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Review

SNAREs and traffic

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Abstract

SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) are now generally accepted to be the major players in the final stage of the docking and the subsequent fusion of diverse vesicle-mediated transport events. The SNARE-mediated process is conserved evolutionally from yeast to human, as well as mechanistically and structurally across different transport events in eukaryotic cells. In the post-genomic era, a fairly complete list of "all" SNAREs in several organisms (including human) can now be made. This review aims to summarize the key properties and the mechanism of action of SNAREs in mammalian cells.

Keywords: Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; Vesicle fusion; Membrane traffic; Synaptic transmission; Exocytosis; Endocytosis

1. Introduction

The highly-organized eukaryotic cell contains many membrane-enclosed intracellular organelles/compartments and it requires precise mechanisms to govern protein transport between different organelles, particularly in the secretory and endocytic pathways. Small shuttling vesicles (such as synaptic vesicles of neurons) or larger transport containers (such as zymogen granules of pancreatic acinar cells) are the major intermediates in anterograde or retrograde translocation of proteins between various compartments in the secretory and endocytic pathways. The basic steps underlying vesicle-mediated transport are vesicle/container formation from a donor compartment, translocation of transport intermediates to a target compartment, tethering of transport intermediates with the target compartment, and, finally, the docking and fusion of vesicles/containers with the target compartment [1].

SNAREs function in the final event of docking of vesicles/containers with the target compartment and catalyze the fusion of the apposing membranes of the transport intermediate and the target compartment [2–4]. Functionally, SNAREs can be classified into v-SNAREs that are associated with the vesicle/container and t-SNAREs that are associated with the target compartment (Table 1). Specific interaction of v-SNARE on the transport intermediate with the cognate t-SNARE on the receiving target compartment underlies the central event of docking and fusion process of vesicle-mediated transport. Our current knowledge is that v-SNARE usually consists of a tail-anchored SNARE having a single SNARE motif, while a t-SNARE consists of either two or three polypeptides [5]. A heterodimeric t-SNARE is usually comprised of a member of the syntaxin (Syn) subfamily, which contributes one SNARE motif as the t-SNARE heavy chain, and a member of the SNAP-25 subfamily, which contributes two SNARE motifs as two t-SNARE light chains. A heterotrimeric t-SNARE is formed by one member of the Syn subfamily (as the heavy chain), one member of the SNARE subfamily related to the N-terminal half of SNAP-25 (as one of the two light chains), and one member of the SNARE subfamily related to the C-terminal half of SNAP-25 (as the other light chain). Interaction between v-SNARE and t-SNARE leads to the formation of the *trans*-SNARE complex (or SNAREpin), in which the

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Table 1
Classification of SNAREs. Functionally, SNAREs can be classified into v-SNAREs associated with the vesicle (or other forms of transport intermediates) and t-SNARE associated with the target compartment

Functionally	v-SNARE	t-SNARE
Sub-classification of t-SNARE	Heavy chain & Light chain	
Structurally according to the central residue of SNARE motif (0 layer)	R-SNARE	Q-SNARE
Sub-classification of Q-SNARE	Qa, Qb & Qc	

A t-SNARE is generally assembled from one heavy chain and two light chains of SNARE domains. The two light chains can come from one or two proteins. Based on the residue in the 0 layer in the four-helical SNARE bundle of the SNARE domain, SNAREs can be structurally divided into Q-SNAREs (those having a Q/Gln residue) and R-SNARE (those having an Arg/R residue). The Q SNAREs can be further subdivided into Qa-, Qb-, and Qc-SNAREs based on amino acid sequence of the SNARE domain.

four SNARE motifs assemble as a twisted parallel four-helical bundle, which catalyzes the apposition and fusion of the vesicle with the target compartment [6–8].

2. The general mode of SNARE action

All newly-made SNAREs are first delivered to their hosting compartment(s) via the secretory and endocytic pathways. The general mode of action of SNAREs in vesicle-mediated transport is highlighted in Fig. 1. First, the v-SNARE is packaged together with other cargo proteins into the budding vesicle so that the resulting transport intermediate is competent to fuse with the target compartment. SNAREs may also play an active role in the formation of the vesicle through direct interaction with coat proteins, as exemplified by the interaction of SNAREs with COPII coat proteins during the formation of vesicles from the endoplasmic reticulum (ER) [9,10]. Interaction of SNAREs with the COPI machinery has also been observed [11]. Similarly, the interaction of Vti1b with epsinR is involved in the formation of shuttling vesicles between the late endosome and trans-Golgi network (TGN) [12]. A role for VAMP2 in rapid endocytosis of synaptic vesicles also suggests that SNAREs function in driving the formation of transport vesicles [13].

