FEBS Letters 583 (2009) 3817–3826

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Multiple roles of the vesicular-SNARE TI-VAMP in post-Golgi and endosomal trafficking

Mathilde Chaineau, Lydia Danglot, Thierry Galli *

Membrane Traffic in Neuronal and Epithelial Morphogenesis', INSERM U950, Paris F-75013, France
Program in Development and Neurobiology, Institut Jacques Monod, CNRS UMR7592, University Denis Diderot/Paris 7, Paris F-75013, France

ARTICLE INFO

Article history:
Received 20 July 2009
Revised 9 October 2009
Accepted 13 October 2009
Available online 20 October 2009

Edited by Antonella De Matteis

Keywords:
SNARE attachment protein receptor
TI-VAMP associated membrane protein
Post-Golgi
Traffic
Endosomal pathway
Secretion

ABSTRACT

SNARE (Soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins are the core machinery of membrane fusion. Vesicular SNAREs (v-SNAREs) interact with their target SNAREs (t-SNAREs) to form SNARE complexes which mediate membrane fusion. Here we review the basic properties and functions of the v-SNARE TI-VAMP/VAMP7 (Tetanus neurotoxin insensitive-vesicle associated membrane protein). TI-VAMP interacts with its t-SNARE partners, particularly plasmalemmal syntaxins, to mediate membrane fusion and with several regulatory proteins especially via its amino-terminal regulatory Longin domain. Partners include AP-3, Hrb/(Human immunodeficiency virus Rev binding) protein, and Varp (Vps9 domain and ankyrin repeats containing protein) and regulate TI-VAMP's function and targeting. TI-VAMP is involved both in secretory and endocytic pathways which mediate neurite outgrowth and synaptic transmission, plasma membrane remodeling and lysosomal secretion.

1. Introduction

Membrane fusion is implicated in several important functions of mammalian cells including hormone and neurotransmitter release, membrane receptors recycling, and viral infection. It is a highly regulated mechanism which enables the merger of two different cellular membrane compartments and the pooling of their contents. SNARE proteins constitute the core machinery of membrane fusion: vesicular SNAREs (v-SNAREs), localized on vesicles, interact with their target SNAREs (t-SNAREs) partners, localized on intracellular membranes, to form a membrane-bridging SNARE complex, responsible for membrane fusion.

1.1. Identification of SNARE proteins

The first SNARE complex was identified in brain extracts [1]. Rothman and coworkers identified the synaptic SNARE complex composed of the t-SNAREs SNAP-25 and syntaxin 1 and the v-SNARE synaptobrevin2/VAMP2. Since this characterization, a very large number of studies have shown the fundamental role played by SNARE proteins in many membrane fusion events in eukaryotes. The SNARE family is composed of about 36 members in human, 25 in the yeast *Saccharomyces cerevisiae* and 54 in the plant *Arabidopsis thaliana*. SNARE proteins are short proteins of 100–300 amino acids, with a highly conserved SNARE domain, directly responsible for the formation of SNARE complexes. Most of them are anchored to membranes via a transmembrane domain located at the C-terminal end of the protein.

Because the general mechanism of SNARE-mediated membrane fusion has been recently described in several reviews [2,3], we will emphasize here on the role of the Tetanus neurotoxin insensitive v-SNARE TI-VAMP (Tetanus neurotoxin insensitive-vesicle associated membrane protein) also called VAMP7, in cell morphogenesis and remodeling. We will first describe the interacting proteins of TI-VAMP and the cargos transported by TI-VAMP vesicles, and finally we will discuss the function of TI-VAMP in the secretory and endocytic pathways.

Abbreviations: SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; t-SNARE, target SNARE; v-SNARE, vesicular-SNARE; TI-VAMP, Tetanus neurotoxin insensitive-vesicle associated membrane protein; SNAP, synaptosome associated protein; VAMP, vesicle associated membrane protein; Hrb, human immunodeficiency virus Rev binding protein; Varp, Vps9 domain and ankyrin repeats containing protein; TGN, trans-Golgi Network; ER, endoplasmic reticulum; SV, synaptic vesicle

* Corresponding author. Address: Institut Jacques Monod, Bat. Buffon, 15 rue Hélène Brion, 75205 Paris Cedex 13, France. Fax: +33 157 278 036.
E-mail address: thierry.galli@inserm.fr (T. Galli).
URL: http://sites.google.com/site/insermu950/ (T. Galli).

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.10.026

2. TI-VAMP

2.1. Gene and protein

V-SNAREs are defined by two main subfamilies: the "Brevin" family, which includes short v-SNAREs (represented by synaptobrevin2/VAMP2, cellubrevin/VAMP3 and endobrevin/VAMP8) and the "Longin" family, characterized by an N-terminal extension called the Longin domain, as in the case of TI-VAMP/VAMP7, Sec22, Ykt6 and the yeast protein Nyv1p [4,5].

TI-VAMP is derived from the SYBL-1 (Synaptobrevin-like 1) gene, localized in the pseudoautosomal region at the termini of the long arm of the X chromosome (XqPARp) [6]. SYBL-1 gene is ubiquitously expressed (http://www.genecards.org/cgi-bin/carddisp.pl?gene=VAMP7) and is extremely conserved. Homologues were identified in rat, mice, non human primates, and also in plants and flies. SYBL-1 undergoes X inactivation but it is also transcriptionally inactive on the Y chromosome [6], due to epigenetic mechanisms [7]. SYBL-1 encodes a 2576 bp cDNA, generating a protein of 220 amino acids and approximately 25 kDa. In contrast to VAMP2 and VAMP3, TI-VAMP is resistant to clostridial neurotoxins such as tetanus and several isotypes of botulinum (B, D, F and G) neurotoxins. The insensitivity to botulinum neurotoxin B is due to at least 12 amino acids changes from the sequence of VAMP2 [8,9]. TI-VAMP is also characterized by an amino-terminal extension of 120 amino acids called the Longin domain [4]. Recently, the corresponding mRNA has been found in pseudopodia of murine fibroblasts responding to migratory stimuli, thus suggesting that TI-VAMP could be synthesized in pseudopodia of migrating cells [10]. SYBL-1 is composed of eight exons and the corresponding protein TI-VAMP is composed of three domains (Fig. 1): the Longin domain, that plays a regulatory role as we will describe later, a SNARE domain, directly involved in the formation of SNARE complex, and a tail-anchor transmembrane domain.

The Longin domain adopts a globular "profilin-like" structure, similar to the profilin domain of the human SEDL protein [4]. Interestingly, the Longin domain of Ykt6 can adopt a closed conformation similar to that of syntaxins, and the Longin domain of Ykt6 folds back onto its SNARE domain to prevent the formation of SNARE complexes [11]. A similar mechanism has recently been observed for the Longin domain of Sec22 [12] and suggested for that of TI-VAMP [13]. The Longin domain of TI-VAMP plays an important role in both the localization and function of the protein. Indeed, it has an inhibitory action on the formation of SNARE complexes composed of TI-VAMP and its t-SNAREs partners [14]. Furthermore, its interaction with the δ subunit of the adaptor AP-3, is responsible for the correct targeting of TI-VAMP to late endosomes in non-neuronal cells [14] and to presynaptic sites of mossy fibers (MF) in hippocampus [15]. Interestingly, some splicing isoforms of TI-VAMP have been described. One of them, TI-VAMPc is deleted from approximately one-third of its Longin domain [4], and does not interact with the δ delta subunit of AP-3 [14]. This short Longin domain, however, still inhibits the interaction with t-SNARE partners like the full-length one. Therefore, the AP-3 dependent targeting and the auto-inhibitory SNARE interaction functions of the Longin domain of TI-VAMP appear

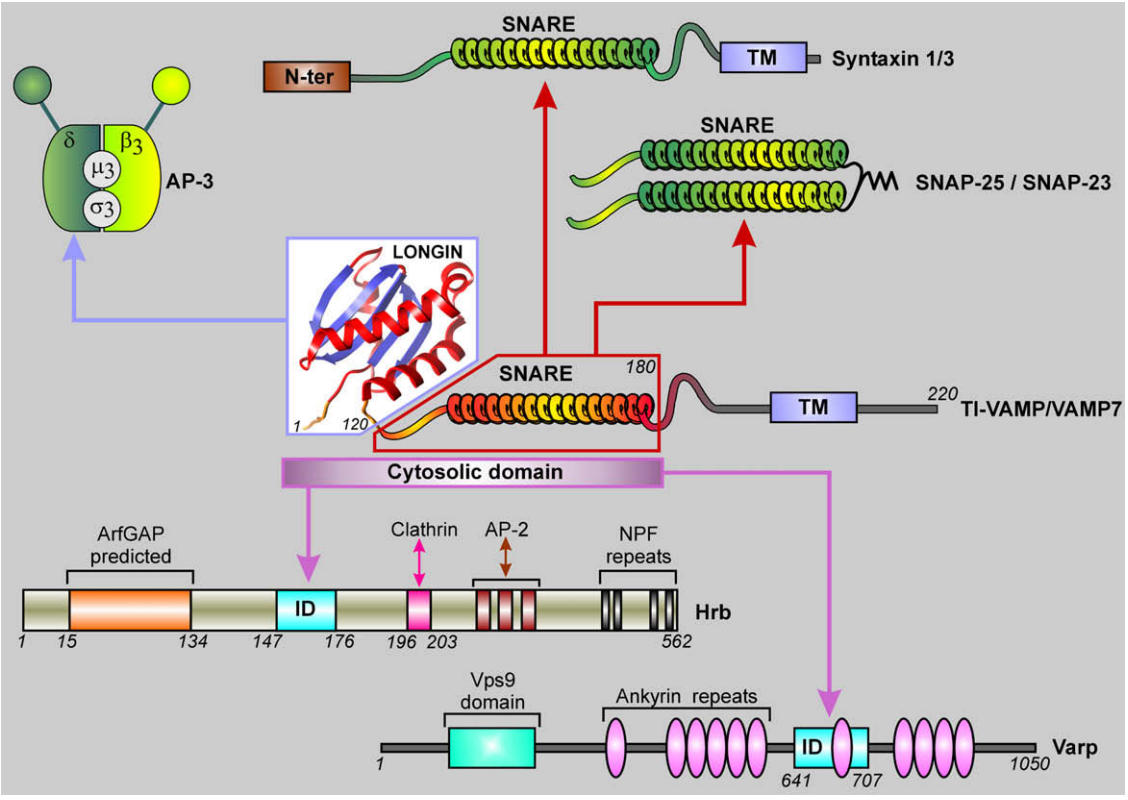

Fig. 1. Partners of TI-VAMP. The different known partners of TI-VAMP are represented. Via its SNARE domain, TI-VAMP interacts with its t-SNARE partners syntaxin 1 and SNAP-25 and syntaxin 3 and SNAP-23 to form SNARE complexes in the brain and in epithelial cells, respectively. The Longin domain of TI-VAMP adopts a "profilin-like" structure, represented here by in silico prediction. Via its Longin domain, TI-VAMP interacts with the δ subunit of the adaptator AP-3 and this interaction regulates the late endosomal localization of TI-VAMP. Via its cytosolic domain, TI-VAMP interacts with Hrb and Varp. Hrb regulates TI-VAMP's endocytosis in a clathrin-dependent pathway. An ArfGAP domain localized at the N-terminal end of Hrb has been predicted from in silico analysis (orange). In addition to its interacting domain with TI-VAMP (ID), Hrb also interact with clathrin (pink), AP-2 (brown) and Eps15 via its NPF repeats (Asp-Pro-Phe, in gray). Varp regulates neurite outgrowth in association with TI-VAMP. Varp is also composed of a Vps9 domain (blue) that it is responsible for the GEF activity of the protein for Rab21 and of 11 ankyrin repeats (pink). For Hrb, the ID has been deduced from [13,33]. TM: transmembrane domain.

