Review TRENDS in Biochemical Sciences Vol.29 No.12 December 2004

Longins and their longin domains: regulated SNAREs and multifunctional SNARE regulators

Valeria Rossi[1], David K. Banfield[2], Marcella Vacca[1,3], Lars E.P. Dietrich[4], Christian Ungermann[4], Maurizio D'Esposito[3], Thierry Galli[5] and Francesco Filippini[1]

[1]Molecular Biology and Bioinformatics Unit (MOLBINFO), Department of Biology, University of Padua, 35131 Padova, Italy
[2]Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, SAR of China
[3]Genome Research Laboratory, Institute of Genetics and Biophysics 'A.Buzzati Traverso' (IGB) – CNR, 80125 Naples, Italy
[4]Biochemie Zentrum der Universität Heidelberg (BZH), 69120 Heidelberg, Germany
[5]Membrane Traffic and Neuronal Plasticity Group, INSERM U536, Institut du Fer-à-Moulin, 75005 Paris, France

Longins are the only R-SNAREs that are common to all eukaryotes and are characterized by a conserved N-terminal domain with a profilin-like fold called a longin domain (LD). These domains seem to be essential for regulating membrane trafficking and they mediate unexpected biochemical functions via a range of protein–protein and intramolecular binding specificities. In addition to the longins, proteins involved in the regulation of intracellular trafficking, such as subunits of the adaptor and transport protein particle complexes, also have LD-like folds. The functions and cellular localization of longins are regulated at several levels and the longin prototypes TI-VAMP, Sec22 and Ykt6 show different distributions among eukaryotes, reflecting their modular and functional diversity. In mammals, TI-VAMP and Ykt6 are crucial for neuronal function, and defects in longin structure or function might underlie some human neurological pathologies.

The general conservation of proteins involved in intracellular vesicle transport is likely to reflect the conservation of molecular mechanisms both within and across species. Indeed, both the homotypic and heterotypic fusion of intracellular membranes along the secretory and endocytic pathway are mediated by a family of SNAREs [soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors] [1]. SNAREs share α-helical coiled-coil domains, called 'SNARE motifs', that probably evolved from a common ancestor and are composed of a repeated hydrophobic heptad register [2] that is interrupted at the so-called 'zero' layer by a conserved arginine or glutamine polar residue [3]. On the basis of this observation, SNAREs are often referred to as R- or Q-SNAREs [4]; alternatively, they are known as v- or t-SNAREs, depending on their localization to either the transport vesicle (v) or the target (t) membrane [1].

SNARE-mediated fusion typically results from the formation of a complex consisting of one R-SNARE and three Q-SNARE motifs [5]. In this context, Q-SNAREs can be subdivided into Qa- (syntaxin), Qb- [25-kDa synaptosomal-associated protein (SNAP-25) N-terminal SNARE motif] or Qc- (SNAP-25 C-terminal SNARE motif) SNAREs [6]. The R-SNAREs can be also subdivided into short VAMPs (vesicle-associated membrane proteins) or 'brevins' (from the Latin word *brevis*, meaning short) and long VAMPs or 'longins', on the basis of whether they contain a short and variable domain or a conserved longin domain (LD) of 120–140 amino acids at their N terminus [7].

Curiously, the longins are the only R-SNAREs that are conserved in all eukaryotes; other R-SNAREs show a more restricted distribution. For example, synaptobrevins, which have been studied extensively, are missing in multicellular eukaryotes such as plants [7–9] and share specific SNARE motifs that are limited to Bilateria [10]. This observation suggests that the LD is likely to be an essential module and that the R-SNARE motif of longins can substitute for that of the brevins [8]. The bioinformatic analysis that defined a profile for the LD also identified three subfamilies of longins [7], tetanus neurotoxin-insensitive VAMP (TI-VAMP; also known as VAMP7) [11,12], Sec22 [13] and Ykt6 [14], all of which are involved in fundamental cell trafficking pathways.

Here we review recent findings on the structure and function of LDs that facilitate a deeper understanding of the regulation and fine-tuning of SNARE functions and other molecular mechanisms that are crucial to eukaryotic cell viability, growth and differentiation.

Molecular organization and functions of LDs

Figure 1 compares the domain architecture of longins to that of other SNAREs. Both longins and syntaxins have N-terminal domains (NTDs) that can downregulate membrane fusion [15–17]. When the 3D structures of the NTDs of the longins Ykt6 and Sec22b were solved, however, the LD was found to show a conserved profilin-like fold that differed considerably from that of the NTD of syntaxins [18,19] (Box 1).

Corresponding author: Francesco Filippini (francesco.filippini@unipd.it).