Next, during the tethering event mediated by various tethering factors [14,15], vesicles are positioned precisely at the region of the target compartment where the t-SNAREs

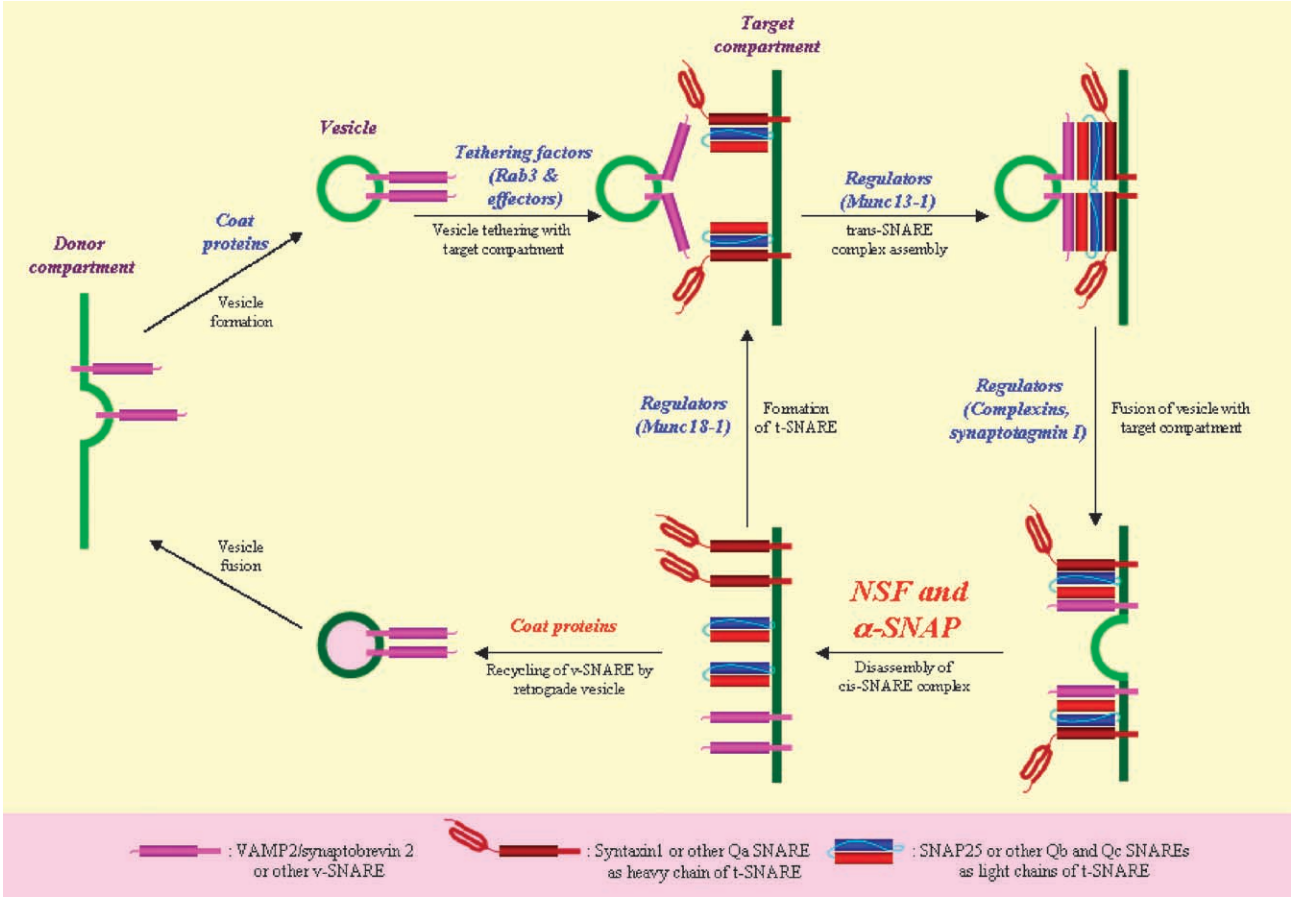


Fig. 1. The general mode of SNARE action using the synaptic SNARE complex as an example.

are located. The tethering factors, which act over a longer distance than the SNAREs, interact with both the vesicle and the target compartment to facilitate the subsequent pairing of the v-SNARE with the cognate t-SNARE. For example, the tethering factor p115 enhances the formation of two SNARE complexes in the early secretory pathway [16].

