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Review

Regulation of SNARE fusion machinery by fatty acids

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Abstract. Vesicle fusion is a ubiquitous biological process involved in membrane trafficking and a variety of specialised events such as exocytosis and neurite outgrowth. The energy to drive biological membrane fusion is provided by fusion proteins called SNAREs. Indeed, SNARE proteins play critical roles in neuronal development as well as neurotransmitter and hormone release. SNARE proteins form a very tight alpha-helical bundle that can pull two membranes together, thereby initiating fusion. Whereas a great deal of attention has been paid to partner proteins that can affect SNARE function, recent genetic and biochemical evidence suggests that local lipid environment may be as important in SNARE regulation. Direct lipid modification of SNARE fusion proteins and their regulation by fatty acids following phospholipase action will be discussed here in detail. Our analysis highlights the fact that lipids are not a passive platform in vesicle fusion but intimately regulate SNARE function.

Keywords. SNARE, fatty acid, phospholipase, exocytosis, palmitoylation.

Introduction

Numerous vesicles fuse with the plasma membrane to mediate many cellular processes including cell growth, plasma membrane repair, axonal branching, recycling of plasma membrane transporters and release of soluble signalling molecules into the extracellular space. Our ability to interfere with any given process relies on full definition of the molecular mechanisms operating during vesicle fusion events. The recent discovery of the general principles of protein-mediated membrane fusion [1, 2] provides a solid basis for achieving this goal. We now know that SNARE proteins present on opposing membranes must form a helical complex for fusion to take place. The generality of the SNARE hypothesis is illustrated by the fact that many fusion events require v-SNAREs on the vesicular (v-) membrane and t-SNAREs on the target (t-) membrane. For example, a prototypical set of fusion proteins involved in neurotransmitter release consists of the vesicular protein synaptobrevin, plasma membrane syntaxin 1 and SNAP-25 (Fig. 1) [3]. The three proteins form, in a zipper-like manner, a slightly twisted four-helical bundle between two approaching membranes [2]. The precise alignment of heptad amino acid repeats between the SNARE interacting regions, also called SNARE motifs, results in a collision of the SNARE transmembrane regions, possibly initiating the fusion event.

Formation of the SNARE ternary complex is traditionally thought to be a protein-regulated event [4]. Indeed, there is solid genetic evidence showing that vesicular synaptotagmin and cytosolic Munc18, Munc13, Rab-interacting molecule (RIM) and complexin are all involved in regulation of SNARE-mediated fusion [5, 6]. However, recent genetic and biochemical work also identified a number of enzymes

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involved in lipid metabolism that impact on protein-driven vesicle fusion [7]. For example, genetic inactivation of a protein closely resembling a diacylglycerol lipase (DAG lipase) has a profound effect on neurotransmitter release in *Drosophila* [8, 9]. An enzyme involved in biosynthesis of long-chain polyunsaturated fatty acids (PUFAs) has been shown to be essential for efficient neurotransmission in *C. elegans* [10]. Phospholipases A_2, C and D, which metabolise fatty acids in phospholipid membranes, have been implicated in vesicle fusion for many decades [11–13]. Multiple kinases/phosphatases change the phosphorylation status of inositol-containing lipids to regulate membrane traffic [14–16]. Furthermore, direct modification of vesicle fusion proteins by fatty acids is a well-known phenomenon [17–19]; for example, SNAP-25 is a major palmitoylation target in neurons [20, 21].

It is becoming clear that the phospholipid bilayer provides not only a physical platform in which the SNARE fusion proteins reside, but also a dynamic reaction environment which can regulate protein function [8, 15, 22, 23]. Microscopic analysis of SNARE protein distribution suggests that t-SNAREs are not uniformly distributed throughout the plasma membrane but co-exist in well-defined domains [24–26]. Since palmitoylated proteins tend to segregate in sphingomyelin/cholesterol-rich rafts, it has been proposed that SNAP-25 palmitoylation can drive accumulation of t-SNAREs in fusion 'hot-spots' [27–32]. Recent studies also highlighted an important role for omega-3 and omega-6 unsaturated fatty acids in activation of the syntaxin molecule [33–36]. In this review, we will consider palmitoylation of the SNARE fusion proteins, the role of lipid-modifying enzymes in vesicle fusion and regulation of SNAREs by free fatty acids. This analysis shows that the current proteocentric view of membrane fusion is rather simplistic and needs to be extended to incorporate the influence of the surrounding lipid environment.