 0968-0004/$ - see front matter doi:10.1016/j.tibs.2004.10.002

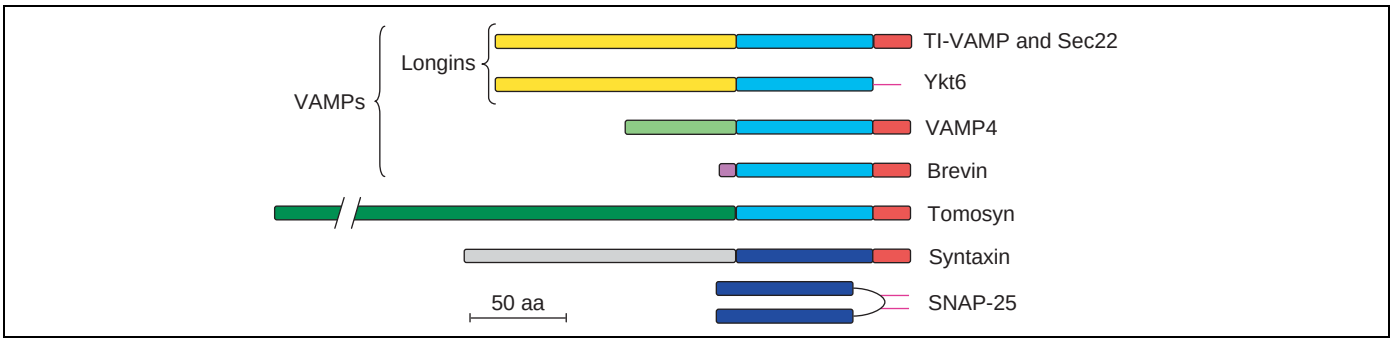

Figure 1. Domain architectures of longins and other types of SNARE. All SNAREs share conserved coiled-coil domains (SNARE motifs) of the R (light blue) or Q (dark blue) type. Among the VAMPs (vesicle-associated membrane proteins), longins share a widely conserved longin domain (LD, yellow), whereas brevins have a short and variable N-terminal domain (NTD, violet) [7]. Other R-SNAREs can have either specific (light green) or conserved (dark green) NTDs [8]. Syntaxins have a domain architecture that is similar to longins but a different NTD (grey). Most SNAREs have transmembrane domains (red), but the SNAP-25 (25-kDa synaptosomal-associated protein) and Ykt6 longins associate with membranes by lipid anchors (magenta).

Intramolecular binding of the NTD of neuronal syntaxin to its SNARE motif mediates the formation of a 'closed conformation' that is necessary to bind Munc18, a protein of the Sec/Munc (SM) family [20]. A similar 'conformation-dependent' modulation of binding capacity has been suggested to occur in some longins [21]; however, not all syntaxins use the same mechanism to bind SM proteins [20], and so far intramolecular binding among the longins has been demonstrated only for Ykt6 [18]. The LD of TI-VAMP can inhibit the formation of a fusion complex [17] and, although intramolecular binding has not been confirmed, the occurrence of low-affinity intramolecular interactions in TI-VAMP cannot be ruled out [22].

Box 1. Structures of longin and longin-like domains

The longin domains (LDs) of Ykt6 [18] and Sec22b [19] show the same globular fold (Figure I), comprising a five-stranded β-sheet core sandwiched between an α-helix on one side and two α-helices on the other. This fold differs from the N-terminal domain (NTD) of syntaxins [20] and resembles a circular permutation of GAF/PAS domains, a large and widespread family of small-molecule binding regulatory modules, and profilin, a widely expressed protein that can bind diverse ligands including polyphosphoinositides [23–25]. The LD of Ykt6 contains a hydrophobic patch that can inhibit the formation of a fusion complex by binding intramolecularly to the coiled-coil domain (SNARE motif); mutation of a conserved phenylalanine residue in this patch abrogates this interaction [18]. Moreover, a recently identified putative binding groove for palmitoyl groups possibly ensures the solubility of the lipid-modified cytoplasmic form of Ykt6 [66].

Despite a lack of sequence similarity to longins, the human protein SEDL (also known as Sedlin) has been recently shown to have an LD-like fold, and mutations that alter its structure (Figure I) are causative for a cartilage disorder [71]. The SEDL orthologue in yeast is part of the transport protein particle termed TRAPP, which contributes to the fidelity of membrane fusion at the ER to Golgi compartment by controlling the tethering process. TRAPP can exchange the nucleotide of Ypt1p GTPase (an event that occurs upstream of the interactions between v- and t-SNAREs) [72].

The InterPro database [73] contains a signature for longin-like domains (accession number IPR011012) that are shared by more than 350 proteins, including longins, SEDL and the assembly domain of adaptins μ2 and σ2. These adaptins are two of the proteins that make up the core of AP2, a complex that functions in clathrin-mediated endocytosis and binds polyphosphoinositides [74]. Despite a lack of significant sequence homology, the folds of σ2 (Figure I) and the NTD of μ2 are structurally almost identical to each other and also share a strong similarity with the LD fold [75]. The structure of AP2 suggests that the LD-like domains of σ2 and μ2 have a predominantly structural role, stabilizing the core of the tetrameric adaptor assembly [74,75]. Evidence that proteins and protein domains from an adaptor complex have an LD-like fold is intriguing, considering that the LD can target TI-VAMP to the late endosomal complex through a direct interaction with an adaptor complex protein [22].