largely independent from each other [14]. Other Longin domains are also important for the correct subcellular localization of SNARE proteins [16,17].

2.2. TI-VAMP partners and cargos

2.2.1. Partners

TI-VAMP interacts with several partners. Most of them have been identified in yeast-two-hybrid screens and confirmed by biochemical experiments. TI-VAMP interacts with several t-SNAREs (depending upon the cell/tissue type) and with several proteins involved in its regulation (Fig. 1).

2.2.1.1. t-SNAREs. TI-VAMP interacts with both plasma membrane and endosomal t-SNAREs. In neurons, TI-VAMP interacts with syntaxin 1 and SNAP-25 [14,18]. In fibroblasts, TI-VAMP interacts with syntaxin 4 and SNAP-23 [14,19]. The calcium-sensor protein synaptotagmin VII likely interacts with the resulting SNARE complex in a calcium-dependent manner [19]. TI-VAMP also forms stable complexes with syntaxin 4 and SNAP-23 at the plasma membrane of human mastocytes [20], with syntaxin 3 in rat mastocytes [21], and with syntaxin 3 and SNAP-23 at the apical side of the epithelial Caco2 cells [8]. Finally, TI-VAMP also interacts with the endosomal t-SNARE complex composed of syntaxin 7, syntaxin 8 and vti1b in fibroblasts and brain extracts [18,22,23].

2.2.1.2. δ AP-3. Yeast two hybrid screens proved to be particularly efficient to identify TI-VAMP partners, particularly the δ subunit of the clathrin adaptor AP-3 via its interaction with the Longin domain [14]. This finding was confirmed by Luzio and coworkers [13]. The molecular coat AP-3 is expressed in endosomes and is involved in the biogenesis of specialized lysosomes related compartments such as melanosomes, platelet granules, or azurophilic granules as well as in the biogenesis of synaptic vesicles (see [24] for review).

AP-3 is composed of four subunits: δ, β_3, μ_3 and σ_3. There are two isoforms of β_3, μ_3 and σ_3 called A and B. β_3B and μ_3B are specifically expressed in neurons and neuroendocrine cells, and define the AP3-B complex. An ubiquitous AP3-A, composed of β_3A and μ_3A also exists. Interestingly, although the clathrin binding domain of the β_3 subunit is conserved, it is not yet clear whether β_3 binds to clathrin in vitro [25,26]. AP-3 is localized in early endosomes derived tubules [27] and is involved in the traffic through late endosomes and lysosomes [28]. AP-3 depleted cells show defects in the sorting of several lysosomal proteins such as LAMP-1, -2 and -3/CD63 and LIMP-2 [27,29,30].

In non-neuronal cells, the interaction of TI-VAMP with AP-3 is responsible for the correct subcellular localization of TI-VAMP in late endosomes, where TI-VAMP colocalizes with CD63 [14] and LAMP-1 [31]. In *mocha* cells, which don't express the δ subunit of AP-3, TI-VAMP is abnormally retained in early and recycling endosomes, but it reaches its correct localization following re-expression of the δ subunit [14]. An important role of AP-3 in the regulation of synaptic vesicles exocytosis in hippocampal mossy fibers terminals has been proposed from electrophysiological recordings of spontaneous and evoked release in *mocha* hippocampal slices [15]. The interaction between TI-VAMP and AP-3 therefore appears as a prototypical example of SNARE-adaptor interactions similar to that observed between VAMP4 and AP-1 [32]. The generality of such mechanism for v-SNARE targeting remains however to be demonstrated.

2.2.1.3. Hrb: Human immunodeficiency virus Rev Binding protein. Our yeast-two hybrid screens [14] also revealed that TI-VAMP interacts with Hrb, also called hRIP for human Rev Interacting Protein or RAB for Rev/Rex Activation-domain Binding protein [33] as was also found by Pryor et al. [13]. Hrb was first identified as a cellular cofactor of the Rev viral protein [34,35]. Structurally, from the N-terminal to the C-terminal end of the protein, Hrb is composed of (i) an in silico ArfGAP predicted domain (Fig. 1), (ii) domains mediating interactions with the endocytic proteins clathrin and AP-2 [13], (iii) four NPF repeats, responsible for the interaction of Hrb with Eps15 [36,37] and (iv) several FG repeats, classically found on nucleoporins. Despite being an important potential function of Hrb, the ArfGAP activity still remains to be experimentally demonstrated. Hrb has also been characterized for its role in the fusion of Golgi-derived vesicles to form the acrosome during spermiogenesis. Male mice lacking Hrb were sterile because vesicles were not able to fuse, resulting in spermatozoids lacking acrosome [38]. Hrb is expressed in different cell types and in brain during development. In HeLa cells, Hrb is expressed in the nucleus and in the cytoplasm where it shows a vesicular pattern, and colocalizes with clathrin, AP-2 and Eps15. Hrb thus appears as a component of clathrin-coated structures [33]. The interaction of Hrb and TI-VAMP is labile and of weak affinity [13,33] thus it is likely to be transient. RNAi experiments showed that Hrb is involved in the endocytosis of TI-VAMP [13,33]. It is also of interest to note that a role of AP-2 in TI-VAMP subcellular localization was further found in *Dictyostelium* [39]. Hrb is more generally involved in clathrin-dependent endocytosis. In fact, we have observed an inhibition on transferrin endocytosis in Hrb silenced cells [33], that depends on the level of expression of Hrb, further demonstrating that Hrb is a regulator and not an indispensable factor of clathrin-dependent endocytosis, in agreement with the viability of Hrb −/− mice. A competition between the SNARE domain and Hrb for binding to the Longin domain of TI-VAMP has been proposed because the same amino acids of the Longin domain would be involved in both interactions [13]. According to this model, the interaction of Hrb with TI-VAMP would be possible only when TI-VAMP is already complexed with its t-SNARE partners, thus exposing the Longin domain and allowing it to interact with Hrb [13]. We found however Hrb in yeast two hybrid screens and GST-pull down experiments using the whole cytoplasmic domain of TI-VAMP as well as the Longin domain alone [33]. Therefore, an alternative hypothesis would be that Hrb interacts with TI-VAMP cis-multimers in which Longin domains are also exposed. In any case, the role of Hrb in clathrin-dependent endocytosis of TI-VAMP and that of AP-3 in TI-VAMP sorting further supports the notion that molecular coats and their regulators play a role in SNARE targeting.

2.2.1.4. Varp: Vps9 domain and ankyrin repeats containing protein. In addition to AP-3 [14] and Hrb [33], we also found the Varp protein (for Vps9 domain and ankyrin repeats containing protein) in our yeast two hybrid screens, using the full cytoplasmic domain of TI-VAMP as a bait [40]. Varp is a Guanine Exchange Factor (GEF) for Rab21 [41] and shows a ubiquitous pattern of expression. As TI-VAMP, Rab21 is involved in phagocytosis, cell migration, mitosis [42–44] and neurite growth [40]. Interaction between Varp and TI-VAMP was confirmed by biochemical experiments in vitro and in vivo [40]. The interaction domain of Varp with TI-VAMP is localized within a region containing ankyrin repeats [45], downstream of the Vps9 domain which is responsible for the GEF activity of Varp for Rab21 (see Fig. 1). Interestingly, Rab21 and TI-VAMP colocalize in the Trans Golgi Network (TGN), suggesting that Varp could regulate a functional link between TI-VAMP and Rab21 at the exit of the TGN [40]. Indeed, silencing the expression of Varp by siRNA leads to a decrease of the colocalization of TI-VAMP and Rab21 in this perinuclear area. Live cell imaging experiments further suggest a co-transport of Varp and TI-VAMP in the same vesicles in the neurites of mouse hippocampal neurons [40]. Therefore the TI-VAMP/Varp interaction could regulate vesicular transport from the soma to growing neurites. Furthermore, silencing

the expression of Varp, or expressing solely its domain of interaction with TI-VAMP results in decreased neurite and axonal growth in cultured PC12 cells and mouse hippocampal neurons, respectively [40]. Thereby, Varp is, like TI-VAMP, an important regulator of neurite growth. Since Varp is also an effector of Rab32 and Rab38 [46,47], it appears as a molecular adaptor connecting several Rab proteins (Rab 21, 32, 38) with the v-SNARE TI-VAMP.

Altogether, yeast two hybrid screens enabled us to establish molecular links between TI-VAMP and molecular coats (directly with AP-3 and through Hrb with AP-2) as well as between TI-VAMP and Rab proteins (through Varp). This is particularly interesting because SNAREs, coats and Rabs constitute some of the crucial elements in vesicular trafficking [48].