Direct lipid modification of SNAREs

Many SNARE proteins are modified by addition of the lipid moiety palmitate, a saturated 16-carbon fatty acid [17]. This reversible modification occurs on cysteine residues and results in formation of a thioester bond [37]. *In vitro*, exposure of cysteine-containing proteins to coenzyme A-palmitate is sufficient for palmitoylation to take place [38]; however, *in vivo* palmitoyl transferases are likely to be responsible for palmitoylation of specific protein sites [19, 39].

SNARE palmitoylation and membrane anchoring. The traditional view of protein modification by hydrophobic fatty acids is that the attached lipid helps to anchor proteins to membranes. However, in the case of palmitoylated SNARE proteins containing a transmembrane region (TMR), this assumption is clearly insufficient. Vesicular synaptobrevin resides in the lipid bilayer owing to its TMR, and even the juxtamembrane part is partially embedded [40, 41]. However, synaptobrevin is palmitoylated in its TMR (Fig. 1), and this event is up-regulated during development, as synaptic vesicles from adult but not embryonic rat brains contain palmitoylated synaptobrevin [42]. Palmitoylation also occurs on a large number of TMR-containing yeast SNAREs (Snc1, Snc 2, Sso1, Sso2, Vam3, Tlg1, Tlg2 and Syx8), suggesting that it must serve an important role in SNARE function [17, 43]. It has recently been shown that palmitoylation-deficient Tlg1 is missorted and degraded, indicating that, in this particular case, palmitoylation is necessary for proper SNARE targeting and maintenance [43].

Potential roles for SNAP-25 palmitoylation. Perhaps the most intensively studied palmitoylated protein is neuronal SNAP-25. The primary structure of SNAP-25 is hydrophilic and contains no apparent membrane-binding domains [44]. Between the N- and C-terminal SNARE helices lies a linker region containing a cluster of four cysteine residues that are palmitoylated (Figs. 1, 2) [20, 45]. It has been reported that palmitoylation-deficient SNAP-25 is unable to support vesicle exocytosis [46]. Several functions have been suggested for SNAP-25 palmitoylation. Firstly, it may be required for accumulation of the protein at the plasma membrane, suggesting a conventional lipid anchoring role [20, 47]. However, it has also been argued that the tight association between SNAP-25 and syntaxin is sufficient for membrane localisation [48]. Syntaxin binding may even be a prerequisite for SNAP-25 palmitoylation [38], as SNAP-25 adopts an α-helical conformation only upon interaction with syntaxin [49], possibly exposing cysteine residues for lipid attachment. Secondly, SNAP-25 palmitoylation may help to sort and cluster assembled syntaxin/SNAP-25 heterodimers in sphingolipid/cholesterol-rich domains within the plasma membrane bilayer; however, the existence of such lipid rafts in live cells and their role in SNARE function are still under debate [32, 50–52]. Thirdly, palmitoylation of SNAP-25 may optimise the SNARE complex dissociation by α-SNAP and NSF ATPase following membrane fusion [46, 53], but the mechanistic basis for such a role is not clear.