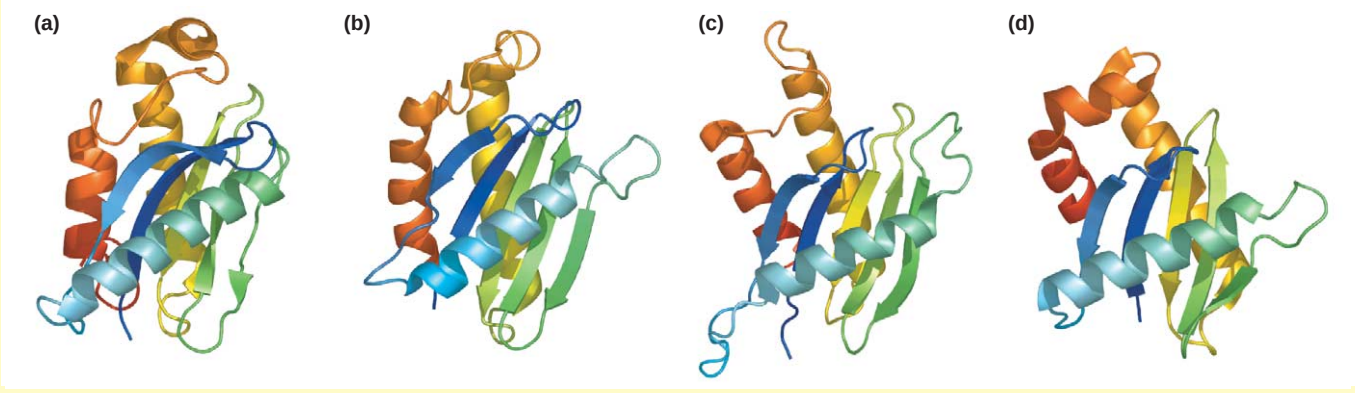

Figure I. Profilin-like folds of longin domains (LDs). Shown are the Ykt6 LD **(a)**, the Sec22 LD **(b)**, the SEDL protein **(c)** and the σ2 chain of the AP2 complex **(d)**, generated from the atomic coordinates of the Protein Data Bank (http://www.rcsb.org/pdb/) entries 1H8M [18], 1IFQ [19], 1H3Q [70] and 1GW5 [75], respectively. Structural representations were prepared by PyMOL (http://pymol.sourceforge.net/) using its 'rainbow' option (fading colour transition for secondary structure elements) and rendering plug-in. The N-terminal β-sheet of each molecule is coloured in dark blue, the C-terminal α-helix is red.

Indeed, recent evidence suggests that LDs are likely to mediate several shared as well as specific molecular mechanisms, in agreement with evidence indicating that profilin-like domains have multiple functions [23–25]. For example, the LD of human TI-VAMP has dual roles: it can negatively regulate the ability of either TI-VAMP or a LD-synaptobrevin chimera to participate in SNARE complexes, and it can target TI-VAMP to the late endosomal compartment [22]. The LD of Ykt6 (but not that of Sec22) can target the protein to a specialized compartment in neurons [26], and the LD of yeast Ykt6 mediates acylation of the fusion factor Vac8 during an early step of homotypic vacuole fusion [27,28].

The fact that TI-VAMP can bind to a subunit of a clathrin coat adaptor complex (AP) [22] and that Ykt6 is involved in distinct steps of the membrane fusion reaction including tethering (in which a transport vesicle is docked onto its target membrane before the pairing of SNAREs) [27,29,30] is not surprising, considering that some tethering factors and AP subunits have a longin-like fold (Box 1) and that the NTDs of other SNAREs can bind tethering complexes [31,32].

Although a few longins show slightly degenerate sequences that are not recognized by the LD profile, most correspond to the TI-VAMP, Sec22 and Ykt6 prototypes and show both shared and specific moieties and molecular and cellular functions.

TI-VAMP

Depending on the type of cell, TI-VAMP has been found to interact *in vivo* with several t-SNARE heavy chains (plasmalemmal syntaxin 1, 3 and 4, and late endosomal syntaxin 7) and light chains (SNAP-23, -25 and -29, syntaxins 6, 8 and 10, and Vti1b), but not with syntaxin 16 (in the Golgi) or syntaxin 13 (in early endosomes) [11,17,22,29,33–36]. Although physiological roles for all of these associations remain to be determined, these interactions clearly indicate that TI-VAMP is involved in plasmalemmal and late endosomal membrane fusion events [37]. In addition, the involvement of TI-VAMP in the degradation pathway of epithelial growth factor [38] and in vesicle fusion in alveolar macrophages [39] is consistent with a role in transport from late endosomes to lysosomes. Endobrevin (also known as VAMP8) has been also proposed to have this role, but whereas TI-VAMP probably mediates heterotypic fusions, endobrevin mediates homotypic fusion involving late endosomal vesicles [39–41].

The evidence obtained so far strongly suggests that TI-VAMP might have a pivotal role in cell morphogenesis and remodelling. In fact, TI-VAMP is required for transport to the apical plasma membrane of epithelial cells and is implicated (together with synaptotagmin VII) in lysosome secretion and membrane repair in fibroblasts [11,33,42,43]. In addition, TI-VAMP is involved in the Ca^{2+}-stimulated degranulation of mastocytic cells and in phagosome sealing in macrophages during phagocytosis, because knockdown of the expression of TI-VAMP leads to a reduction in the exocytosis of late endocytic vesicles and an early blockade of pseudopod extension [44,45].

TI-VAMP is also central to neuronal morphogenesis: it concentrates at the tips of growing axons and dendrites, particularly at contact sites, and is required for vesicular transport that mediates neuritogenesis [34,37,46]. Molecular dissection of TI-VAMP and ectopic expression experiments have started to yield insights into the role of its LD in neurite outgrowth; in fact, overexpression of the TI-VAMP LD inhibits neuritogenesis in neuroendocrine cells and both axonal and dendritic outgrowth in hippocampal neurons [17,47]. By contrast, expression of an LD deletion mutant of TI-VAMP increases the formation of SNARE complexes and strongly stimulates neurite outgrowth [17,47].

Evidence on the specific compartmentalization of mammalian TI-VAMP underlines the specificity of v-SNAREs in distinct trafficking pathways; moreover, the enrichment of such longins in cerebral tissues and the specific role of TI-VAMP in neurite outgrowth suggest that longins, rather than brevins, are 'developmental VAMPs'. Indeed, VAMP2 is essential for neurotransmitter release but, in contrast to TI-VAMP, it is dispensable for neuronal differentiation [48], as has been confirmed *in vivo* by VAMP2 knockout mice, which show an almost complete block in neurotransmitter release but apparently normal brain development [49]. Knockout, knockdown and gain-of-function experiments *in vivo* are expected to improve our knowledge of the morphogenetic and developmental role of this longin, which, together with Ykt6, is likely to have important roles in brain function (Box 2).

Sec22 and Ykt6

The proteome of yeast *Saccharomyces cerevisiae* contains five R-SNAREs: its two 'brevins' [7,8] (Snc1 and Snc2) function in trafficking to the cell surface, both within the endosomal system and between endosomes and the Golgi [50]; Nyv1 is the largest of the five R-SNAREs and the least similar to the other four [51]; and the two longins (Sec22 and Ykt6) are evolutionarily conserved proteins, but only Ykt6 is essential [14].

