNAP-25 membrane interactions

Palmitoylation of SNAP-25 undoubtedly performs an essential function in the membrane targeting and binding of these proteins. Nevertheless, SNAP-25 is not displaced from the plasma membrane by chemical deacylation [59], implying that palmitoylation of SNAP-25 is not required for stable membrane association. Continued binding of SNAP-25 to membranes following depalmitoylation suggests that this protein may remain membrane-bound as a result of a tight association with another protein or lipid. In agreement with this idea, the SNAP-25 homologue of yeast, Sec9p, is abundant at the plasma membrane in the absence of either a TMD or lipid anchor [70].

An obvious possibility is that SNAP-25 is membrane-bound through an interaction with its t-SNARE partner, syntaxin 1. However, the extent of SNAP-25 association with syntaxin 1 at steady state is unclear. In adrenal chromaffin cells, syntaxin 1 and SNAP-25 co-cluster extensively at specific sites of the plasma membrane. Disruption of SNAP-25 binding to syntaxin leads to a loss of co-localization and SNAP-25 is partially redistributed to the cytosol [71]. Similarly, most SNAP-25 in primary neurons is complexed with syntaxin 1A [72]. In contrast, SNAP-25 and syntaxin 1 in PC12 cells display only a limited co-clustering at the plasma membrane [73], and most SNAP-25

is not bound to syntaxin in these cells [72,74]. Interestingly, deacylation of SNAP-25 in PC12 cells does not displace it from membranes suggesting, in this cell type at least, factors other than syntaxin may facilitate SNAP-25 membrane association.

As with studies on syntaxin and munc18 (Section 1), the specific dependence of SNAP-25 trafficking on syntaxin is difficult to assess in cells endogenously expressing both of these proteins. As a result, a number of investigators have analyzed membrane targeting of these SNARE proteins in nonneuronal cell lines that do not express endogenous syntaxin 1 or SNAP-25. These studies clearly demonstrate that syntaxin affects the membrane targeting of newly synthesized SNAP-25. When expressed in HeLa cells, newly synthesized SNAP-25 associates inefficiently with cell membranes; however, co-expression of syntaxin 1 markedly increases the binding of newly synthesized SNAP-25 to membranes [75]. Furthermore, a normally cytosolic SNAP-25 mutant lacking the palmitoylation domain was efficiently targeted to membranes when co-expressed with syntaxin 1 [75]. Analysis of SNAP-25 membrane targeting in BHK and COS7 cells also suggests that syntaxin functions in trafficking of newly synthesized SNAP-25 [52]. In these cells, SNAP-25 accumulates at the plasma membrane when expressed alone, whereas syntaxin is targeted to the Golgi region. However, co-expression of syntaxin 1 and SNAP-25 leads to accumulation of SNAP-25 in the Golgi region of the cells. In addition, syntaxin 1 co-expression targeted normally cytosolic mutants of SNAP-25 to this intracellular compartment. Syntaxin 1 has also been shown to partially restore membrane localization of SNAP-25 cysteine mutants in pancreatic beta cells [76]. The dominant effect of syntaxin on SNAP-25 intracellular distribution was further emphasized when wild-type SNAP-25 was co-expressed with a syntaxin mutant lacking the TMD: this resulted in SNAP-25 remaining in the cytosol along with mutant syntaxin. These results suggest the possibility that syntaxin 1 functions as a molecular chaperone for SNAP-25, mediating its initial targeting to membranes, and hence facilitating its subsequent palmitoylation [75]. Indeed, newly synthesized SNAP-25 has been detected associated with syntaxin 1 in the cytosol [75].

Nevertheless, there are a number of inconsistencies with a model in which syntaxin 1 directs SNAP-25's intracellular traffic. In particular, a 36-amino-acid membrane binding domain of SNAP-25 (residues 85–120, which include the palmitoylation site, but not the syntaxin binding site) has been shown to target GFP to the plasma membrane in neuronal cell types [49]. Additionally, a SNAP-25 protein with a single point mutation that abolished binding to syntaxin was also correctly targeted to the plasma membrane in neuronal cells [51]. These results suggest that in cells endogenously expressing SNAP-25 the membrane targeting of this protein is independent of syntaxin. Nonneuronal cells may lack a specific factor required for the membrane targeting or palmitoylation of SNAP-25 [51]. Another concern with this model of SNAP-25 plasma membrane trafficking relates to the relative expression levels of syntaxin and SNAP-25. Quantification of SNAP-25/syntaxin1 levels in synaptosomes [77], cerebellar neurons [72], PC12 cells [72] and SNAP-23/syntaxin 4 levels in adipocytes [78] demonstrated that SNAP-25/23 were present at greater levels than their syntaxin counterpart. Although the expression levels of SNAP-25 were only slightly greater than syntaxin in synaptosomes and cerebellar neurons, SNAP-25 was expressed at five- to sixfold higher levels than syntaxin in PC12 cells [72], and SNAP-23 was expressed at threefold higher levels than syntaxin 4 in adipocytes [78]. As the half-life of SNAP-25 is shorter than syntaxin [53,75,79], these observations suggest that at steady state there will be a significant (or substantial) excess of SNAP-25 synthesized relative to syntaxin. Furthermore, SNAP-25 is efficiently targeted to the plasma membrane even when overexpressed in neuronal/neuroendocrine cells, which may increase the ratio of SNAP-25/syntaxin by several orders of magnitude (Ref. [51]; authors' unpublished observation). These observations suggest that unless syntaxin 1A recycles from the plasma membrane to the cytosol, syntaxin 1A is unlikely to be present at sufficient levels to support membrane targeting of newly synthesized SNAP-25.