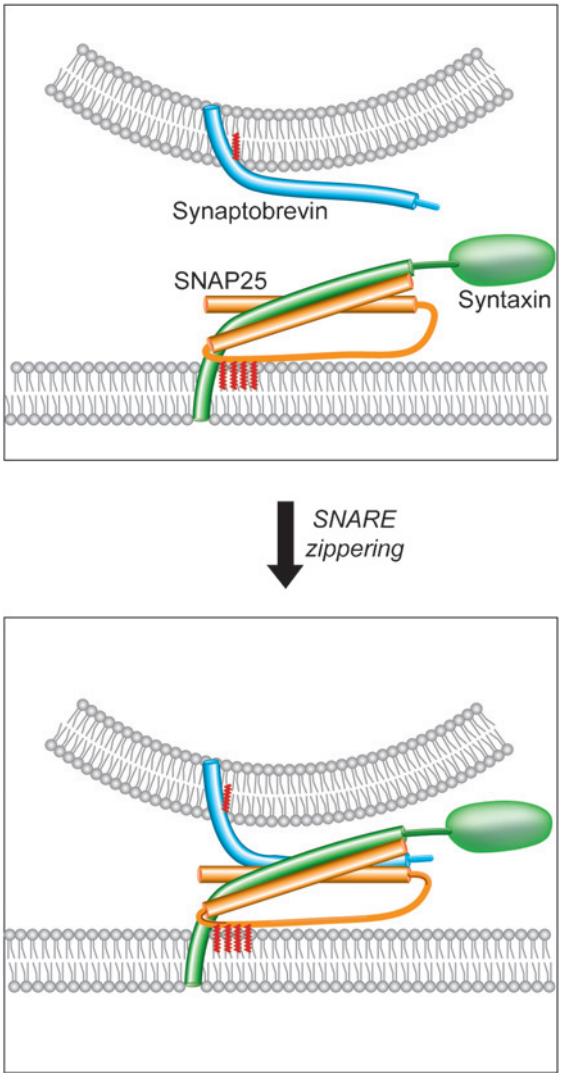

Figure 1. Three neuronal SNARE proteins assemble into the ternary SNARE complex to drive membrane fusion. Syntaxin (*green*) and SNAP-25 (*orange*) assemble into a stable heterodimer on the plasma membrane. Both vesicular synaptobrevin (*blue*) and SNAP-25 are lipid-modified through attachment of saturated palmitic acid (red). Following vesicle approach, synaptobrevin 'zippers up' with the trihelical syntaxin/SNAP-25 assembly to form a tight four-helix bundle, also called the SNARE complex. This complex facilitates fusion of two opposing membranes (not shown).

A role for palmitates in membrane fusion? When syntaxin and SNAP-25 associate on the plasma membrane to form a stable t-SNARE heterodimer, the SNAP-25 palmitates are located in the immediate vicinity of the syntaxin TMR (Fig. 1); this resembles the arrangement of palmitates and TMRs in many palmitoylated TMR-containing SNAREs, as discussed above. SNAP-25 exists as two splice isoforms, A and B, which differ in the relative positions of palmitoylated cysteines (Fig. 2) and in several adjacent residues that may also interact with the phospholipid bilayer [54, 55]. During initial embryonic brain development, the SNAP-25A isoform is highly expressed, but shortly after birth, when most cortical synapses mature, there is a rapid increase in SNAP-25B expression [54]. Crucially, impairment of the switch between the two isoforms leads to premature mortality and a change in short-term plasticity in hippocampal neurons [56]. SNAP-25B is essential for fast inducible vesicle fusion at the synapse; indeed, the most obvious positive correlate for maturation of a nerve terminal is a pronounced rise in SNAP-25B levels [54, 57]. The importance of the switch between SNAP-25A and SNAP-25B has been demonstrated recently in rescue experiments [58]: the authors found that in a SNAP-25-null background, exogenous expression of SNAP-25B leads to significantly enhanced vesicle exocytosis compared with SNAP-25A. In addition, SNAP-23, which is not palmitoylated to the same extent, is less effective in mediating exocytosis than either SNAP-25 isoform [58, 59], suggesting that vesicle fusion might be assisted not only by SNARE TMRs but also by palmitates located close by. The remarkable conservation of the small rearrangement of palmitoylated cysteines in SNAP-25A and SNAP-25B throughout vertebrate evolution (Fig. 2) provides additional support for the importance of SNARE palmitoylation.

It is worth mentioning that biochemical reconstitution of SNARE-mediated membrane fusion using liposomes originally involved syntaxin as well as unpalmitoylated SNAP-25 and synaptobrevin [60]. Only a low level of fusion was observed, and addition of other factors, such as Munc18 and synaptotagmin, was required for acceleration of this process [60–64]. Taking into account the conserved nature of SNARE palmitoylation, it may be useful to further investigate its role using such a fully defined reconstitutional approach.

Regulation of SNAREs by polyunsaturated fatty acids (PUFAs)

Whereas direct lipid modification of SNARE proteins is a very well-studied event, emerging evidence suggests that transient interactions of SNARE proteins with fatty acids may also be of critical importance in regulation of vesicle fusion. Unsaturated fatty acids are released into the cytosol through membrane depolarisation or receptor-mediated reactions triggered by growth factors, hormones and neurotransmitters [65–70]. Two types of PUFAs, omega-6 and omega-3, are essential for vertebrates, as they cannot be formed *de novo* and need to be ingested. These PUFAs play a special role in membrane fluidity due to their motional freedom [71, 72]. Omega-6