Structure and function

The functional conservation of Ykt6 is reflected in the observations that Ykt6-depleted yeast strains can be complemented by human Ykt6 [14] and that yeast Ykt6 contains the targeting information necessary for its correct localization in mammalian cells and for a specific role in neurons [26] (Box 2). Although Ykt6 and Sec22 are strikingly similar proteins – both in their SNARE motifs [10] and in the structures of their LDs [18,19] – they differ from one another in several significant aspects.

First, Sec22 is anchored via a transmembrane domain (TMD) and functions in both anterograde [52] and retrograde [53] trafficking between the endoplasmic reticulum (ER) and the Golgi. By contrast, Ykt6 is lipid-anchored (Figure 1) and is required for protein trafficking at the Golgi, endosomes and the yeast vacuole [54–56]. Second, the primary function of Sec22 is attributed to its role as a SNARE, whereas Ykt6 is known to have at least one additional non-SNARE function in homotypic vacuolar fusion [27,29,30]. Third, even though Ykt6 and Sec22 have very similar LD structures, it has been proposed that the

Box 2. Longin involvement in neuronal function and brain disease

Longins are involved in exocytotic pathways underlying neuronal functions, as indicated by their localization in the nervous system and their effect on neuronal plasticity. Thus, malfunctions in longins or their binding partners might be involved in genetic pathologies, especially those of a neurological or behavioural origin.

TI-VAMP localizes to axons and dendrites in developing cerebral neurons [37,46] and is found at high concentrations in a subset of nerve terminals (particularly the mossy fibres in the CA3 region of the hippocampus) in the adult brain [76]. By contrast, Ykt6 is not widely expressed, but it is highly enriched in neurons from the hippocampus and the cerebral cortex, where it is targeted to a newly identified compartment via its longin domain (LD) [26]. Intracellular targeting of TI-VAMP is also regulated by its LD, which interacts with the δ-subunit of the clathrin-coat adaptor complex AP3 (AP3δ); in mutant *mocha* cells, which lack AP3δ, the localization of TI-VAMP is shifted from late to early endosomes [22]. Phenotypically, *mocha* mice show neurological and platelet dysfunctions. Localization of TI-VAMP is also altered in neuroepithelial cells from *hyh* (hydrocephalus with hop gait) mice that have mutations in the gene encoding the regulator of SNARE-mediated vesicle fusion, α-SNAP [77].

In studies using knockdown by RNA interference, TI-VAMP has been found to mediate both the intracellular transport of L1 – an important cell adhesion molecule (CAM) that also guides and stimulates axonal elongation – and the formation of L1-CAM-dependent cell contacts [34]. This observation suggests that TI-VAMP might participate in neuronal morphogenesis by delivering CAMs to the plasma membrane. Mutations in L1-CAM are responsible for a wide spectrum of neurological abnormalities, including CRASH syndrome (for corpus callosum hypoplasia, mental retardation, adducted thumbs, spastic paraplegia and hydrocephalus) [78].

Genetic linkage has pinpointed *SYBL1* [79], which encodes human TI-VAMP, as a causative candidate gene in bipolar disorder [80,81]. Intriguingly, SNAP-29, a homologue of SNAP-25 (25-kDa synaptosomal-associated protein) that has been associated with both schizophrenia and bipolar disorder [82], physically interacts with TI-VAMP in *Homo sapiens* and *Drosophila melanogaster* [22].

LD of Sec22, unlike that of Ykt6, does not fold back and interact with its SNARE motif to adopt a closed conformation [19]. It remains, however, to be established whether Sec22 can adopt such a conformation during its incorporation into coat protein II (COPII)-coated vesicles [21,57], and whether the LD of Sec22 might be involved in interactions with other coat proteins.

By contrast, the LD-mediated folded-back conformation of Ykt6 is known to be important for both the stability of the protein *in vivo* [18] and its correct localization in some but not all cell types [26,58]. In addition, the folded-back conformation of Ykt6 is likely to be necessary to chaperone both the prenyl-modified C terminus of the protein, thereby ensuring the solubility of the lipid-modified cytoplasmically localized form of the protein [26], and its SNARE motif to prevent promiscuous interactions with non-cognate SNAREs. Indeed, a mutation that disrupts the interaction between the LD and SNARE motif of yeast Ykt6 alters both its binding specificity for other SNAREs and the kinetics of SNARE complex assembly *in vitro*, albeit modestly [18]. The LD is therefore crucial for the function of Ykt6.

Because Sec22 is anchored in the membrane bilayer via its TMD, the extent to which Sec22 can move throughout the secretory pathway and participate in multiple transport steps might be restricted by the composition and length of its TMD [59], as well as by signals on Sec22 that direct its efficient recycling from the Golgi to the ER in COPI-coated vesicles [60]. Ykt6 can functionally substitute as a SNARE for Sec22 in ER-to-Golgi traffic in yeast, which perhaps explains why deletion of the *SEC22* gene is not lethal in this organism [53]; however, Sec22 cannot functionally substitute for Ykt6 [61].

Overexpression of the R-SNARE Nyv1p can compensate for specific defects in transport steps in *ykt6* mutant cells [55], but it remains to be determined whether Sec22 can (conditionally) substitute for any of the trafficking pathways in which Ykt6 is otherwise required. Nonetheless, there seems to be an obligate requirement for these two LD-containing proteins in trafficking between the ER and Golgi compartments: Sec22 is part of the t-SNARE complex (Sed5–Bos1–Sec22) that acts at the *cis*-Golgi [62,63], and Ykt6 is the R-SNARE component of the corresponding t-SNARE complex (Sed5–Gos1–Ykt6) that functions at the *trans*-Golgi [14,62,64,65]. Thus, each Golgi t-SNARE complex contains an LD protein. Whether the LD of Sec22 fulfils a specific role in the control of membrane fusion reactions has not been addressed so far; however, this domain is not required for SNARE complex formation with Sed5, Bos1 and Bet1 *in vivo* [57] and thus in this case it does not contribute to the specificity of SNARE–SNARE interactions. By contrast, the LD of Ykt6 is likely to be involved in both facilitating and preventing interactions between Ykt6 and other SNAREs *in vivo*.