2.3. Cargos

2.3.1. L1-cam

The Cell Adhesion Molecule (CAM) L1 is involved in axonal growth and cell motility [49]. During axonal growth, L1 is actively recycled at neuronal plasma membrane [50]. The formation of L1-dependent homophilic contacts depends on both lateral diffusion of L1 molecules pre-existing at the cell surface, and transport of new molecules from an endosomal pool [51]. In embryonic rat brain and PC12 cells, L1 colocalizes with TI-VAMP. In PC12 cells, there is a pool of L1 localized in vesicular structures where TI-VAMP is also expressed, in the cell body as well as at the tip of growing neurons. In addition, in antibody uptake experiment of Fab directed against L1, it has been shown that the endocytosed L1 fraction is localized in a perinuclear structure where TI-VAMP is also present [18]. Finally, in primary neurons cultures, the endocytosed L1 fraction colocalizes with TI-VAMP structures along the axon. Altogether, these results show that L1 is a cargo of TI-VAMP, specifically endocytosed in TI-VAMP compartments. It is also important to note that a strong functional link between both proteins exists because silencing the expression of TI-VAMP leads to a defect in cell adhesion, due to a specific lack of L1- but not N-cadherin-dependent contact formation [18].

2.3.2. MT1-MMP

The proteolytic degradation of extracellular matrix is an intrinsic property of metastatic cells. This process involves actin enriched protrusions localized at the plasma membrane called invadopodia [52]. MT1-MMP (for Membrane Type 1-Matrix Metalloproteinase Protein) is one of the proteases that allow the invadopodia to degrade the matrix and thereby cell colonization of other tissues (see [53,54] for reviews). MT1-MMP colocalizes with TI-VAMP in late endocytic and lysosomal structures and is carried by TI-VAMP vesicles to degradation sites [55]. Silencing the expression of either MT1-MMP or TI-VAMP in different cell types inhibits the degradation capacity of these cells on extracellular matrix [55]. Finally, TI-VAMP is also involved in a positive feedback on the invadopodia formation by regulating the transport and the exocytosis of MT1-MMP that is also involved in the formation and in the maturation of invadopodia [55].

2.3.3. LewisX

LewisX (Le^X) is a fucosylated carbohydrate synthesized by the fucosyltransferase IX [56] and it is one of the most abundant carbohydrates expressed in human brain. Its expression is temporally and spatially regulated during development of the central nervous system and Le^X is involved in neuronal adhesion and neurite outgrowth [57,58]. In neuronal NT2N cells, its expression is enhanced during neuronal differentiation and Le^X is expressed at the cell surface, in lysosomes, and in a TI-VAMP positive compartment. In rat hippocampal neurons, Le^X is found in TI-VAMP structures after 7 days in vitro (*div*). Later, at 14 *div*, it appears in synaptic and extrasynaptic sites of glutamatergic and GABAergic neurons, respectively. In these neurons, Le^X colocalizes with TI-VAMP in the soma, and most extensively in punctate structures along the neurites. A colocalization of Le^X and TI-VAMP has also been reported to a lesser extent, in the growth cone. These results suggest that Le^X is carried by one or several cargos of TI-VAMP [59].

2.3.4. CD82

The tetraspanin CD82, also known as KAI-1, is a wide-spectrum tumor metastasis suppressor [116]. Expression of this gene has been shown to be down regulated in the progression of human tumors, and the loss of expression of CD82 is associated with poor survival for prostate cancer patients [117,118]. CD82 is localized at the plasma membrane, interacts with other tetraspanins and associates with proteins involved in cell migration, such as cell adhesion molecule, growth factor receptors, and signaling molecules. CD82 directly associates with the EGF receptor (EGFR) [119]. We find that depletion of TI-VAMP is correlated with decreased amounts of CD82 at the plasma membrane, with increased endocytosis of EGFR, and impaired signaling of MAPK. Fast confocal video microscopy indicates that TI-VAMP positive vesicles colocalize in time and space with CD82 molecules suggesting that they are transported by the same vesicles [60].

We do not have a full view of TI-VAMP cargos at this point but it appears, from the above cited studies, that they include molecules which are involved in, or regulate cell adhesion and signaling at the cell surface. This further indicates that TI-VAMP is primarily a secretory v-SNARE, and suggests important connections to cell signaling.

3. TI-VAMP pathways

3.1. TI-VAMP distribution in intracellular compartments

At the optical level, TI-VAMP was shown to colocalize with late endosome/lysosomal markers (CD63 and LAMP-1, respectively), and to slightly colocalize with early endosomes, labeled with the Transferrin Receptor (TfR) in HeLa and NIH3T3 cells [14,31]. However, the localization of TI-VAMP shift to TfR positive compartments when the Longin domain of TI-VAMP was deleted, suggesting a role of the Longin domain in TI-VAMP localization [14]. Accordingly, in *mocha* cells lacking AP-3δ, TI-VAMP is retained in an early endosomal compartment indicating that TI-VAMP needs to interact with the adaptor complex AP-3 to reach late endosomes [14,24]. TI-VAMP immunogold labeling was detected in tubulovesicular structures of 20–50 nm in diameter (60.8% of the labeling), in LDCVs (Large Dense Core Vesicles, 31.0% of the labeling), and occasionally on Golgi stacks in PC12 cells [61]. Within the endosomal compartments, TI-VAMP localizes mainly in late endosomes (50%), and also in early endosomes (5%) and lysosomes (5%) [31]. In both studies, Golgi stacks staining was detected in a proportion of 8%, but Advani and coworkers [31] also reported a 30% of labeling within the TGN region.

In PC12 cells, TI-VAMP shows a punctate pattern in the cytoplasm, colocalizing with the lysosomal marker CD63. After nerve growth factor (NGF) treatment, it is found in neuritic extensions. In primary hippocampal cultures of neuronal cells, TI-VAMP localizes in vesicles in the axonal and dendritic compartments and is mainly expressed at the tip of the growth cone after 2 *div* [61]. The localization of TI-VAMP in the actin-rich region of growth cones is regulated by Cdc42 [62], a key player in neuronal cell polarity (see [63] for review).

After the onset of synaptogenesis, TI-VAMP is mainly found at extrasynaptic sites in the somatodendritic compartments of pyramidal cells [15]. In contrast, the protein was expressed at the presynaptic level in cultured granules neurons of the dentate gyrus,

indicating that its localization can vary from one neuronal type to another [15]. In the adult brain, TI-VAMP is widely distributed, and mainly somatodendritic. However, here again, a subset of neuronal cell types express TI-VAMP in axonal compartments and presynaptic sites [64]. Four distinctive populations of labeled axon terminals were identified: (i) the hippocampal mossy fibers (MF) of the granule cells of the dentate gyrus, (ii) the striatal peridendritic terminal plexuses in the globus pallidus (GP), substantia nigra pars reticulata (SNr), (iii) the peridendritic plexuses in the central nucleus of the amygdala, and (iv) the primary sensory afferents in the dorsal horn of the spinal cord. Interestingly, we found that in *mocha* mice, the synaptic localization of TI-VAMP in MF is lost. Indeed, TI-VAMP was blocked in the peri-Golgi region of the granule cells [15,24]. These results are reminiscent from the one in fibroblasts, where TI-VAMP is blocked in the early endososomes in the absence of AP-3 [14]. Altogether, these results indicate that AP-3 is important for the targeting of TI-VAMP in different compartments, depending upon cell types, which are late endosomes in epithelial cells, and synaptic vesicles in certain neuronal cells. The potential relationship between non-neuronal late endosomes and neuronal synaptic vesicles is still unclear at this point.

Overall, TI-VAMP localizes into different post-Golgi compartments and its subcellular localization varies in different cell types. Further studies at the ultrastructural level and in live cells are required to further understand the life cycle of TI-VAMP.

3.2. TI-VAMP dependent membrane trafficking pathways

3.2.1. Prechylomicron trafficking

TI-VAMP has been shown to be expressed in the endoplasmic reticulum (ER) in rat enterocytes, whereas it is absent in ER of liver or kidney [65]. The brush border of intestinal cells is dedicated to the absorption of lipids and nutrients. Tri-acyl glycerol (TAG) is absorbed and reaches the ER of enterocytes. TAG is then incorporated into lipoproteins, called chylomicrons. The chylomicrons then exit the ER in specialized vesicles (PCTV, prechylomicron transport vesicle) which travel to the cis-Golgi by anterograde transport. TI-VAMP has been shown to be concentrated in PCTVs and to colocalize with ER proteins like Sar1 and rBet1. Anti-TI-VAMP antibodies reduce the transfer of TAG from the ER to the Golgi by 85%. The SNARE complex associated to PCTV docked on cis-Golgi was shown to contained TI-VAMP, Syntaxin 5, Vti1a and rBet1 [66]. This function of TI-VAMP appears very specific of enterocytes but it is puzzling in the context of post-Golgi roles in most other cell types.

3.2.2. Golgi to cell surface

As discussed above, TI-VAMP is present mainly in the Golgi apparatus (40%), and in late endosomes/lysosomes (55%) in most cells. Thus one could envision that TI-VAMP may have a role both in trafficking to and/or from the Golgi apparatus and also in the endocytic pathway (Fig. 2). The mammalian post-Golgi system involves at least five v-SNAREs: VAMP2, VAMP3, VAMP4, TI-VAMP, and VAMP8. VAMP3 localizes to early and recycling endosomes and is involved in plasmalemma receptor recycling [67–69]. VAMP4 localizes to the TGN and is involved in early endosome to TGN transport [70,71], and possibly in homotypic fusion of early endosomes [72]. VAMP8 is involved in secretory mechanisms in specialized cells as well as in the endosomal system [73–76].