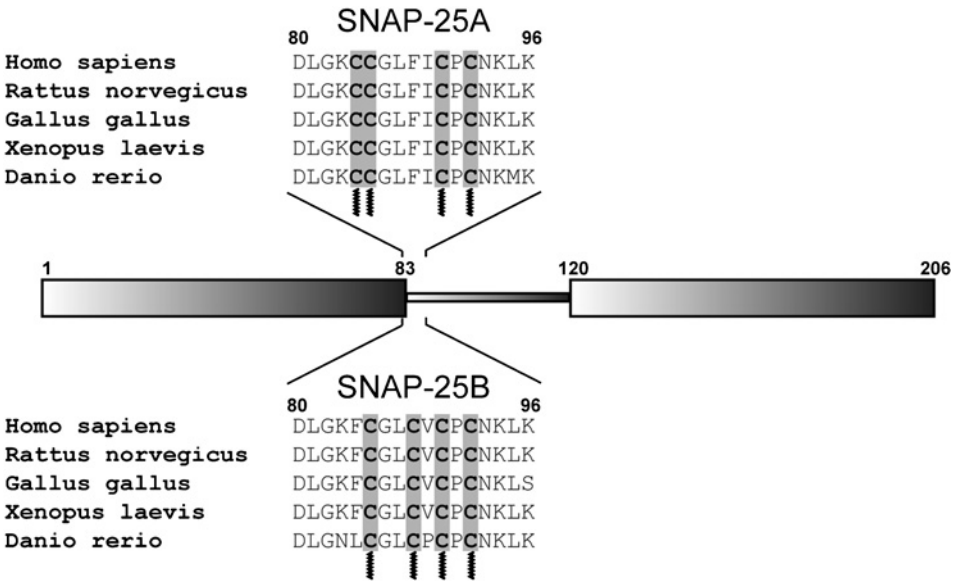

Figure 2. Full conservation of the order of palmitoylated cysteines in the linker region of two SNAP-25 splice isoforms throughout vertebrate evolution. SNAP-25 cysteine-containing sequences shown are human, rat, chicken, frog and fish (from top to bottom). The SNAP-25B isoform is predominant in the adult brain, while SNAP-25A is the main embryonic isoform in neurons. Up-regulation of synaptic vesicle exocytosis during development requires a change in the pattern of SNAP-25 palmitoylation.

arachidonic and omega-3 docosahexaenoic acid are major building blocks of biological membranes, accounting for 6% dry weight of the cerebral cortex [73]. In addition, due to their favourable biophysical properties and solubility, they are involved in the regulation of many cellular processes and in the production of multiple eicosanoids, including prostaglandins [74]. The importance of PUFAs for neuronal function has been highlighted in many studies [71, 75–77]. Mutations in an enzyme involved in PUFA production cause neuronal impairment in *C. elegans*, which can be rescued by external application of arachidonic or docosahexaenoic acid [10, 78, 79]. Furthermore, mutations in PUFA-related enzymes cause mental retardation in humans [80], and diets deficient in essential PUFAs are associated with deficits in infant brain function [76, 81]. Interestingly, PUFA-rich diets result in down-regulation of only a few genes; among them is the syntaxin-binding protein Munc18, suggesting a SNARE link [82].

Involvement of fatty acid-releasing phospholipases in vesicle fusion. Many phospholipases target phospholipids to release PUFAs, such as arachidonic acid, into the cytosol [83–85]. Unsaturated fatty acids have intrinsically high rates of dissociation from membranes and, in contrast to phospholipids, can diffuse to intracellular sites of action without any transporter molecules [86, 87]. Phospholipase A2s (PLA_2s) are one group of enzymes that catalyze the breakdown of phospholipids. They release arachidonic acid from the phospholipid molecule (Fig. 3a) and are postulated to be involved in vesicle fusion in many biological systems, including neurotransmitter release, insulin secretion, sperm acrosome exocytosis and neurite outgrowth [12, 88–100]. There was some uncertainty regarding the effect of arachidonic acid on catecholamine secretion from chromaffin cells [101–103], but a recent study demonstrated arachidonic acid-induced up-regulation of secretion in both permeabilised and intact cell models [36]. Revealingly, snake neurotoxins that disrupt synaptic function also contain PLA_2s [11], and their secretogogue action can be blocked by application of a SNARE-cleaving botulinum toxin, suggesting that PLA_2s act upstream of SNARE assembly [104, 105].