Role of post-translational modifications

Mammalian Ykt6 is farnesylated and palmitoylated [58]. Both modifications are essential in HeLa cells, possibly because both are required for stable association with the membrane; accordingly, soluble Ykt6 was found to lack a palmitate anchor [58]. Thus, Ykt6 might cycle between a palmitoylated membrane-bound form and a de-palmitoylated but farnesylated and folded-back cytosolic form. Such cycling could regulate the participation of Ykt6 in several fusion reactions and transport steps.

By contrast, the cytoplasmic pool of neuronal Ykt6 has been postulated to be palmitoylated and prenylated. This doubly modified form of Ykt6 is crucial to direct the protein to the numerous punctate and presumably membranous structures that are occupied by the wild-type protein and that do not colocalize with any available organelle markers [26,66]. It has been suggested that the folded-back conformation of Ykt6 is necessary to interact with the C-terminal lipids, because substitutions of evolutionarily conserved hydrophobic residues in the LD that are predicted to mediate such interactions alter the localization pattern of the protein [66]. The incongruent data on the lipid-modified status of the cytoplasmic pool of Ykt6 in HeLa and neuronal cells are difficult to reconcile, partly because the experiments described in each case are not directly comparable. But although it is not possible to exclude the possibility that Ykt6 might be differentially modified in different types of cell or species, the

requirement that Ykt6 must be both prenylated and palmitoylated to be fully functional is at least consistent.

Not only is Ykt6 itself palmitoylated, but it seems to have a direct role in the palmitoylation of other target proteins. It has been recently shown that Ykt6 mediates acylation of the fusion protein Vac8 at yeast vacuoles [27]. This unexpected feature of Ykt6 could explain why five SNAREs are required for vacuole fusion [30]. It remains to be determined whether Ykt6 has a general role in palmitoylation reactions during intracellular membrane fusion events that are not restricted to the vacuole.

Distribution and domain variation

Although longins represent the most conserved and widely distributed R-SNAREs in terms of both their LDs and their SNARE motifs [7,10], a comparison of the R-SNARE proteomic complements from model eukaryotes with completely sequenced genomes demonstrates that the longin subfamilies show different distributions and varying numbers of members among taxa (Table 1).

The Sec22 and Ykt6 longins have an apparently homogeneous distribution and number of genes, whereas the TI-VAMP longins and brevins are either absent or amplified in their numbers in different taxa. For example, *Arabidopsis thaliana* lacks brevins and has more than ten TI-VAMPs, whereas *S. cerevisiae* and *Schizosaccharomyces pombe* have brevins but lack TI-VAMP. Protists and metazoans (including *Homo sapiens*) seem to have only one representative of the TI-VAMP longin subfamily, but some variants, which might result in functional specialization, are generated at the post-transcriptional level (Figure 2).

Mammalian cells contain two Sec22 isoforms (termed Sec22a and Sec22c), both of which contain LDs and hydrophobic C termini but lack the SNARE motifs. Both Sec22a and Sec22c localize to the ER and their overexpression leads to the mislocalization of some Golgi SNAREs, suggesting that they might be involved in protein trafficking [67]. Because these isoforms lack a SNARE motif, however, they are unlikely to function as fusion-promoting SNAREs. Additional studies will be necessary to determine the precise role of Sec22a and Sec22c, which in turn might also provide more clues to the role of the LD.

Splicing variants have been reported for TI-VAMP, including TI-VAMPc (showing an in-frame central truncation of the LD [22]) and TI-VAMPb (having a truncated SNARE motif, followed by an extra domain substituting for the TMD; TrEMBL accession code Q9H4A7; http://www.expasy.org/sprot/). In addition to the longins, non-SNARE proteins containing a longin-like domain can have splice variants [68]; this is not surprising, considering that LDs have profilin-like folds and that alternative splicing of profilins in mammals results in differentially expressed isoforms with distinct biochemical properties [25].

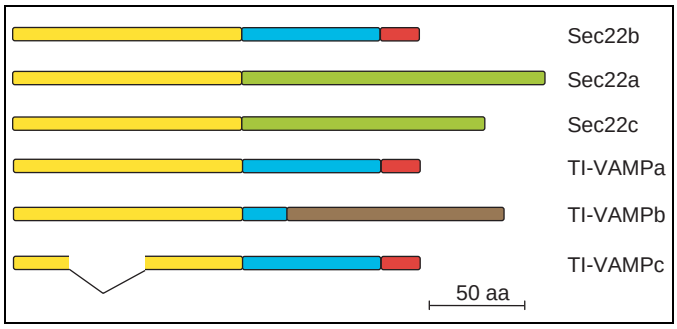

Figure 2. Domain variation in longins. Shown is a comparison of the domain architecture of longin isoforms (Sec22b and TI-VAMPa are the main isoforms). The R-SNARE coiled-coil (light blue) and transmembrane (red) domains are both either missing or altered in the Sec22a, Sec22c [67] and TI-VAMPb isoforms, which show novel C-terminal regions (green or brown). TI-VAMPc is characterized by a variant longin domain (yellow) [22].

Concluding remarks

The SNARE family has remained mostly unchanged in yeast, flies and worms, but has significantly increased in the number of representatives in mammals [6] and plants [9]. These features suggest that multicellular organisms, first, do not have an inherently more complex secretory pathway and, second, use additional SNAREs for the tissue-specific specialization of membrane trafficking [6]. The possibility that longins and brevins might have different roles in differentiation, development and 'cellucellular' exocytosis is suggested by their differential distributions among taxa: plants (i.e. organisms with a never-ending development and growth programme) show an increase in the longin family and lack brevins, whereas yeast species lack TI-VAMP longins. Given that the specificity of SNARE compartmentalization and role in neuronal differentiation seems to depend on the LD, this domain as well as LD-like domains might be chief factors in the control of mammalian (including human) brain development and function.