In the third stage, the interaction of v-SNAREs and t-SNAREs on the two opposing membranes mediates the short-range docking of the vesicle with the target compartment followed by the formation of a *trans*-SNARE complex [17–20]. The SNARE motifs are believed to be "unstructural" before complex assembly and become highly organized into a four-helical bundle during the formation of the *trans*-SNARE complex. The energy released during the SNARE complex assembly (which functions like a zipper and the zippering starts from the N-terminal side and progresses toward the C-terminal end) may overcome the energy barrier for membrane opposition created largely by the negative charges of phospholipid headgroups of the lipid bilayers. The *trans*-SNARE complex may thus directly catalyze the fusion of the two apposing membranes. After fusion, the complex becomes a cis-SNARE complex in the target compartment.

Finally, to be ready for the subsequent rounds of transport, the cis-SNARE complex needs to be disassembled. This is catalyzed by the combined action of α-SNAP (*s*oluble *N*-ethylmaleimide-sensitive factor *a*ttachment *p*rotein) and NSF (*N*-ethylmaleimide-*s*ensitive *f*actor) which is an ATPase. Interaction of NSF (in the form of a hexamer) and three α-SNAPs with the cis-SNARE complex leads to the formation of a transient 20 S complex [21–24]. ATP hydrolysis by NSF leads to the disassembly of the 20 S complex as well as the cis-SNARE complex. The freed v-SNAREs can then be recycled to the donor compartment by retrograde transport, while the t-SNARE subunits can be re-organized into functional t-SNAREs for the next round of docking and fusion events.

3. General structural features of SNAREs

3.1. Most SNAREs are anchored to the cytoplasmic side of the membrane via a carboxyl (C)-terminal membrane anchor

SNAREs are generally small proteins of around 100–300 amino acids in length (Fig. 2, Table 1). The core