PLA_2 action leads to production of free arachidonic acid and lysophospholipid (Fig. 3b). After phospholipid hydrolysis, free arachidonic acid can diffuse out of the lipid bilayer to either be metabolized into a multitude of biologically active compounds or converted to arachidonyl-coenzyme A for reincorporation into phospholipids. In contrast to flexible arachidonic acid, which adopts a hairpin structure when in solution [74], lysophospholipids tend to remain in the membrane due to the lower solubility of their saturated carbon chains. Retention of lysophospholipids with cone-shaped morphology in the bilayer might allow formation of 'dimples', which may be conducive to membrane hemifusion and thus contribute to vesicle exocytosis [106]. In a recent study, application of a lysophospholipid and monounsaturated oleic acid was shown to reconstitute snake PLA_2

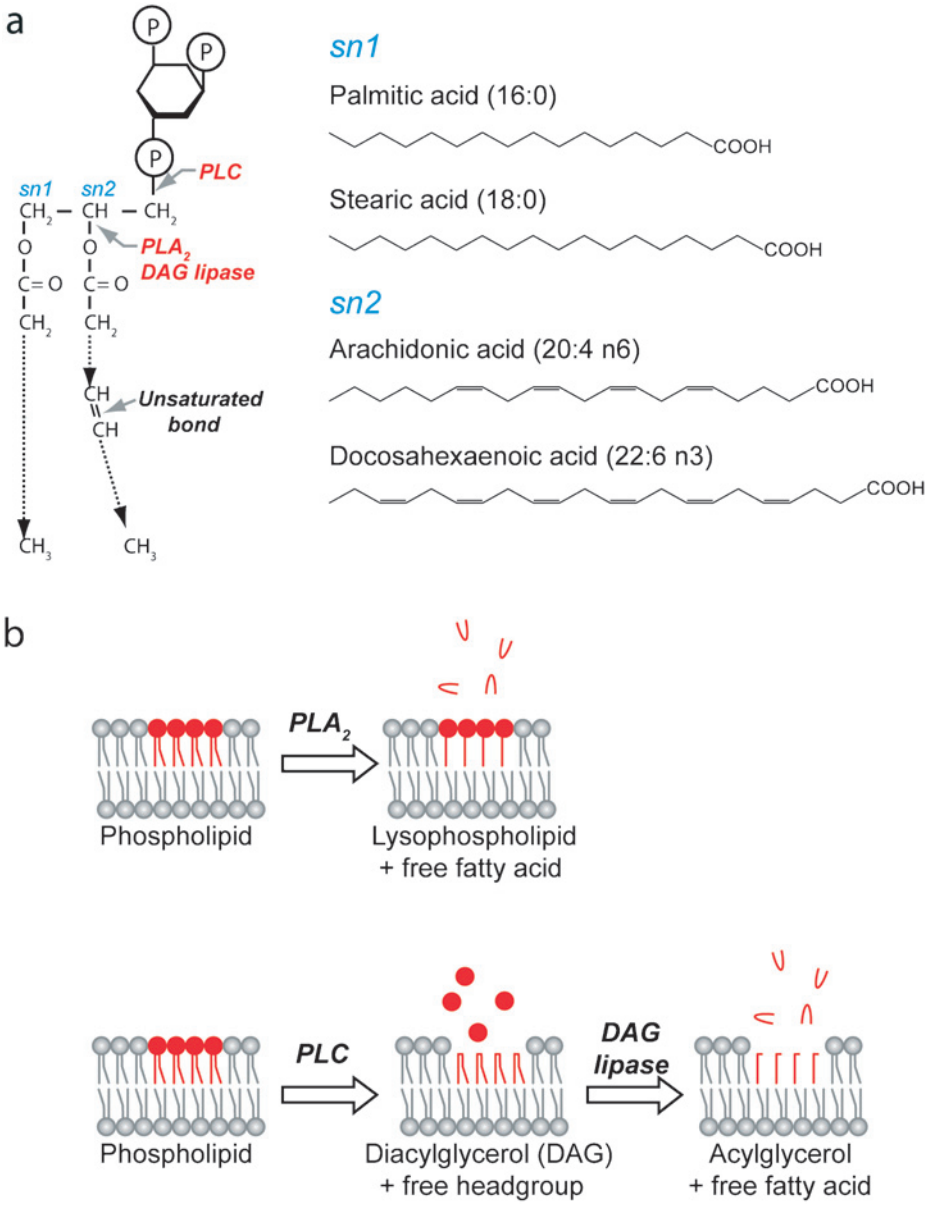

Figure 3. Alternative pathways leading to the release of mobile polyunsaturated fatty acids (PUFAs) from the lipid bilayer into the cytosol. (*a*) A typical phospholipid consists of saturated palmitic acid (C16) or, more commonly, stearic acid (C18) attached to the sn1 position of the glycerol backbone, while unsaturated omega-6 arachidonic acid (C20:4) or omega-3 docosahexaenoic acid (C22:6) occupy its sn2 position. The glycerol backbone also carries a hydrophilic headgroup shown here as a phosphorylated inositol; other common headgroups are choline, ethanolamine and serine. Sites of phospholipase action are shown by *grey arrows*. Note the presence of four and six unsaturated double bonds in arachidonic acid and docosahexaenoic acid, respectively. (*b*) Hydrolysis of the phospholipid molecule by phospholipase A_2 (PLA_2) produces lysophospholipid, which is preferentially retained in the membrane, and more soluble arachidonic acid (red hairpin), which, following diffusion into the cytosol, is either metabolised by cyclooxygenases into different eicosanoid products or incorporated back into the sn2 position of phospholipids (not shown). Phospholipase C (PLC) acts on the lipid bilayer to release the soluble headgroup (red circle), leaving diacylglycerol (DAG) in the membrane. Further action of a DAG lipase results in the release of PUFAs into the cytosol. Monoacylated glycerol remains in the membrane due to straight saturated stearic, or palmitic, fatty acids.

action in depletion of synaptic vesicle pools, once again highlighting the importance of lipids in vesicle fusion [107].