One model used to study VAMP post-Golgi trafficking has been the insulin-stimulated translocation of the facilitative glucose transporter (GLUT4) from intracellular vesicular compartments to the plasma membrane in myocytes [77] or adipocytes [78]. At basal state, the glucose transporter is mainly located in intracellular compartments. Upon stimulation by insulin, there is a marked increased exocytosis of GLUT4 at the plasma membrane, allowing glucose to be internalized more efficiently [79,80]. Insulin and hypertonicity each increase the content of GLUT4 glucose transporters at the surface of muscle cells. GLUT4 has been shown to be solicited by insulin and hypertonicity to recruit overlapping but distinct sources defined by VAMP2 and TI-VAMP, respectively [81,82]. Requirement of VAMP2 and TI-VAMP for insulin and os-

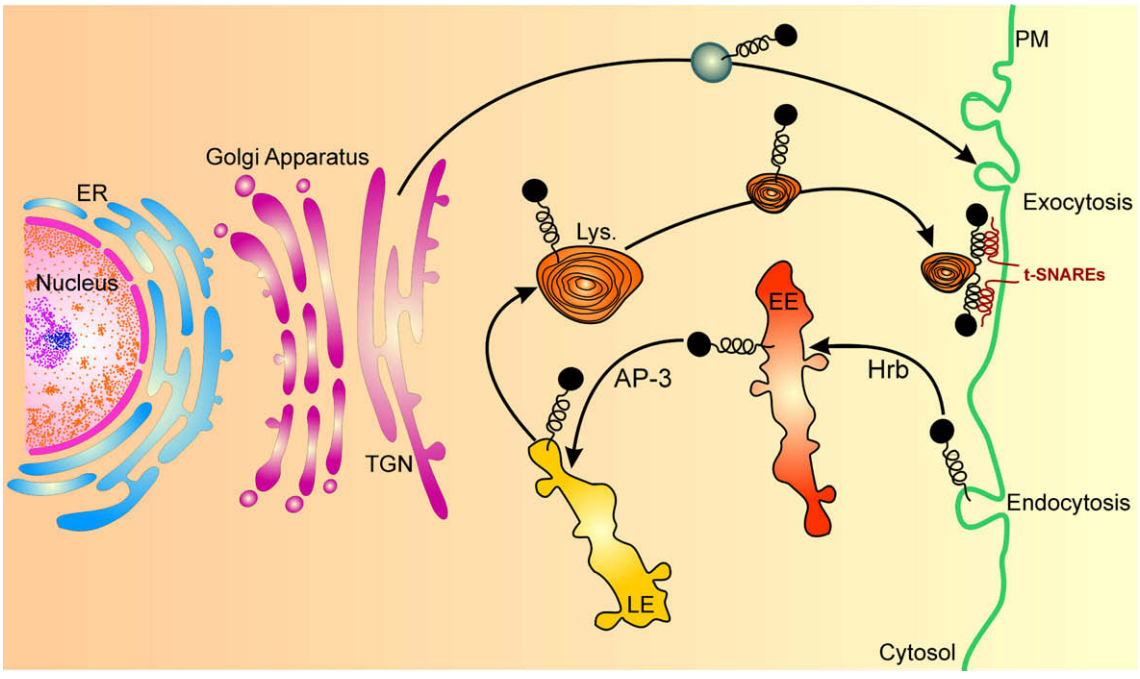

Fig. 2. TI-VAMP routes. The main TI-VAMP routes are represented. TI-VAMP is involved in the transport from the Golgi to the cell surface. After mediating exocytic events via pairing with its t-SNAREs at the plasma membrane, TI-VAMP is endocytosed in a clathrin-dependent manner by interacting with Hrb, to be included in clathrin-coated structures. In early endosomes, TI-VAMP interacts with AP-3 to reach late endosomes and lysosomes. Then, TI-VAMP mediates lysosomal secretion. ER: endoplasmic reticulum, TGN: trans-Golgi Network, Lys: lysosome, EE: early endosome, LE: late endosome.

motic shock respectively was confirmed in adipocytes [78]. Moreover, VAMP4 was required for the initial biosynthetic entry of GLUT4 from the Golgi apparatus into the insulin-responsive vesicle compartments, VAMP8 for plasma membrane endocytosis, and VAMP2 for sorting to the specialized insulin-responsive compartment after plasma membrane endocytosis. TI-VAMP was also shown to mediate constitutive exocytosis of growth hormone from Golgi apparatus in human parotid epithelial HSY cell line [83].

Recently, we showed that TI-VAMP is necessary for the general exocytosis from the Golgi apparatus to the cell surface in HeLa cells. We used the VSVG-GFP thermosensitive mutant and we showed that the appearance of vesicular stomatitis virus G-protein (VSVG) at the cell surface is significantly delayed upon depletion of TI-VAMP by siRNA. These results suggest that TI-VAMP may mediate a fusion process between secretory vesicles derived from the Golgi apparatus and the plasma membrane [60]. TI-VAMP depletion thus leads to decreased cell surface expression of proteins including the tetraspanin CD82 and the CAM L1. TI-VAMP was also shown to mediate the direct apical delivery of both raft- and non-raft-associated proteins in epithelial polarized cells, whereas VAMP8 was involved in the transcytotic pathway sorting [84]. Finally, pHluorin imaging experiments showed that TI-VAMP mediated exocytosis is positively regulated by Cdc42 and actin dynamics [62].

3.2.3. Endocytic pathway: from late endosomes to lysosomes

TI-VAMP was previously proposed to mediate the transport from endosomes to lysosomes on the basis of the 25% inhibitory effect of anti-TI-VAMP polyclonal antibodies on EGF degradation in permeablized cells [31]. From in vitro studies, it was proposed that TI-VAMP mediates heterotypic fusion between endosomes and lysosomes, whereas VAMP8 mediates homotypic fusion [23,85]. In our hands, TI-VAMP depletion by siRNA in HeLa cells enhances EGF endocytosis but only slightly delay degradation of the EGFR, suggesting that EGFR is still able to reach the lysosomal degradative compartments in the absence of TI-VAMP. These results seem to indicate that, if TI-VAMP mediates the fusion between endosomes and lysosomes as suggested by in vitro experiments [23,31], TI-VAMP's function may be redundant in this pathway (possibly with VAMP8), thus enabling EGF to still reach lysosomes in the absence of TI-VAMP in vivo.

3.2.4. From late endosome to the Golgi apparatus

TI-VAMP was also shown to co-immunoprecipitate with syntaxin 7 in neurons [18], in melanoma cells [86] and in solubilized rat liver membranes [23]. Syntaxin 7 is part of a t-SNARE complex comporting also syntaxin 8 and vti1b [87]. Interestingly, a role for syntaxin 8 in the early endosomes to the late endosome transport was suggested by the effect of anti-syntaxin 8 antibodies on EGFR trafficking in Streptolysin-O permeabilized HeLa cells [88]. Moreover, syntaxin 8 has been localized mainly in the TGN and in late endosomes and lysosomes [88]. From this, it was conceivable that TI-VAMP might mediate TGN-endosome transport. However no data so far support this hypothesis. Mannose-6–phosphate receptors (M6PR) are transported from endosomes to Golgi after delivering lysosomal enzymes to the endocytic pathway. This pathway has been recently shown to require SNARE complexes composed of syntaxin 10, 16, Vti1a and VAMP3. Soluble syntaxin 5, 6, 11, 13, Vti1b, TI-VAMP and VAMP8 were without effect on in vitro transport reaction recapitulating this route [89]. These data suggest that TI-VAMP may not be implicated in M6PR retrograde transport. Moreover TI-VAMP transport vesicles examined by electron microscopy were previously shown to be devoid of mannose M6PR [31]. In mammalian cells, two SNARE complexes have been implicated in the transport of Shiga toxin from early and/or recycling endosomes to the TGN. The first one is composed of GS15 (Golgi SNARE of 15 kDa), syntaxin 5, GS28 (Golgi SNARE of 28 kDa) and Ykt6 [90]. The second one is composed of the v-SNAREs VAMP4 or VAMP3 and the t-SNAREs syntaxin 16, syntaxin 6 and Vti1a [70]. Syntaxin 6/syntaxin 16/Vti1a interacts with VAMP3 and VAMP4, but not with TI-VAMP or VAMP8. VAMP3 and VAMP4 have been suggested to form separate molecular complexes. Thus, we can conclude that TI-VAMP does not participate to retrograde transport of M6PR or Shiga toxin toward the TGN. Therefore, only a redundant function with VAMP8 in late endosome to lysosome transport could be assigned to TI-VAMP in the late endosomal system.

3.2.5. Lysosomal secretion

TI-VAMP has also been implicated in lysosomal secretion in different cell types. For example, TI-VAMP has been involved in fusion of vesicles with plasma membrane necessary for phagocytosis [91] and in lysosomal and granule secretion in polarized cells [83,84,92–96]. TI-VAMP is also involved in secretion in more specialized cells (see Section 4.3).

Overall, TI-VAMP appears mainly as a secretory v-SNARE and it may function in a redundant manner in other pathways, particularly with VAMP8 in the endosomal system.

4. Cellular functions

By silencing experiments using RNA-mediated interference or by using the expression of its Longin domain as a dominant negative, multiple roles of TI-VAMP in different cell types have been deduced (Fig. 3). Molecular mechanism involved in the inhibitory action of an exogenous Longin domain is not yet known but it could result from the trapping of partners like AP-3, Hrb and other still unknown partners, or from its binding to endogenous TI-VAMP itself.

4.1. Apical transport

Composition and function of apical and basal plasma membrane of polarized epithelial cells are different and maintained by specific apical and basolateral transport routes (see [97] for review). A role for TI-VAMP in the apical transport has been reported in Caco-2 and MDCK cells. In polarized Caco-2 cells, TI-VAMP forms SNARE complexes with its t-SNAREs partners syntaxin 3 and SNAP-23 [8], and antibodies against TI-VAMP inhibit transport to the apical surface in polarized MDCK cells [98]. More recently, it has been shown by RNAi experiments that the knock-down of TI-VAMP results in the mislocalisation to the basolateral side of several GPI-anchored proteins which are normally expressed at the apical surface [84].