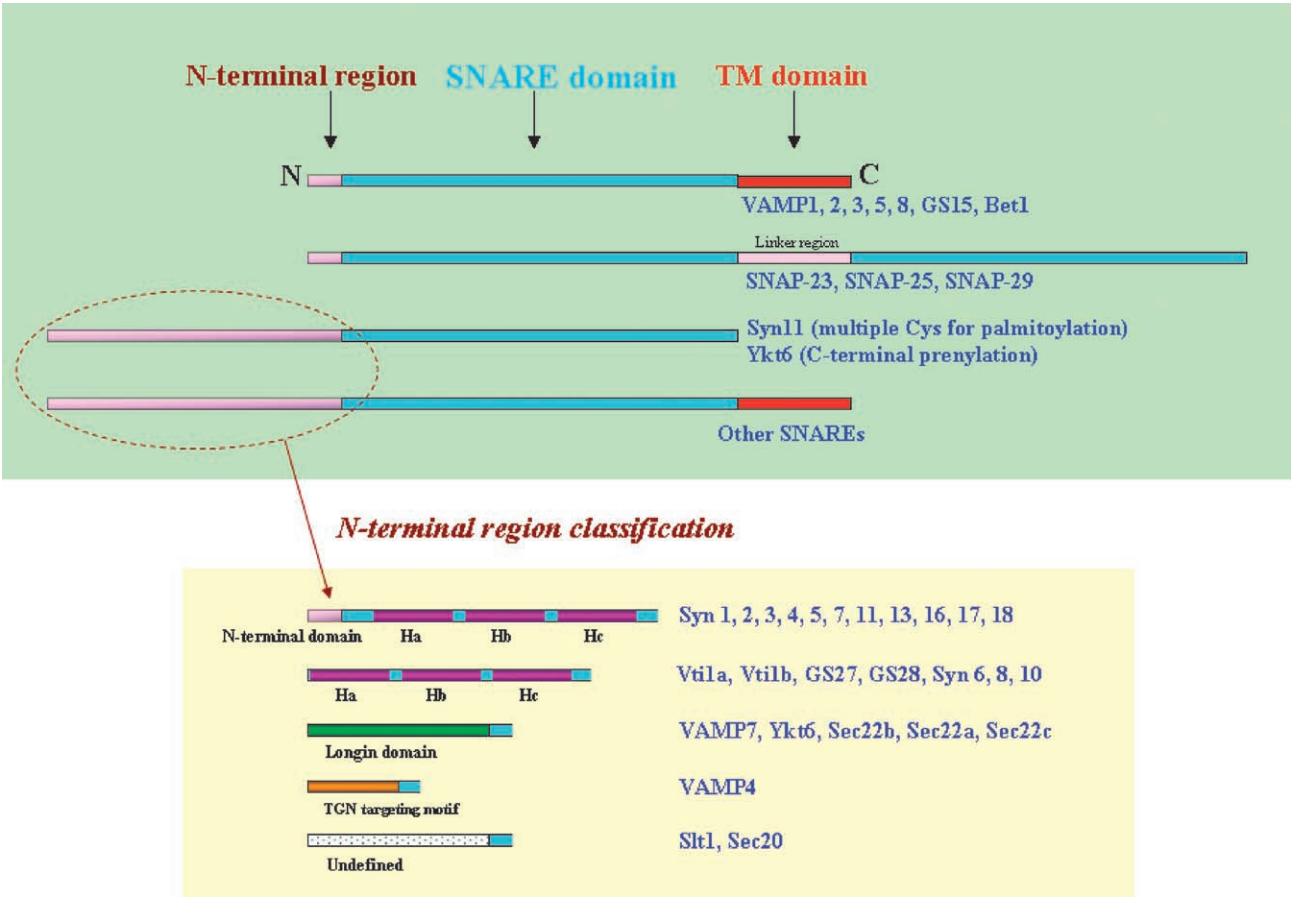


Fig. 2. The general structural frameworks of different SNAREs.

structural feature of SNAREs is an evolutionally-conserved SNARE motif of about 60 residues that is present in all SNAREs (Figs. 2 and 4) [25,26]. Around 36 distinct SNAREs are known in mammalian cells and the major features of the 36 human SNAREs are summarized in Table 2 [17,27,28]. The majority (31 out of the 36 SNAREs) is also characterized by a C-terminal hydrophobic region that functions as a membrane anchor (Fig. 1), anchoring the polypeptide to the cytoplasmic side of the membrane and oriented the rest of the polypeptide towards the cytoplasm. The other 5 SNAREs (SNAP-23, SNAP-25, SNAP-29, Syn11, and Ykt6) do not have a C-terminal membrane spanning domain, but are instead attached to the membrane by prenylation (Ykt6) [29], palmitoylation of Cys residues (SNAP-25, Ykt6, and Syn11) [29–31], and/or interaction with other SNAREs that are anchored by C-terminal tails [32].

3.2. Segregation of SNARE motifs into Qa/Syn, Qb(S25N), Qc(S25C), and R(VAMP) subfamilies

Although SNAREs are functionally classified as v-SNAREs or t-SNAREs, they can be structurally also distinguished as Q or R types [26] (Table 1). Most SNAREs (33 out of the 36) contain only one SNARE motif near the C-terminal tail anchor or the C-terminus, but 3 of these (SNAP-23, SNAP-25, and SNAP-29) contain two tandem SNARE motifs separated by a linker region. The N-terminal SNARE motifs of these three SNAREs are more homologous to each other than to the

Table 2
List of human SNAREs and their properties

Name	Yeast homolog	Locations	AA	SNARE motif	TM domain	GenBank acc #	Synonyms	Type
Syntaxin1	Sso1p/Sso2p	PM	288	202–254	266–288	Q16623	HPC-1	Qa
Syntaxin2		PM	288	201–253	265–286	P32856	Epimorphin	Qa
Syntaxin3		PM	289	201–253	264–288	NM_004177		Qa
Syntaxin4		PM	297	210–262	274–296	Q12846		Qa
Syntaxin5	Sed5p	Go	301	219–271	280–301	U26648		Qa
Syntaxin6	Tlg1p	TGN and End	255	173–225	235–255	AJ002078		Qc
Syntaxin7	Pep12p	EE and LE	261	175–227	238–259	U77942		Qa
Syntaxin8	Syn8p	EE and LE	236	155–207	216–233	NP_004844		Qc
Syntaxin10		TGN	249	167–219	229–249	AF035531		Qc
Syntaxin11		TGN and LE	287	214–266	No	O75558		Qa
Syntaxin13		EE	276	188–240	251–273	NP_803173	Syntaxin12	Qa
Syntaxin16	Tlg2p	TGN	325	240–292	302–322	NP_001001433		Qa
Syntaxin17		ER	302	172–224	230–250	NP_060389		Qa
Syntaxin18	Ufe1p	ER	335	253–305	314–330	Q9P2W9		Qa
SNAP-23	Sec9p	PM	211	24–76 and 156–208	No	NP_003816	Syndet	Qb and Qc
SNAP-25	Spo20p	PM	206	29–81 and 150–202	No	NP_003072		Qb and Qc
SNAP-29		Go and End	258	60–112 and 206–258	No	O95721	GS32	Qb and Qc
VAMP1	Snc1p/Snc2p	SV	118	34–86	97–117	P23763	Synaptobrevin1	R
VAMP2		SV	116	32–84	95–114	NP_055047	Synaptobrevin2	R
VAMP3		EE and RE	100	15–67	78–98	NP_004772	Cellubrevin	R
VAMP4		TGN and EE	141	53–105	119–137	NP_973723		R
VAMP5		PM	116	6–58	73–93	NP_006625		R
VAMP7	Nyv1p	LE and Ly and PM	220	126–178	189–214	NP_005629	Ti-VAMP	R
VAMP8		EE and LE	100	13–65	76–99	NP_003752	Endobrevin	R
Ykt6	Ykt6p	Go	198	139–191	Prenyl	AAB81131		R
Sec22a		ER and IC	282	135–187	190–208	AAD43013		?
Sec22b	Sec22p	IC and cis-Go	215	135–187	196–215	NP_004883	ERS-24	R
Sec22c		ER and IC	250	135–185	186–204	AAD02171		?
Bet1	Bet1p	IC, cis-Go	118	36–88	96–115	NP_005859		Qc
GS15	Sft1p	Go	111	25–77	87–106	AAF37877		Qc
GS27	Bos1p	IC and Go	212	130–182	192–212	O14653	Membrin, Gos-27	Qb
GS28	Gos1p	Go	250	170–222	231–250	O95249	GOS-28	Qb
Vti1a	Vti1p	trans-Go	217	132–184	193–214	BI830707 and BF805294	Vti1-rp2	Qb
Vti1b		EE and LE	232	146–198	207–229	NP_006361	Vti1-rp1	Qb
Slt1	Slt1p/Use1p	ER	259	173–225	232–252	BC008455	Use1, p31	Qc
Sec20	Sec20p	ER	228	132–184	203–220	NP_001196	Bnip1	?