1.2.4. A model for SNAP-25 trafficking and membrane association

Studies on SNAP-25 membrane trafficking in neuronal/neuroendocrine cells (i.e. cells that normally express SNAP-25) demonstrate that interaction with syntaxin is not required for either palmitoylation or plasma membrane localization of SNAP-25 [49,51]. In nonneuronal cell lines, syntaxin expression can interfere with the plasma membrane binding of SNAP-25 and can also drive the membrane association of cysteine-less SNAP-25 mutants [52]. However, in these cell types syntaxin is not correctly targeted; it accumulates in the Golgi region rather than at the plasma membrane. Thus, it is likely that Golgi-localized syntaxin 1A can interact with SNAP-25 during its transport through the secretory pathway, and retain SNAP-25 at this intracellular location. Interestingly, when syntaxin 1A is expressed in MDCK cells it accumulates on lysosomal membranes [80]; at this cellular location syntaxin 1A is unable to interfere with SNAP-25 trafficking, which targets efficiently to the plasma membrane in the presence or absence of syntaxin co-expression [81]. A further point of note is that (as discussed in Section 1.2) syntaxin 1A requires co-expression of munc18-1 for effective plasma membrane localization and protein stability [22–24,26]. Munc18-1 binding to syntaxin 1A holds syntaxin in a closed conformation; in this conformation syntaxin is unable to bind to SNAP-25. Thus, under normal conditions the requirement of syntaxin 1 to bind munc18-1 for plasma membrane targeting should prevent the interaction of newly synthesized syntaxin with SNAP-25 (Fig. 1).

An important area of research will involve the identification of the membrane-bound factors that allow the continued association of SNAP-25 with the plasma membrane following chemical depalmitoylation [59]. Clues to this question are likely to come from further analysis of membrane targeting and binding of the SNAP-25(85–120)-GFP chimera; for example, does this chimera remain membrane-bound following chemical depalmitoylation? Additionally, as SNAP-25 only becomes palmitoylated during or after transport through the secretory pathway [59], there must be other targeting information present within the protein sequence that serves to direct newly synthesized SNAP-25 to the secretory pathway. It is known that prenylation of H- and N-ras target these proteins to the secretory pathway, however, SNAP-25 does not contain a consensus site for such modification; so what directs it to the secretory pathway? Finally, it is formally possible that SNAP-25 does not traffic through the secretory pathway and that the effects of BFA on SNAP-25 stability/palmitoylation/targeting reflect the requirement for another protein that does traffic through the secretory pathway [59].

2. Concluding remarks and future perspective

If indeed SNARE proteins are membrane fusion catalysts, then it is sensible to assume that their intracellular trafficking is tightly regulated, and that the proteins are transported in an ‘inactive’ form. Undoubtedly, the controlled conversion of syntaxin proteins from an open to a closed conformation is key to their cellular functions; however, the conformational state of syntaxins is also likely to impact on their intracellular trafficking. Syntaxin proteins are likely to be chaperoned during their transport within the cell. In cases such as the yeast syntaxin, Ssop, the N-terminal domain of the protein that folds over the SNARE-binding domain would make the protein functionally impotent and hence facilitate plasma membrane targeting, by acting as an intramolecular chaperone. However, for syntaxin proteins that favour an open conformation, there is likely to be a requirement for other factors which either stabilize the closed conformation (munc18) or that shield the SNARE binding domain. Similar factors may also be required for SNAP-25 trafficking to the plasma membrane; however, the proposal that syntaxin trafficking depends upon a closed conformation would rule out this protein as a SNAP-25 chaperone. In addition, it appears unlikely that cells would transport a functional Q-SNARE complex (with affinity for R-SNAREs) through various membranes of the secretory pathway. Clearly, there are many more questions than answers, but the development of techniques that permit the simple ‘knock-down’ of specific cellular proteins will be a useful tool to dissect the precise molecular mechanisms involved in the plasma membrane targeting of exocytic SNAREs.

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