Mounting evidence also implicates phospholipase C (PLC), which produces diacylglycerol (DAG), in SNARE-driven vesicle fusion and membrane expansion [108, 109]. PLC hydrolyses phospholipids to release the soluble headgroup, while DAG remains embedded in the lipid bilayer (Fig. 3b). The presence of DAG changes membrane properties, and DAG recruits a number of enzymes, such as protein kinase C and DAG lipase. DAG lipase releases arachidonic acid

from the phospholipid sn2 position, leaving monoacylated glycerol in the lipid bilayer [74]. Interestingly, a DAG lipase inhibitor has been shown to block neurite outgrowth and insulin release [110, 111], and in addition, the PLC inhibitor U73122 potently blocks synaptic vesicle exocytosis in many systems, even in the case of the most powerful secretagogue known – latrotoxin from the black widow spider [112, 113]. Recently it has been shown that a DAG lipase-like protein is obligatory for neurotransmitter release in *Drosophila* and may be genetically linked to syntaxin function [8, 9]. It is currently unclear whether the alternative PLA_2 and PLC pathways producing free fatty acids operate together or independently in targeting specific vesicular fusion events.

PUFA activation of the SNARE fusion machinery

It has been shown that PLA_2 acts to 'prime' fusion machinery on the plasma membrane, suggesting up-regulation of SNAREs or SNARE-associated molecules [114]. Interestingly, this priming of vesicle fusion is achieved through the production of arachidonic acid and not lysophospholipid [114]. Recent publications reported immediate effects of arachidonic acid on the SNARE fusion machinery [33–35]. Addition of arachidonic acid to synaptic membranes or membrane treatment with PLA_2s was sufficient to potentiate SNARE complex formation [33, 34]. Analysis of individual SNARE components revealed that it is syntaxin that is sensitive to the presence of two major PUFAs enriched in brain, arachidonic and docosahexaenoic acid [33, 35]. A strong correlation was observed between changes in the structural properties of syntaxin and neuronal growth, providing mechanistic insights into PUFA action in brain development [33]. Although this was demonstrated for syntaxin 3, which mediates vesicle fusion during neuronal growth, it is now clear that syntaxin 1, the major brain isoform, is also sensitive to arachidonic acid [35, 36], suggesting a conserved role for phospholipases in regulating the SNARE fusion machinery.