As predicted by the SNARE hypothesis, membrane flow patterns are encoded and recapitulated by SNAREs [69],

Table 1. Longins and brevins in model eukaryotes

Organism	Number of longins (different genes) in each subfamily			Brevins (presence)
	TI-VAMP	Sec22	Ykt6	
Homo sapiens	1	3	1	Yes
Mus musculus	1	3	1	Yes
Caenorhabditis elegans	1[a]	1	1	Yes
Anopheles gambiae	1	1	1	Yes
Drosophila melanogaster	1	1	1	Yes
Plasmodium falciparum	1	1	2	No
Encephalitozoon cuniculi	1	–	1	Yes
Arabidopsis thaliana	12	2	2	No
Neurospora crassa	1	1	1	Yes
Saccharomyces cerevisiae	–	1	1	Yes
Schizosaccharomyces pombe	–	1	1	Yes

[a]Slightly degenerate longin domain, not recognized by profile PS50859 from the PROSITE database (http://www.expasy.org/prosite/).

suggesting that combinatorial interactions and specific asymmetric requirements might generate a sufficient number of complexes to enable a single SNARE complex to act in only one transport step [70]. In addition to specificity, however, spatial and temporal regulation are crucial to ensure correct cellular trafficking. Future studies on such layers of control need to focus on how SNAREs and their regulatory domains, tethering proteins and adaptor complexes are associated in a finely integrated network. Several SNAREs have 'regulatory' NTDs that can fold independently [8], and recent evidence indicates that the LD has various functions. Moreover, longins are SNAREs that, at the same time, are related to (and interact with) both domains and proteins of tethering and adaptor complexes; intriguingly, some of these non-SNARE trafficking regulators also adopt LD-like folds. Such a sharing of the LD fold among SNARE and non-SNARE proteins might account for a common basic interaction code in the spatial and temporal control of membrane trafficking, ensuring both the fine-tuning of membrane fusion and the proper subcellular localization and tethering of vesicles to the target membrane.

In conclusion, the evidence obtained so far strongly suggests that LDs and longins (as well as LD-like domains and proteins) mediate and regulate fundamental trafficking steps as diverse as vacuolar sorting in yeast and brain function and development in mammals. Determining and comparing further longin and LD-like structures, functions and interactions, as well as knockdown or knockout gene expression experiments, are expected to shed more light on the cellular, developmental and physiopathological processes that are dependent on longins and their LDs.

Acknowledgements

This manuscript is dedicated to the memory of Giuliana Ferrarone. V.R. and M.V. are supported, respectively, by a research associate and a postdoctoral fellowship funded by the University of Padua. L.E.P.D. is a recipient of a predoctoral fellowship of the Boehringer Ingelheim Fonds. D.K.B.'s laboratory is supported by the Research Grants Council of Hong Kong; C.U.'s laboratory by the DFG, the SFB638, the Fonds der Chemischen Industrie and the EMBO young investigator programme; M.D.E.'s laboratory by a Telethon grant (GGP02308) and the EC Sixth FP ('SAFE' Network of Excellence, contract LSHB-CT-2004–503243); T.G.'s laboratory by INSERM, the AFM, the ARC, the HFSP and the EC Sixth FP (Signaling and Trafficking STREP 503229); and F.F.'s laboratory by MIUR (ex 60%).