4.2. Neuronal outgrowth and synaptic transmission

Consistent with its localization in neuronal growth cones, TI-VAMP plays a role in neurite outgrowth. Indeed, the expression of exogenous Longin domain of TI-VAMP has a dominant negative effect on neuritogenesis, contrary to a mutant deleted from the Longin domain which overexpression enhances neurite outgrowth in PC12 cells [94]. Finally, these mutants affect the outgrowth of both axons and dendrites in rat hippocampal neurons cultures. These results indicate that TI-VAMP is involved in neurite outgrowth and that its Longin domain plays a regulatory function in this process. This is further confirmed by the effect of the silencing of TI-VAMP by RNAi, which result in a decreased neurite outgrowth in both PC12 cells and in rat hippocampal neurons [18]. Quantitative analysis and mathematical modelisation of vesicular transport in growing neurites showed that the flux of TI-VAMP but not that

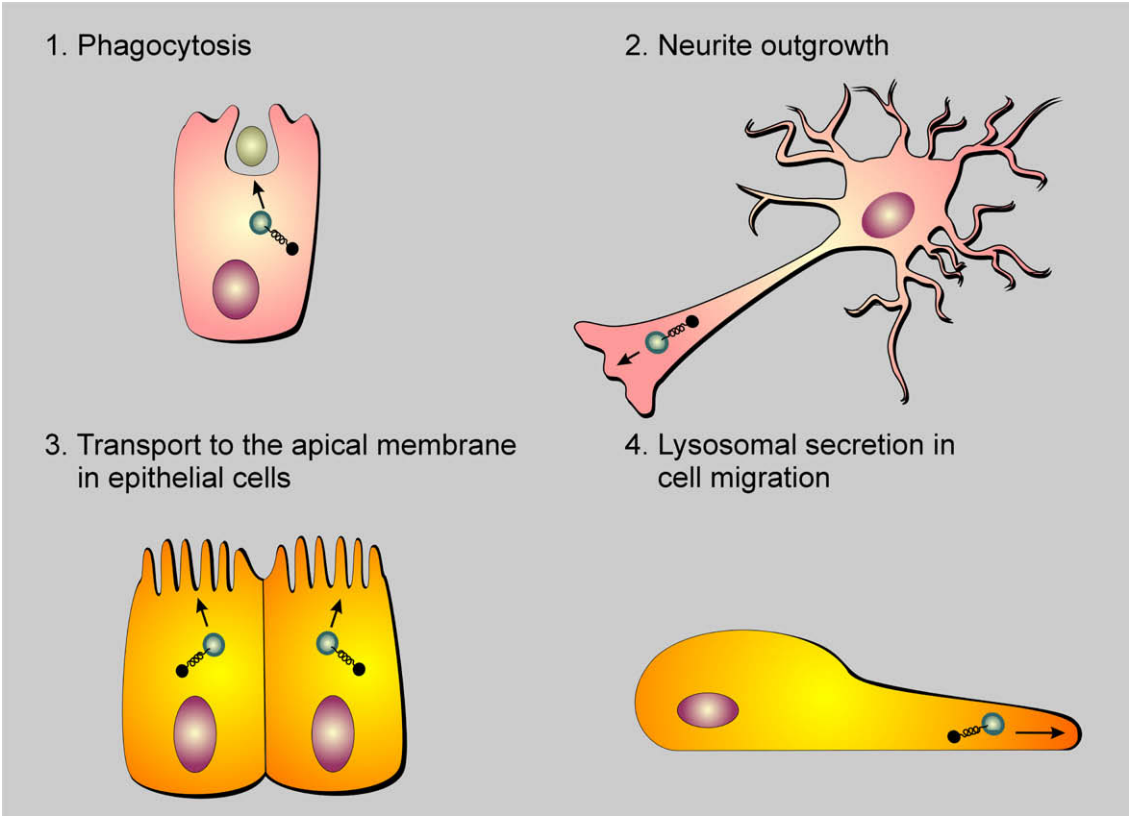

Fig. 3. Cellular functions of TI-VAMP. Depending on the considered cell type, TI-VAMP is involved in different cellular functions. TI-VAMP is involved in plasma membrane remodeling with a role in phagocytosis in macrophages (1), in neurite outgrowth in neurons and PC12 cells (2), in apical transport in epithelial cells (3) and in lysosomal secretion, particularly in cell migration (4).

of VAMP2 accounted for the extent of growth [99]. Synaptotagmin VII, a partner of TI-VAMP [19], involved in lysosomal secretion in non-neuronal cells [100] is also involved in neurite growth, at least in cultured neurons [101]. A function of syntaxin 3 but not syntaxin 1 in neurite growth has also been proposed [102]. Therefore, TI-VAMP could operate in this pathway together with syntaxin 3 as t-SNARE and synaptotagmin VII.

As previously discussed, TI-VAMP is expressed in granule cells of rodent dentate gyrus. In wild-type mice, which express AP-3, TI-VAMP is concentrated in nerve terminals contacting CA3 pyramidal cells (the so-called MF terminals). Thus MF synaptic vesicles (SVs) contain two v-SNAREs, VAMP2 and TI-VAMP. In *mocha* mice, TI-VAMP is blocked in the soma of granule cells and is absent from MF SVs. We thus proposed that AP-3 is necessary for the export of TI-VAMP from the soma to the SVs present in the axon terminal.

Using *mocha* mice, we identified an asynchronous release that can be evoked at hippocampal MF synapses and which is resistant to tetanus neurotoxin [15]. Since these results are correlated with a loss of the presynaptic localization of TI-VAMP, whereas the localization of VAMP2 is unaffected, we proposed the implication of TI-VAMP in this particular regulation. In addition, quantal release in *mocha* cultures is more frequent and more sensitive to sucrose. Therefore, we proposed that the presence of TI-VAMP in MF SVs provides a regulation affecting both basal and evoked release. Since TI-VAMP has a lower capacity to assemble into SNARE complexes than VAMP2 [14], this could explain, at least in part, the weaker docking capacity of control SVs and lead to smaller Readily Releasable Pool (RRP) in control MF terminals [24].

Altogether, these data suggest that TI-VAMP plays a role in both developing and mature neurons.

4.3. Lysosomal secretion

Conventional lysosomes are major organelles of the calcium-dependent exocytosis. In NRK cells, TI-VAMP interacts with the calcium-sensor protein synaptotagmin VII and forms SNARE complexes with syntaxin 4 and SNAP-23 following an elevation of the intracellular calcium concentration. In these cells, overexpression of the SNARE domain of both TI-VAMP and syntaxin 4 is responsible for an inhibition of the secretion of the enzyme β-hexosaminidase [19].

Membrane contribution is required at the leading edge of migrating cells. VAMP3 is directly involved in cell migration of MDCK cells [103–106] but interestingly, TI-VAMP is also expressed in the lamellipodia of these cells. It has been shown that the overexpression of the Longin domain in MDCK cells inhibits the velocity of migrating cells, due to an effect on lysosomal secretion [107]. Finally, VAMP3 is necessary for fusion between autophagosomes and multivesicular bodies (MVBs) whereas TI-VAMP is needed for fusion of autophagosomes with lysosomes and for the release of exosomes [108].

Recently, more and more studies have focused on the role of SNARE proteins in immunology. TI-VAMP has been involved in local inflammatory responses mediated by specialized cells such as mastocytes, basophils, eosinophils or neutrophils. These cells contain granules that secrete histamine, serotonin, or other inflammatory mediators by exocytic events. For example, rat or human mastocytes expressed the t-SNAREs syntaxin 2, 3 and 4 and SNAP-23 and the v-SNAREs TI-VAMP and VAMP8 [20,109]. In these cells, TI-VAMP is localized in intracellular compartments, partially colocalizing with CD63 [21], thus in good agreement with a late

endosomal/lysosomal origin of these compartments and the fact that TI-VAMP is localized in lysosomes in other cell types [19,91,110,111]. TI-VAMP plays also a role in regulated exocytosis in human neutrophils and eosinophils [92,96], in which VAMP8 is also involved; each SNARE playing specific roles in the secretion of inflammatory mediators by specific granules. Finally, TI-VAMP is also involved in the secretion of natural killer (NK) cells that contain secretory lysosome-related granules, responsible for the secretion of lytic enzymes like perforine and granzyme B [93].

Altogether, these results suggest that TI-VAMP is a v-SNARE of lysosomal secretion in different cell types.

4.3. Plasma membrane remodeling

TI-VAMP also plays a role in membrane remodeling at the cell surface, in phagocytosis, and in mitosis [91,112] for instance. Phagocytosis implies the formation of plasma membrane extensions at the phagocytic site. These extensions, called pseudopods, depend on actin polymerization and membrane contribution during the formation of the phagocytic cup [113]. The addition of tetanus neurotoxin, which cleaves VAMP2 and VAMP3, inhibits phagocytosis [114] but it has been shown that silenced macrophages for the expression of VAMP3 were still able to phagocyte latex beads [115]. Niedergang and coworkers have shown that TI-VAMP is localized in a late endocytic compartment in macrophages at steady state and is recruited to phagosomes during Fc mediated phagocytosis. Furthermore, phagocytosis is inhibited by silencing TI-VAMP expression by RNAi, or by overexpressing its Longin domain. Finally, TI-VAMP silencing is also responsible for an inhibition of the exocytosis of late endosomal compartments derived vesicles, resulting in an early blocking of pseudopods extension [91].

Similarly, during mitosis, cells change their shape. Kirchhausen and its coworkers have correlated plasma membrane surface modifications and dynamics of exocytosis and endocytosis during the different stages of cell division [112]. During mitosis, endocytosis is not affected but recycling of internalized membrane is considerably slow down at the beginning of mitosis and reaches its normal rate at anaphase, due to a fusion of endosomes with the plasma membrane [112]. The expression of dominant negative forms of cellubrevin and TI-VAMP inhibits respectively the reappearance of TfR and LAMP-1 at cell surface [112], showing a role of both v-SNARE in this plasma membrane remodeling process.

Therefore, the TI-VAMP compartment is mobilized to the cell surface when rapid expansion and remodeling of plasma membrane is needed.

5. Conclusion

In conclusion, TI-VAMP is an original v-SNARE involved in secretion. It is ubiquitously expressed, and is involved in many cellular functions. TI-VAMP is present both in the Golgi apparatus and in the endosomal system, and is thus at the cross-roads of various secretory and endocytic pathways. The function of TI-VAMP is particularly important in dynamic cellular processes which involve plasma membrane growth and remodeling. Of notes, TI-VAMP-mediated exocytosis is needed during morphogenesis in epithelial cell migration, phagocytosis, as well as neurite outgrowth. While its SNARE domain mediates membrane fusion through interactions with plasma membrane and endosomal t-SNAREs, its Longin domain plays a crucial role in the regulation of TI-VAMP, through both intra and intermolecular interactions with AP-3 and Hrb. Its interaction with Varp connects TI-VAMP to several Rab proteins, particularly Rab21, which is also involved in epithelial cell migration, phagocytosis and neurite outgrowth. In vitro approaches will be required to understand the molecular functions and the regulation of TI-VAMP and its partners. More in vivo experiments will be also necessary to determine the importance of the vesicular trafficking pathway involving TI-VAMP at the level of the organism.