How does arachidonic acid promote SNARE interactions? The syntaxin structure is conserved throughout evolution [5, 115] and consists of a membrane anchor, SNARE motif and an autonomously folded N-terminal domain (Fig. 1). This latter N-terminal domain folds back over the helical SNARE motif, thereby inhibiting SNARE complex assembly [5, 116, 117]. In this folded conformation, syntaxin binds a cytosolic protein, Munc18, leading to further down-regulation of SNARE assembly [5, 118]. Remarkably, arachidonic acid is able to activate syntaxin in tight association with Munc18, allowing syntaxin engagement of its SNARE partners without abolition of native syntaxin/Munc18 association [34, 35]. It is possible that flexible unsaturated fatty acids can penetrate into hydrophobic grooves between the amphipathic syntaxin helices, loosening the 'closed' syntaxin structure and thereby allowing SNARE complex formation. A similar mode of interaction has been suggested for several fatty acid-binding proteins [86]. Structural studies, *e.g.* a nuclear magnetic resonance approach, are now required to provide an atomic level description of the dynamic interaction between arachidonic acid and syntaxin.

PUFAs exhibit high specificity in up-regulation of syntaxin for entry into the SNARE complex, while saturated fatty acids are ineffective [34, 35]. Since saturated fatty acids form micelles at lower concentrations than PUFAs [87], micelle formation *per se* is not likely to underlie syntaxin activation [35]. The half-maximal effective concentration for activation of syntaxin by arachidonic acid was reported to be around 50 μM [34]. Is such concentration achievable within the cellular environment? A high percentage of phospholipid molecules carry esterified PUFA, meaning that inside the tightly packed lipid bilayer, PUFA concentrations will be in a molar range. Local phospholipase action on phospholipid membranes is likely to result in a transiently high concentration of arachidonic acid at the membrane where syntaxin resides before arachidonic acid diffuses deeper into the cytosol [74]. Direct measurements in endocrine insulin-secreting cells demonstrated that upon stimulation of exocytosis, the global arachidonic acid concentration can reach 50–75 μM [90]. Furthermore, a very high turnover of arachidonic acid has been demonstrated in isolated nerve terminals and neuronal growth cones, which are enriched in PLA_2 [119]. The small volume of these specialized neuronal compartments containing the SNARE fusion machinery suggests that syntaxins can encounter transiently high PUFA concentrations that would be sufficient to stimulate SNARE interactions. It should be noted here that syntaxins may not be the only protein targets of PUFAs in the exocytotic pathway, as the cytoskeleton and potassium channels are sensitive as well [120–125]. The mechanisms underlying PUFA action on ion channels are still not clear and may involve PUFA binding to the channel itself or to auxiliary proteins, or a local change in the lipid environment immediately surrounding the channel [126, 127].

Lipid/protein interface in vesicle fusion

Although we have focused our analysis on SNARE/fatty acid interplay, it may also be useful to consider the

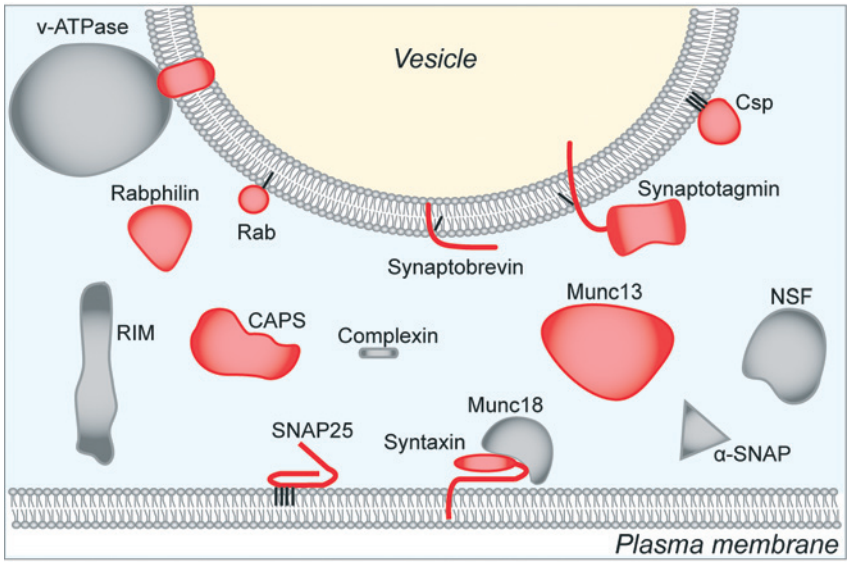

Figure 4. Lipids regulate the function of a wide array of proteins involved in vesicle fusion. Proteins implicated in SNARE-mediated membrane fusion at the synapse are shown. Lipid-modified and/or lipid-interacting proteins are in red. Rabphilin interacts with Rab proteins but also binds phospholipid membranes in calcium-dependent manner owing to the presence of C2 domains. Rab proteins carry a lipid moiety, geranylgeranyl. CAPS (Ca^{2+}-dependent activator protein for secretion) binds PIP2 lipids due to the presence of PH domain. Synaptotagmin is palmitoylated and carries calcium/phospholipid-binding C2 domains that can also interact with PIP2 constitutively. Cysteine-string protein (Csp) carries multiple palmitates. Complexin, Munc18, α-SNAP, RIM and NSF ATPase are not known to be lipid regulated. Munc18 preferentially binds the 'closed' conformation of syntaxin.