References

1 Ungar, D. and Hughson, F.M. (2003) SNARE protein structure and function. *Annu. Rev. Cell Dev. Biol.* 19, 493–517
2 Weimbs, T. *et al*. (1998) A model for structural similarity between different SNARE complexes based on sequence relationships. *Trends Cell Biol.* 8, 260–262
3 Sutton, R.B. *et al*. (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature* 395, 347–353
4 Fasshauer, D. *et al*. (1998) Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15781–15786
5 Ossig, R. *et al*. (2000) Exocytosis requires asymmetry in the central layer of the SNARE complex. *EMBO J.* 19, 6000–6010
6 Bock, J.B. *et al*. (2001) A genomic perspective on membrane compartment organization. *Nature* 409, 839–841
7 Filippini, F. *et al*. (2001) Longins: a new evolutionary conserved VAMP family sharing a novel SNARE domain. *Trends Biochem. Sci.* 26, 407–409
8 Dietrich, L.E.P. *et al*. (2003) Control of eukaryotic membrane fusion by N-terminal domains of SNARE proteins. *Biochim. Biophys. Acta* 1641, 111–119
9 Pratelli, R. *et al*. (2004) A new catch in the SNARE. *Trends Plant Sci.* 9, 187–195
10 Rossi, V. *et al*. (2004) VAMP subfamilies identified by specific R-SNARE motifs. *Biol. Cell* 96, 251–256
11 Galli, T. *et al*. (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
12 Advani, R.J. *et al*. (1998) Seven novel mammalian SNARE proteins localize to distinct membrane compartments. *J. Biol. Chem.* 273, 10317–10324
13 Sacher, M. *et al*. (1997) The synaptobrevin-related domains of Bos1p and Sec22p bind to the syntaxin-like region of Sed5p. *J. Biol. Chem.* 272, 17134–17138
14 McNew, J.A. *et al*. (1997) Ykt6p, a prenylated SNARE essential for endoplasmic reticulum–Golgi transport. *J. Biol. Chem.* 272, 17776–17783
15 Nicholson, K.L. *et al*. (1998) Regulation of SNARE complex assembly by an N-terminal domain of the t-SNARE Sso1p. *Nat. Struct. Biol.* 5, 793–802
16 Parlati, F. *et al*. (1999) Rapid and efficient fusion of phospholipid vesicles by the α-helical core of a SNARE complex in the absence of an N-terminal regulatory domain. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12565–12570
17 Martinez-Arca, S. *et al*. (2000) Role of tetanus neurotoxin insensitive vesicle-associated membrane protein (TI-VAMP) in vesicular transport mediating neurite outgrowth. *J. Cell Biol.* 149, 889–899
18 Tochio, H. *et al*. (2001) An autoinhibitory mechanism for nonsyntaxin SNARE proteins revealed by the structure of Ykt6p. *Science* 293, 698–702
19 Gonzalez, L.C., Jr. *et al*. (2001) A novel SNARE N-terminal domain revealed by the crystal structure of Sec22b. *J. Biol. Chem.* 276, 24203–24211
20 Toonen, R.F. and Verhage, M. (2003) Vesicle trafficking: pleasure and pain from SM genes. *Trends Cell Biol.* 13, 177–186
21 Mossessova, E. *et al*. (2003) SNARE selectivity of the COPII coat. *Cell* 114, 483–495
22 Martinez-Arca, S. *et al*. (2003) A dual mechanism controlling the localization and function of exocytic v-SNAREs. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9011–9016
23 Aravind, L. and Ponting, C.P. (1997) The GAF domain: an evolutionary link between diverse phototransducing proteins. *Trends Biochem. Sci.* 22, 458–459
24 Hefti, M.H. *et al*. (2004) The PAS fold. A redefinition of the PAS domain based upon structural prediction. *Eur. J. Biochem.* 271, 1198–1208
25 Lambrechts, A. *et al*. (2000) Profilin II is alternatively spliced, resulting in profilin isoforms that are differentially expressed and have distinct biochemical properties. *Mol. Cell. Biol.* 20, 8209–8219
26 Hasegawa, H. *et al*. (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
27 Dietrich, L.E.P. *et al*. (2004) The SNARE Ykt6 mediates protein palmitoylation during an early stage of homotypic vacuole fusion. *EMBO J.* 23, 45–53
28 Veit, M. *et al*. (2001) Vac8p release from the SNARE complex and its palmitoylation are coupled and essential for vacuole fusion. *EMBO J.* 20, 3145–3155
29 Ungermann, C. *et al*. (1998) A vacuolar v-t-SNARE complex, the predominant form *in vivo* and on isolated vacuoles, is disassembled and activated for docking and fusion. *J. Cell Biol.* 140, 61–69
30 Ungermann, C. *et al*. (1999) Three v-SNAREs and two t-SNAREs, present in a pentameric *cis*-SNARE complex on isolated vacuoles, are essential for homotypic fusion. *J. Cell Biol.* 145, 1435–1442
31 Laage, R. and Ungermann, C. (2001) The N-terminal domain of the t-SNARE Vam3p coordinates priming and docking in yeast vacuole fusion. *Mol. Biol. Cell* 12, 3375–3385

32 Siniossoglou, S. and Pelham, H.R. (2001) An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J.* 20, 5991–5998

33 Rao, S.K. *et al*. (2004) Identification of SNAREs involved in Synaptotagmin VII-regulated lysosomal exocytosis. *J. Biol. Chem.* 279, 20471–20479

34 Alberts, P. *et al*. (2003) Cross talk between tetanus neurotoxin-insensitive vesicle-associated membrane protein-mediated transport and L1-mediated adhesion. *Mol. Biol. Cell* 14, 4207–4220

35 Martinez-Arca, S. *et al*. (2003) Ectopic expression of syntaxin 1 in the ER redirects TI-VAMP and cellubrevin-containing vesicles. *J. Cell Sci.* 116, 2805–2816

36 Wade, N. *et al*. (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

37 Coco, S. *et al*. (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

38 Advani, R.J. *et al*. (1999) VAMP-7 mediates vesicular transport from endosomes to lysosomes. *J. Cell Biol.* 146, 765–775

39 Ward, D.M. *et al*. (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

40 Pryor, P.R. *et al*. (2004) Combinatorial SNARE complexes with VAMP7 or VAMP8 define different late endocytic fusion events. *EMBO Rep.* 5, 590–595

41 Antonin, W. *et al*. (2000) The R-SNARE endobrevin/VAMP-8 mediates homotypic fusion of early endosomes and late endosomes. *Mol. Biol. Cell* 11, 3289–3298

42 Lafont, F. *et al*. (1999) Raft association of SNAP receptors acting in apical trafficking in Madin–Darby canine kidney cells. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3734–3748

43 Reddy, A. *et al*. (2001) Plasma membrane repair is mediated by Ca^{2+}-regulated exocytosis of lysosomes. *Cell* 106, 157–169

44 Hibi, T. *et al*. (2000) Rat basophilic leukemia cells express syntaxin-3 and VAMP-7 in granule membranes. *Biochem. Biophys. Res. Commun.* 271, 36–41

45 Braun, V. *et al.* TI-VAMP/VAMP7 is required for optimal phagocytosis of opsonised particles in macrophages. *EMBO J.* (in press)

46 Alberts, P. and Galli, T. (2003) The cell outgrowth secretory endosome (COSE): a specialized compartment involved in neuronal morphogenesis. *Biol. Cell.* 95, 419–424

47 Martinez-Arca, S. *et al*. (2001) A common exocytotic mechanism mediates axonal and dendritic outgrowth. *J. Neurosci.* 21, 3830–3838

48 Osen-Sand, A. *et al*. (1996) Common and distinct fusion proteins in axonal growth and transmitter release. *J. Comp. Neurol.* 367, 222–234