Acknowledgements

We are grateful to Jean-Pierre Laigneau (INSERM, ADR Paris 7, Paris, France) for illustrating this review. Work in the group was funded in part by grants from INSERM, the European Commission ('Signalling and Traffic' STREP 503229), the Association pour la Recherche sur le Cancer, the Agence Nationale pour la Recherche, the Mairie de Paris Medical Research and Health Program, the Fondation pour la Recherche Médicale (to T.G.). L.D. and M.C. were supported by Association pour la Recherche sur le Cancer.

References

[1] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324.
[2] Hong, W. (2005) SNAREs and traffic. Biochim. Biophys. Acta 1744, 120–144.
[3] Jahn, R. and Scheller, R.H. (2006) SNAREs – engines for membrane fusion. Nat. Rev. Mol. Cell Biol. 7, 631–643.
[4] Rossi, V., Banfield, D.K., Vacca, M., Dietrich, L.E., Ungermann, C., D'Esposito, M., Galli, T. and Filippini, F. (2004) Longins and their longin domains: regulated SNAREs and multifunctional SNARE regulators. Trends Biochem. Sci. 29, 682–688.
[5] Wen, W., Chen, L., Wu, H., Sun, X., Zhang, M. and Banfield, D.K. (2006) Identification of the yeast R-SNARE Nyv1p as a novel longin domain-containing protein. Mol. Biol. Cell 17, 4282–4299.
[6] D'Esposito, M., Ciccodicola, A., Gianfrancesco, F., Esposito, T., Flagiello, L., Mazzarella, R., Schlessinger, D. and D'Urso, M. (1996) A synaptobrevin-like gene in the Xq28 pseudoautosomal region undergoes X inactivation. Nat. Genet. 13, 227–229.
[7] Matarazzo, M.R., De Bonis, M.L., Gregory, R.I., Vacca, M., Hansen, R.S., Mercadante, G., D'Urso, M., Feil, R. and D'Esposito, M. (2002) Allelic inactivation of the pseudoautosomal gene SYBL1 is controlled by epigenetic mechanisms common to the X and Y chromosomes. Hum. Mol. Genet. 11, 3191–3198.
[8] Galli, T., Zahraoui, A., Vaidyanathan, V.V., Raposo, G., Tian, J.M., Karin, M., Niemann, H. and Louvard, D. (1998) A novel tetanus neurotoxin-insensitive vesicle-associated membrane protein in SNARE complexes of the apical plasma membrane of epithelial cells. Mol. Biol. Cell 9, 1437–1448.
[9] Sikorra, S., Henke, T., Swaminathan, S., Galli, T. and Binz, T. (2006) Identification of the amino acid residues rendering TI-VAMP insensitive toward botulinum neurotoxin B. J. Mol. Biol. 357, 574–582.
[10] Mili, S., Moissoglu, K. and Macara, I.G. (2008) Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. Nature 453, 115–119.
[11] Tochio, H., Tsui, M.M.K., Banfield, D.K. and Zhang, M.J. (2001) An autoinhibitory mechanism for nonsyntaxin SNARE proteins revealed by the structure of Ykt6p. Science 293, 698–702.
[12] Mancias, J.D. and Goldberg, J. (2007) The transport signal on Sec22 for packaging into COPII-coated vesicles is a conformational epitope. Mol. Cell 26, 403–414.
[13] Pryor, P.R., Jackson, L., Gray, S.R., Edeling, M.A., Thompson, A., Sanderson, C.M., Evans, P.R., Owen, D.J. and Luzio, J.P. (2008) Molecular basis for the sorting of the SNARE VAMP7 into endocytic clathrin-coated vesicles by the ArfGAP Hrb. Cell 134, 817–827.
[14] Martinez-Arca, S., Rudge, R., Vacca, M., Raposo, G., Camonis, J., Proux-Gillardeaux, V., Daviet, L., Formstecher, E., Hamburger, A., Filippini, F., D'Esposito, M. and Galli, T. (2003) A dual mechanism controlling the localization and function of exocytic v-SNAREs. Proc. Natl. Acad. Sci. USA 100, 9011–9016.
[15] Scheuber, A., Rudge, R., Danglot, L., Raposo, G., Binz, T., Poncer, J.C. and Galli, T. (2006) Loss of AP-3 function affects spontaneous and evoked release at hippocampal mossy fiber synapses. Proc. Natl. Acad. Sci. USA 103, 16562–16567.
[16] Fukasawa, M., Varlamov, O., Eng, W.S., Sollner, T.H. and Rothman, J.E. (2004) Localization and activity of the SNARE Ykt6 determined by its regulatory domain and palmitoylation. Proc. Natl. Acad. Sci. USA 101, 4815–4820.
[17] Hasegawa, H., Zinsser, S., Rhee, Y., VikMo, E.O., Davanger, S. and Hay, J.C. (2003) Mammalian Ykt6 is a neuronal SNARE targeted to a specialized compartment by its profilin-like amino terminal domain. Mol. Biol. Cell 14, 698–720.
[18] Alberts, P., Rudge, R., Hinners, I., Muzerelle, A., MartinezArca, S., Irinopoulou, T., Marthiens, V., Tooze, S., Rathjen, F., Gaspar, P. and Galli, T. (2003) Cross talk between tetanus neurotoxin-insensitive vesicle-associated membrane protein-mediated transport and L1-mediated adhesion. Mol. Biol. Cell 14, 4207–4220.