wider lipid/protein interface during vesicle fusion. This is especially important, as currently there is belief that proteins are sufficient to both regulate and execute membrane fusion [4]. It is well known that lipids modify protein function in many biological processes, including signal transduction and cell cycle events, tumour growth and cardiovascular disease [128–130]. As the protein requirements for SNARE-mediated vesicle fusion are gradually unravelled, it is becoming clear that many lipid species play important and diverse roles in this process (Fig. 4). In addition to the above-mentioned Munc18, a large multi-domain protein, Munc13, is essential for exocytosis. Munc13 functionally interacts with RIM and syntaxins for SNARE assembly to take place [131–133]. Munc13 binds DAG, generated by PLC, and this binding represents an obligatory step in neurotransmission [134]. DAG binding is mediated by the C1 domain, which is shared between many proteins involved in vesicle trafficking, including protein kinase C [135]. Another common domain in trafficking proteins is the C2 domain, which is responsible for calcium-dependent phospholipid binding and, in some cases, PIP2 binding [136–138]. Vesicular synaptotagmin, which associates with the syntaxin/SNAP-25 heterodimer, carries tandem C2 domains and exhibits phospholipid-binding abilities [139, 140]. In addition, this integral vesicular protein is palmitoylated in the juxtamembrane region [20, 42]. Rabphilin, which also carries phospholipid-binding C2 domains, can interact with both Rab proteins and SNAP-25 to up-regulate exocytosis [141, 142]. Another protein implicated in calcium-triggered SNARE activation is 'calcium-activated protein in secretion', or CAPS for short [143, 144]. CAPS was shown to bind PIP2 with high specificity and affinity via its PH (pleckstrin homology) domain [145]. The yeast SNARE protein Vam7 also contains a phosphoinositide-binding domain called PX [146]. Furthermore, mammalian synaptobrevin has been shown to functionally interact with the phospholipid bilayer through its amphipathic SNARE motif, adding another level of lipid regulation of vesicle fusion [22, 41]. While the above-mentioned trafficking proteins can dynamically interact with phospholipids, another class of proteins is directly modified by soluble lipid moieties, such as palmitoylated SNARE proteins themselves. For example, Vesicular cysteine-string protein (csp), which is involved in regulating exocytosis, carries multiple palmitates [147]. Rab proteins have also been shown to be intimately involved in regulation of SNARE-mediated fusion [148–150], and they are modified by the geranylgeranyl lipid moiety. It is possible that during different stages of vesicle cycling, exposure of the rab geranylgeranyl lipid tail, mediated by a host of rab-associated proteins [151, 152], determines the state of vesicle priming. Clearly not only SNAREs themselves but a whole spectrum of regulatory proteins require lipids to carry out their function in SNARE-mediated vesicle fusion.

Concluding remarks

The last decade has witnessed remarkable progress in our understanding of the protein machinery executing vesicle fusion. We now know that SNARE proteins, which are well conserved in evolution, form four-helical bundles to drive membrane fusion. Until recently, popular models for vesicle fusion relegated membrane lipids to a passive platform. However, it is

now becoming clear that specific lipid components of membrane bilayers play important roles in modulating the activity of proteins that either constitute the membrane fusion machinery itself or regulate its core components (Fig. 4). Accumulating evidence indicates that lipids enter into intimate collaboration with proteins to drive the pivotal step in membrane transport, vesicle fusion. Emerging data emphasize the fact that a dynamic interface exists between activity of the secretory pathway and regulation of lipid metabolism. It still remains to be determined exactly which phospholipase and palmitoyltransferase isoforms assist the core proteins in vesicle fusion. The recent discovery that a ceramidase involved in sphingomyelin metabolism is essential for vesicle fusion [153] further highlights the fact that new aspects of the intimate interplay between the protein fusion machinery and lipids are yet to be elucidated.

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