49 Schoch, S. *et al*. (2001) SNARE function analyzed in synaptobrevin/VAMP knockout mice. *Science* 294, 1117–1122

50 Gerst, J.E. (1999) SNAREs and SNARE regulators in membrane fusion and exocytosis. *Cell. Mol. Life Sci.* 55, 707–734

51 Nichols, B.J. *et al*. (1997) Homotypic vacuolar fusion mediated by v- and t- SNAREs. *Nature* 387, 199–202

52 Liu, Y. and Barlowe, C. (2002) Analysis of Sec22p in endoplasmic reticulum/Golgi transport reveals cellular redundancy in SNARE protein function. *Mol. Biol. Cell* 13, 3314–3324

53 Burri, L. *et al*. (2003) A SNARE required for retrograde transport to the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9873–9877

54 Tsui, M.M.K. and Banfield, D.K. (2000) Yeast Golgi SNARE interactions are promiscuous. *J. Cell Sci.* 113, 145–152

55 Dilcher, M. *et al*. (2001) Genetic interactions with the yeast Q-SNARE VTI1 reveal novel functions for the R-SNARE YKT6. *J. Biol. Chem.* 276, 34537–34544

56 Kweon, Y. *et al*. (2003) Ykt6p is a multifunctional yeast R-SNARE that is required for multiple membrane transport pathways to the vacuole. *Mol. Biol. Cell* 14, 1868–1881

57 Liu, Y. *et al*. (2004) Sec22p export from the endoplasmic reticulum is independent of SNARE pairing. *J. Biol. Chem.* 279, 27225–27232

58 Fukasawa, M. *et al*. (2004) Localization and activity of the SNARE Ykt6 determined by its regulatory domain and palmitoylation. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4815–4820

59 Bretscher, M.S. and Munro, S. (1993) Cholesterol and the Golgi apparatus. *Science* 261, 1280–1281

60 Ballensiefen, W. *et al*. (1998) Recycling of the yeast v-SNARE Sec22p involves COPI-proteins and the ER transmembrane proteins Ufe1p and Sec20p. *J. Cell Sci.* 111, 1507–1520

61 Tsui, M.M.K. *et al*. (2001) The selective formation of Sed5p-containing SNARE complexes is mediated by combinatorial binding interactions. *Mol. Biol. Cell* 12, 521–538

62 Parlati, F. *et al*. (2002) Distinct SNARE complexes mediating membrane fusion in Golgi transport based on combinatorial specificity. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5424–5429

63 Volchuk, A. *et al*. (2004) Countercurrent distribution of two distinct SNARE complexes mediating transport within the Golgi stack. *Mol. Biol. Cell* 15, 1506–1518

64 Xu, Y. *et al*. (2002) GS15 forms a SNARE complex with syntaxin 5, GS28, and Ykt6 and is implicated in traffic in the early cisternae of the Golgi apparatus. *Mol. Biol. Cell* 13, 3493–3507

65 Zhang, T. and Hong, W. (2001) Ykt6 forms a SNARE complex with syntaxin 5, GS28, and Bet1 and participates in a late stage in endoplasmic reticulum–Golgi transport. *J. Biol. Chem.* 276, 27480–27487

66 Hasegawa, H. *et al*. (2004) Intramolecular protein–protein and protein–lipid interactions control the conformation and subcellular targeting of neuronal Ykt6. *J. Cell Sci.* 117, 4495–4508

67 Tang, B.L. *et al*. (1998) Hsec22c: a homolog of yeast Sec22p and mammalian rsec22a and msec22b/ERS-24. *Biochem. Biophys. Res. Commun.* 243, 885–891

68 Mumm, S. *et al*. (2001) The sedlin gene for spondyloepiphyseal dysplasia tarda escapes X-inactivation and contains a non-canonical splice site. *Gene* 273, 285–293

69 McNew, J.A. *et al*. (2000) Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* 407, 153–159

70 Banfield, D.K. (2001) SNARE complexes – is there sufficient complexity for vesicle targeting specificity? *Trends Biochem. Sci.* 26, 67–68

71 Jang, S.B. *et al*. (2002) Crystal structure of SEDL and its implications for a genetic disease spondyloepiphyseal dysplasia tarda. *J. Biol. Chem.* 277, 49863–49869

72 Gécz, J. *et al*. (2003) Human wild-type SEDL protein functionally complements yeast Trs20p but some naturally occurring SEDL mutants do not. *Gene* 320, 137–144

73 Apweiler, R. *et al*. (2001) The InterPro database, an integrated documentation resource for protein families, domains and functional sites. *Nucleic Acids Res.* 29, 37–40

74 Robinson, M.S. (2004) Adaptable adaptors for coated vesicles. *Trends Cell Biol.* 14, 167–174

75 Collins, B.M. *et al*. (2002) Molecular architecture and functional model of the endocytic AP2 complex. *Cell* 109, 523–535

76 Muzerelle, A. *et al*. (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

77 Chae, T.H. *et al*. (2004) The *hyh* mutation uncovers roles for α-SNAP in apical protein localization and control of neural cell fate. *Nat. Genet.* 36, 264–270

78 Fransen, E. *et al*. (1997) L1-associated diseases: clinical geneticists divide, molecular geneticists unite. *Hum. Mol. Genet.* 6, 1625–1632

79 D'Esposito, M. *et al*. (1996) A synaptobrevin-like gene in the Xq28 pseudoautosomal region undergoes X inactivation. *Nat. Genet.* 13, 227–229

80 Saito, T. *et al*. (2000) Analysis of the pseudoautosomal X-linked gene *SYBL1* in bipolar affective disorder: description of a new candidate allele for psychiatric disorders. *Am. J. Med. Genet.* 96, 317–323

81 Muller, D.J. *et al*. (2002) Association between a polymorphism in the pseudoautosomal X-linked gene *SYBL1* and bipolar affective disorder. *Am. J. Med. Genet.* 114, 74–78

82 Saito, T. *et al*. (2001) Polymorphism in SNAP29 gene promoter region associated with schizophrenia. *Mol. Psychiatry* 6, 193–201