[19] Rao, S.K., Huynh, C., Proux-Gillardeaux, V., Galli, T. and Andrews, N.W. (2004) Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis. J. Biol. Chem. 279, 20471–20479.
[20] Sander, L.E., Frank, S.P., Bolat, S., Blank, U., Galli, T., Bigalke, H., Bischoff, S.C. and Lorentz, A. (2008) Vesicle associated membrane protein (VAMP)-7 and VAMP-8, but not VAMP-2 or VAMP-3, are required for activation-induced degranulation of mature human mast cells. Eur. J. Immunol. 38, 855–863.
[21] Puri, N., Kruhlak, M.J., Whiteheart, S.W. and Roche, P.A. (2003) Mast cell degranulation requires N-ethylmaleimide-sensitive factor-mediated SNARE disassembly. J. Immunol. 171, 5345–5352.
[22] Bogdanovic, A., Bennett, N., Kieffer, S., Louwagie, M., Morio, T., Garin, J., Satre, M. and Bruckert, F. (2002) Syntaxin 7, syntaxin 8, Vti1 and VAMP7 (vesicle-associated membrane protein 7) form an active SNARE complex for early macropinocytic compartment fusion in *Dictyostelium discoideum*. Biochem. J. 368, 29–39.
[23] Pryor, P.R., Mullock, B.M., Bright, N.A., Lindsay, M.R., Gray, S.R., Richardson, S.C., Stewart, A., James, D.E., Piper, R.C. and Luzio, J.P. (2004) Combinatorial SNARE complexes with VAMP7 or VAMP8 define different late endocytic fusion events. EMBO Rep. 5, 590–595.
[24] Danglot, L. and Galli, T. (2007) What is the function of neuronal AP-3? Biol. Cell 99, 349–361.
[25] Dell'Angelica, E.C., Klumperman, J., Stoorvogel, W. and Bonifacino, J.S. (1998) Association of the AP-3 adaptor complex with clathrin. Science 280, 431–434.
[26] Simpson, F., Peden, A.A., Christopoulou, L. and Robinson, M.S. (1997) Characterization of the adaptor-related protein complex, AP-3. J. Cell Biol. 137, 835–845.
[27] Peden, A.A., Oorschot, V., Hesser, B.A., Austin, C.D., Scheller, R.H. and Klumperman, J. (2004) Localization of the AP-3 adaptor complex defines a novel endosomal exit site for lysosomal membrane proteins. J. Cell Biol. 164, 1065–1076.
[28] Bonifacino, J.S. and Traub, L.M. (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu. Rev. Biochem. 72, 395–447.
[29] Dell'Angelica, E.C., Shotelersuk, V., Aguilar, R.C., Gahl, W.A. and Bonifacino, J.S. (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. Mol. Cell 3, 11–21.
[30] Le Borgne, R., Alconada, A., Bauer, U. and Hoflack, B. (1998) The mammalian AP-3 adaptor-like complex mediates the intracellular transport of lysosomal membrane glycoproteins. J. Biol. Chem. 273, 29451–29461.
[31] Advani, R.J., Yang, B., Prekeris, R., Lee, K.C., Klumperman, J. and Scheller, R.H. (1999) VAMP-7 mediates vesicular transport from endosomes to lysosomes. J. Cell Biol. 146, 765–775.
[32] Peden, A.A., Park, G.Y. and Scheller, R.H. (2001) The di-leucine motif of vesicle-associated membrane protein 4 is required for its localization and AP-1 binding. J. Biol. Chem. 276, 49183–49187.
[33] Chaineau, M., Danglot, L., Proux-Gillardeaux, V. and Galli, T. (2008) Role of HRB in clathrin-dependent endocytosis. J. Biol. Chem. 283, 34365–34373.
[34] Bogerd, H.P., Fridell, R.A., Madore, S. and Cullen, B.R. (1995) Identification of a novel cellular cofactor for the Rev/Rex class of retroviral regulatory proteins. Cell 82, 485–494.
[35] Fritz, C.C., Zapp, M.L. and Green, M.R. (1995) A human nucleoporin-like protein that specifically interacts with HIV Rev. Nature 376, 530–533.
[36] de Beer, T., Hoofnagle, A.N., Enmon, J.L., Bowers, R.C., Yamabhai, M., Kay, B.K. and Overduin, M. (2000) Molecular mechanism of NPF recognition by EH domains. Nat. Struct. Biol. 7, 1018–1022.
[37] Doria, M., Salcini, A.E., Colombo, E., Parslow, T.G., Pelicci, P.G. and Di Fiore, P.P. (1999) The eps15 homology (EH) domain-based interaction between eps15 and hrb connects the molecular machinery of endocytosis to that of nucleocytosolic transport. J. Cell Biol. 147, 1379–1384.
[38] Kang-Decker, N., Mantchev, G.T., Juneja, S.C., McNiven, M.A. and van Deursen, J.M. (2001) Lack of acrosome formation in Hrb-deficient mice. Science 294, 1531–1533.
[39] Bennett, N., Letourneur, F., Ragno, M. and Louwagie, M. (2008) Sorting of the v-SNARE VAMP7 in *Dictyostelium discoideum*: a role for more than one Adaptor Protein (AP) complex. Exp. Cell Res. 314, 2822–2833.
[40] Burgo, A., Sotirakis, E., Simmler, M.C., Verraes, A., Chamot, C., Simpson, J.C., Lanzetti, L., Proux-Gillardeaux, V. and Galli, T. (2009) Role of Varp, a Rab21 exchange factor and TI-VAMP/VAMP7 partner, in neurite growth. EMBO Rep. 10, 1117–1124.
[41] Zhang, X., He, X., Fu, X.Y. and Chang, Z. (2006) Varp is a Rab21 guanine nucleotide exchange factor and regulates endosome dynamics. J. Cell Sci. 119, 1053–1062.
[42] Khurana, T., Brzostowski, J.A. and Kimmel, A.R. (2005) A Rab21/LIM-only/CH-LIM complex regulates phagocytosis via both activating and inhibitory mechanisms. EMBO J. 24, 2254–2264.
[43] Pellinen, T., Arjonen, A., Vuoriluoto, K., Kallio, K., Fransen, J.A. and Ivaska, J. (2006) Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of beta1-integrins. J. Cell Biol. 173, 767–780.
[44] Pellinen, T., Tuomi, S., Arjonen, A., Wolf, M., Edgren, H., Meyer, H., Grosse, R., Kitzing, T., Rantala, J.K., Kallioniemi, O., Fassler, R., Kallio, M. and Ivaska, J. (2008) Integrin trafficking regulated by Rab21 is necessary for cytokinesis. Dev. Cell 15, 371–385.
[45] Mosavi, L.K., Cammett, T.J., Desrosiers, D.C. and Peng, Z.Y. (2004) The ankyrin repeat as molecular architecture for protein recognition. Protein Sci. 13, 1435–1448.
[46] Tamura, K., Ohbayashi, N., Maruta, Y., Kanno, E., Itoh, T. and Fukuda, M. (2009) Varp is a novel Rab32/38-binding protein that regulates Tyrp1 trafficking in melanocytes. Mol. Biol. Cell 20, 2900–2908.
[47] Wang, F., Zhang, H., Zhang, X., Wang, Y., Ren, F., Zhang, X., Zhai, Y. and Chang, Z. (2008) Varp interacts with Rab38 and functions as its potential effector. Biochem. Biophys. Res. Commun. 372, 162–167.
[48] Cai, H., Reinisch, K. and Ferro-Novick, S. (2007) Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. Dev. Cell 12, 671–682.
[49] Brummendorf, T., Kenwrick, S. and Rathjen, F.G. (1998) Neural cell recognition molecule L1: from cell biology to human hereditary brain malformations. Curr. Opin. Neurobiol. 8, 87–97.
[50] Kamiguchi, H. and Yoshihara, F. (2001) The role of endocytic l1 trafficking in polarized adhesion and migration of nerve growth cones. J. Neurosci. 21, 9194–9203.
[51] Dequidt, C., Danglot, L., Alberts, P., Galli, T., Choquet, D. and Thoumine, O. (2007) Fast turnover of l1 adhesions in neuronal growth cones involving both surface diffusion and exo/endocytosis of l1 molecules. Mol. Biol. Cell 18, 3131–3143.
[52] Linder, S. (2007) The matrix corroded: podosomes and invadopodia in extracellular matrix degradation. Trends Cell Biol. 17, 107–117.
[53] Hotary, K., Li, X.Y., Allen, E., Stevens, S.L. and Weiss, S.J. (2006) A cancer cell metalloprotease triad regulates the basement membrane transmigration program. Genes Dev. 20, 2673–2686.
[54] Sato, H., Okada, Y. and Seiki, M. (1997) Membrane-type matrix metalloproteinases (MT-MMPs) in cell invasion. Thromb. Haemost. 78, 497–500.
[55] Steffen, A., Le Dez, G., Poincloux, R., Recchi, C., Nassoy, P., Rottner, K., Galli, T. and Chavrier, P. (2008) MT1-MMP-dependent invasion is regulated by TI-VAMP/VAMP7. Curr. Biol. 18, 926–931.
[56] Nishihara, S., Iwasaki, H., Nakajima, K., Togayachi, A., Ikehara, Y., Kudo, T., Kushi, Y., Furuya, A., Shitara, K. and Narimatsu, H. (2003) Alpha1, 3-fucosyltransferase IX (Fut9) determines Lewis X expression in brain. Glycobiology 13, 445–455.
[57] Brito, C., Escrevente, C., Reis, C.A., Lee, V.M., Trojanowski, J.Q. and Costa, J. (2007) Increased levels of fucosyltransferase IX and carbohydrate Lewis(x) adhesion determinant in human NT2N neurons. J. Neurosci. Res. 85, 1260–1270.
[58] Gotz, M., Wizenmann, A., Reinhardt, S., Lumsden, A. and Price, J. (1996) Selective adhesion of cells from different telencephalic regions. Neuron 16, 551–564.
[59] Brito, C., Danglot, L., Galli, T. and Costa, J. (2009) Subcellular localization of the carbohydrate Lewis(x) adhesion structure in hippocampus cell cultures. Brain Res. 1287, 39–46.
[60] Danglot, L., Chaineau, M., Dahan, M., Gendron, M.C., Boggetto, N., Perez, F. and Galli, T. (Unpublished observations). Role of TI-VAMP and CD82 in EGF receptor cell surface dynamics and signaling.
[61] Coco, S., Raposo, G., Martinez, S., Fontaine, J.J., Takamori, S., Zahraoui, A., Jahn, R., Matteoli, M., Louvard, D. and Galli, T. (1999) Subcellular localization of tetanus neurotoxin-insensitive vesicle-associated membrane protein (VAMP)/VAMP7 in neuronal cells: Evidence for a novel membrane compartment. J. Neurosci. 19, 9803–9812.
[62] Alberts, P., Rudge, R., Irinopoulou, T., Danglot, L., Gauthier-Rouviere, C. and Galli, T. (2006) Cdc42 and actin control polarized expression of TI-VAMP vesicles to neuronal growth cones and their fusion with the plasma membrane. Mol. Biol. Cell 17, 1194–1203.
[63] Fukata, M., Nakagawa, M. and Kaibuchi, K. (2003) Roles of Rho-family GTPases in cell polarisation and directional migration. Curr. Opin. Cell Biol. 15, 590–597.
[64] Muzerelle, A., Alberts, P., Martinez-Arca, S., Jeannequin, O., Lafaye, P., Mazie, J.-C., Galli, T. and Gaspar, P. (2003) Tetanus neurotoxin-insensitive vesicle-associated membrane protein localizes to a presynaptic membrane compartment in selected terminal subsets of the rat brain. Neuroscience 122, 59–75.
[65] Siddiqi, S.A., Mahan, J., Siddiqi, S., Gorelick, F.S. and Mansbach 2nd, C.M. (2006) Vesicle-associated membrane protein 7 is expressed in intestinal ER. J. Cell Sci. 119, 943–950.
[66] Siddiqi, S.A., Siddiqi, S., Mahan, J., Peggs, K., Gorelick, F.S. and Mansbach 2nd, C.M. (2006) The identification of a novel endoplasmic reticulum to Golgi SNARE complex used by the prechylomicron transport vesicle. J. Biol. Chem. 281, 20974–20982.
[67] Das, V., Nal, B., Dujeancourt, A., Thoulouze, M.I., Galli, T., Roux, P., Dautry-Varsat, A. and Alcover, A. (2004) Activation-induced polarized recycling targets T cell antigen receptors to the immunological synapse; involvement of SNARE complexes. Immunity 20, 577–588.
[68] Galli, T. and Haucke, V. (2004). Cycling of synaptic vesicles: how far? How fast! Sci STKE 2004, re19.
[69] Proux-Gillardeaux, V., Rudge, R. and Galli, T. (2005) The tetanus neurotoxin-sensitive and insensitive routes to and from the plasma membrane: fast and slow pathways? Traffic 6, 366–373.
[70] Mallard, F., Tang, B.L., Galli, T., Tenza, D., Saint-Pol, A., Yue, X., Antony, C., Hong, W., Goud, B. and Johannes, L. (2002) Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. J. Cell Biol. 156, 653–654.

[71] Tran, T.H., Zeng, Q. and Hong, W. (2007) VAMP4 cycles from the cell surface to the trans-Golgi network via sorting and recycling endosomes. J. Cell Sci. 120, 1028–1041.
[72] Brandhorst, D., Zwilling, D., Rizzoli, S.O., Lippert, U., Lang, T. and Jahn, R. (2006) Homotypic fusion of early endosomes: SNAREs do not determine fusion specificity. Proc. Natl. Acad. Sci. USA 103, 2701–2706.
[73] Lippert, U., Ferrari, D.M. and Jahn, R. (2007) Endobrevin/VAMP8 mediates exocytotic release of hexosaminidase from rat basophilic leukaemia cells. FEBS Lett. 581, 3479–3484.
[74] Ren, Q., Barber, H.K., Crawford, G.L., Karim, Z.A., Zhao, C., Choi, W., Wang, C.C., Hong, W. and Whiteheart, S.W. (2007) Endobrevin/VAMP-8 is the primary v-SNARE for the platelet release reaction. Mol. Biol. Cell 18, 24–33.
[75] Wang, C.C., Shi, H., Guo, K., Ng, C.P., Li, J., Gan, B.Q., Chien Liew, H., Leinonen, J., Rajaniemi, H., Zhou, Z.H., Zeng, Q. and Hong, W. (2007) VAMP8/endobrevin as a general vesicular SNARE for regulated exocytosis of the exocrine system. Mol. Biol. Cell 18, 1056–1063.
[76] Wong, S.H., Zhang, T., Xu, Y., Subramaniam, V.N., Griffiths, G. and Hong, W.J. (1998) Endobrevin, a novel synaptobrevin/VAMP-like protein preferentially associated with the early endosome. Mol. Biol. Cell 9, 1549–1563.
[77] Randhawa, V.K., Thong, F.S., Lim, D.Y., Li, D., Garg, R.R., Rudge, R., Galli, T., Rudich, A. and Klip, A. (2004) Insulin and hypertonicity recruit GLUT4 to the plasma membrane of muscle cells using NSF-dependent SNARE mechanisms but different v-SNAREs: role of TI-VAMP. Mol. Biol. Cell 15, 5565–5573.
[78] Williams, D. and Pessin, J.E. (2008) Mapping of R-SNARE function at distinct intracellular GLUT4 trafficking steps in adipocytes. J. Cell Biol. 180, 375–387.
[79] Holman, G.D. and Sandoval, I.V. (2001) Moving the insulin-regulated glucose transporter GLUT4 into and out of storage. Trends Cell Biol. 11, 173–179.
[80] Pessin, J.E., Thurmond, D.C., Elmendorf, J.S., Coker, K.J. and Okada, S. (1999) Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! J. Biol. Chem. 274, 2593–2596.
[81] Cheatham, B., Volchuk, A., Kahn, C.R., Wang, L., Rhodes, C.J. and Klip, A. (1996) Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. Proc. Natl. Acad. Sci. USA 93, 15169–15173.
[82] Rea, S., Martin, L.B., McIntosh, S., Macaulay, S.L., Ramsdale, T., Baldini, G. and James, D.E. (1998) Syndet, an adipocyte target SNARE involved in the insulin-induced translocation of GLUT4 to the cell surface. J. Biol. Chem. 273, 18784–18792.
[83] Oishi, Y., Arakawa, T., Tanimura, A., Itakura, M., Takahashi, M., Tajima, Y., Mizoguchi, I. and Takuma, T. (2006) Role of VAMP-2, VAMP-7, and VAMP-8 in constitutive exocytosis from HSY cells. Histochem. Cell Biol. 125, 273–281.
[84] Pocard, T., Le Bivic, A., Galli, T. and Zurzolo, C. (2007) Distinct v-SNAREs regulate direct and indirect apical delivery in polarized epithelial cells. J. Cell Sci. 120, 3309–3320.
[85] Antonin, W., Holroyd, C., Tikkanen, R., Honing, S. and Jahn, R. (2000) The R-SNARE Endobrevin/VAMP-8 mediates homotypic fusion of early endosomes and late endosomes. Mol. Biol. Cell 11, 3289–3298.
[86] Wade, N., Bryant, N.J., Connolly, L.M., Simpson, R.J., Luzio, J.P., Piper, R.C. and James, D.E. (2001) Syntaxin 7 complexes with mouse Vps10p tail interactor 1b, Syntaxin 6, vesicle-associated membrane protein (VAMP)8, and VAMP7 in B16 melanoma cells. J. Biol. Chem. 276, 19820–19827.
[87] Antonin, W., Holroyd, C., Fasshauer, D., Pabst, S., Von Mollard, G.F. and Jahn, R. (2000) A SNARE complex mediating fusion of late endosomes defines conserved properties of SNARE structure and function. EMBO J. 19, 6453–6464.
[88] Prekeris, R., Yang, B., Oorschot, V., Klumperman, J. and Scheller, R.H. (1999) Differential roles of syntaxin 7 and syntaxin 8 in endosomal trafficking. Mol. Biol. Cell 10, 3891–3908.
[89] Ganley, I.G., Espinosa, E. and Pfeffer, S.R. (2008) A syntaxin 10-SNARE complex distinguishes two distinct transport routes from endosomes to the trans-Golgi in human cells. J. Cell Biol. 180, 159–172.
[90] Tai, G.H., Lu, L., Wang, T.L., Tang, B.L., Goud, B., Johannes, L. and Hong, W.J. (2004) Participation of the syntaxin 5/Ykt6/GS28/GS15 SNARE complex in transport from the early/recycling endosome to the trans-Golgi network. Mol. Biol. Cell 15, 4011–4022.
[91] Braun, V., Fraisier, V., Raposo, G., Hurbain, I., Sibarita, J.B., Chavrier, P., Galli, T. and Niedergang, F. (2004) TI-VAMP/VAMP7 is required for optimal phagocytosis of opsonised particles in macrophages. EMBO J. 23, 4166–4176.
[92] Logan, M.R., Lacy, P., Odemuyiwa, S.O., Steward, M., Davoine, F., Kita, H. and Moqbel, R. (2006) A critical role for vesicle-associated membrane protein-7 in exocytosis from human eosinophils and neutrophils. Allergy 61, 777–784.
[93] Marcet-Palacios, M., Odemuyiwa, S.O., Coughlin, J.J., Garofoli, D., Ewen, C., Davidson, C.E., Ghaffari, M., Kane, K.P., Lacy, P., Logan, M.R., Befus, A.D., Bleackley, R.C. and Moqbel, R. (2008) Vesicle-associated membrane protein 7 (VAMP-7) is essential for target cell killing in a natural killer cell line. Biochem. Biophys. Res. Commun. 366, 617–623.
[94] Martinez-Arca, S., Alberts, P., Zahraoui, A., Louvard, D. and Galli, T. (2000) Role of tetanus neurotoxin insensitive vesicle-associated membrane protein (TI-VAMP) in vesicular transport mediating neurite outgrowth. J. Cell Biol. 149, 889–899.
[95] Martinez-Arca, S., Coco, S., Mainguy, G., Schenk, U., Alberts, P., Bouille, P., Mezzina, M., Prochiantz, A., Matteoli, M., Louvard, D. and Galli, T. (2001) A common exocytotic mechanism mediates axonal and dendritic outgrowth. J. Neurosci. 21, 3830–3838.
[96] Mollinedo, F., Calafat, J., Janssen, H., Martin-Martin, B., Canchado, J., Nabokina, S.M. and Gajate, C. (2006) Combinatorial SNARE complexes modulate the secretion of cytoplasmic granules in human neutrophils. J. Immunol. 177, 2831–2841.
[97] Folsch, H. (2008) Regulation of membrane trafficking in polarized epithelial cells. Curr. Opin. Cell Biol. 20, 208–213.
[98] Lafont, F., Verkade, P., Galli, T., Wimmer, C., Louvard, D. and Simons, K. (1999) Raft association of SNAP receptors acting in apical trafficking in Madin-Darby canine kidney cells. Proc. Natl. Acad. Sci. USA 96, 3734–3738.
[99] Tsaneva-Atanasova, K., Burgo, A., Galli, T. and Holcman, D. (2009) Quantifying neurite growth mediated by interactions among secretory vesicles, microtubules, and actin networks. Biophys. J. 96, 840–857.
[100] Reddy, A., Caler, E.V. and Andrews, N.W. (2001) Plasma membrane repair is mediated by Ca2+-regulated exocytosis of lysosomes. Cell 106, 157–169.
[101] Arantes, R.M. and Andrews, N.W. (2006) A role for synaptotagmin VII-regulated exocytosis of lysosomes in neurite outgrowth from primary sympathetic neurons. J. Neurosci. 26, 4630–4637.
[102] Darios, F. and Davletov, B. (2006) Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 440, 813–817.
[103] Luftman, K., Hasan, N., Day, P., Hardee, D. and Hu, C. (2009) Silencing of VAMP3 inhibits cell migration and integrin-mediated adhesion. Biochem. Biophys. Res. Commun. 380, 65–70.
[104] Proux-Gillardeaux, V., Gavard, J., Irinopoulou, T., Mege, R.M. and Galli, T. (2005) Tetanus neurotoxin-mediated cleavage of cellubrevin impairs epithelial cell migration and integrin-dependent cell adhesion. Proc. Natl. Acad. Sci. USA 102, 6362–6367.
[105] Skalski, M. and Coppolino, M.G. (2005) SNARE-mediated trafficking of alpha5beta1 integrin is required for spreading in CHO cells. Biochem. Biophys. Res. Commun. 335, 1199–1210.
[106] Tayeb, M.A., Skalski, M., Cha, M.C., Kean, M.J., Scaife, M. and Coppolino, M.G. (2005) Inhibition of SNARE-mediated membrane traffic impairs cell migration. Exp. Cell Res. 305, 63–73.
[107] Proux-Gillardeaux, V., Raposo, G., Irinopoulou, T. and Galli, T. (2007) Expression of the Longin domain of TI-VAMP impairs lysosomal secretion and epithelial cell migration. Biol. Cell 99, 261–271.
[108] Fader, C.M., Sanchez, D.G., Mestre, M.B. and Colombo, M.I. (in press). TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps. Biochim. Biophys. Acta., doi:10.1016/j.bbamer.2009.09.11.
[109] Hibi, T., Hirashima, N. and Nakanishi, M. (2000) Rat basophilic leukemia cells express syntaxin-3 and VAMP-7 in granule membranes. Biochem. Biophys. Res. Commun. 271, 36–41.
[110] Advani, R.J., Bae, H.R., Bock, J.B., Chao, D.S., Doung, Y.C., Prekeris, R., Yoo, J.S. and Scheller, R.H. (1998) Seven novel mammalian SNARE proteins localize to distinct membrane compartments. J. Biol. Chem. 273, 10317–10324.
[111] Ward, D.M., Pevsner, J., Scullion, M.A., Vaughn, M. and Kaplan, J. (2000) Syntaxin 7 and VAMP-7 are soluble N-ethylmaleimide-sensitive factor attachment protein receptors required for late endosome–lysosome and homotypic lysosome fusion in alveolar macrophages. Mol. Biol. Cell 11, 2327–2333.
[112] Boucrot, E. and Kirchhausen, T. (2007) Endosomal recycling controls plasma membrane area during mitosis. Proc. Natl. Acad. Sci. USA 104, 7939–7944.
[113] Booth, J.W., Trimble, W.S. and Grinstein, S. (2001) Membrane dynamics in phagocytosis. Semin. Immunol. 13, 357–364.
[114] Hackam, D.J., Rotstein, O.D., Sjolin, C., Schreiber, A.D., Trimble, W.S. and Grinstein, S. (1998) V-SNARE-dependent secretion is required for phagocytosis. Proc. Natl. Acad. Sci. USA 95, 11691–11696.
[115] Allen, L.A.H., Yang, C.M. and Pessin, J.E. (2002) Rate and extent of phagocytosis in macrophages lacking vamp3. J. Leukocyte Biol. 72, 217–221.
[116] Dong, J.T. et al. (1995) KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. Science 268, 884–886.
[117] Dong, J.T. et al. (1996) Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Res. 56, 4387–4390.
[118] Smith, S.C. and Theodorescu, D. (2009) Learning therapeutic lessons from metastasis suppressor proteins. Nat Rev Cancer 9, 253–264.
[119] Odintsova, E., Sugiura, T. and Berditchevski, F. (2000) Attenuation of EGF receptor signaling by a metastasis suppressor, the tetraspanin CD82/KAI-1. Curr. Biol. 10, 1009–1012.