Mammalian homologues of yeast Vam3p and Vam7p have not been defined yet. Syn5 has a longer version with N-terminal extension which harbors an ER retrieval signal [146] and the longer form of human Syn5 has the GenBank accession number CAD97668. PM: plasma membrane; Go: Golgi apparatus; cis-Go: cis-Golgi compartments; trans-Go: trans-Golgi compartments; TGN: trans-Golgi network; End: endosomes; EE: early endosomes; LE: late endosomes; RE: recycling endosomes; Ly: Lysosomes; ER: endoplasmic reticulum; IC: ER-Golgi intermediate compartments; SV: synaptic vesicles; AA: number of amino acid residues; TM: transmembrane domain.

C-terminal SNARE motif of the same protein. The same is also true for the C-terminal SNARE motif. Accordingly, the N-terminal SNARE motif of SNAP-25 defines a subfamily (S25N) of SNARE domains, while the C-terminal SNARE motif of SNAP-25 defines another subfamily (S25C). The SNARE motifs of GS27, GS28, Vti1a, and Vti1b are more structurally similar to the S25N motif, while the SNARE motifs of Syn6, Syn8, Syn10, GS15, Bet1, and Slt1 conform structurally to the S25C motif. SNARE motifs of the remaining SNAREs belong to either the Syn subfamily (Syn1, 2, 3, 4, 5, 7, 11, 13, 16, 17, and 18) or the VAMP subfamily (VAMP1, 2, 3, 4, 5, 7, 8, Sec22b, and Ykt6). The SNARE-like motifs of Sec22a, Sec22c, and Sec20/BNIP1 segregate together in the phylogenetics analysis and are more divergent (Fig. 3). In summary, the SNARE proteins may be segregated into four major subfamilies based on the amino acid sequence homologies of the SNARE domains, as shown in Fig. 3. The Syn subfamily is also referred to as Qa subfamily, while the S25N and S25C are the Qb and Qc subfamilies, respectively. Members of the VAMP subfamily are all R-SNAREs [7,8,17,28]. The classification of Qa, Qb, Qc, and R SNAREs is based on the residue at the position of the zero ionic layer of the 4-helical bundle (see Section 3.3). Analysis of SNARE complex formation in reconstituted lipid vesicles has provided another designation for t-SNAREs. Qa/Syn SNARE is considered to be the heavy chain, while Qb and Qc SNAREs (either from the same protein such as SNAP-25 or from two different proteins) are considered as the light chains [5,7,8].

3.3. A SNARE complex consists of a four-helical bundle assembled from one member from each of the four subfamilies

The crystal structure of the synaptic SNARE complex consisting of the SNARE domain of Syn1, S25N, S25C, and VAMP2 (Fig. 3B, upper panel) reveals that the four SNARE domains form a twisted parallel 4-helical bundle with each SNARE domain contributing one helix [7]. In this manner, the formation of the *trans*-SNARE complex will serve to "zipper up" (from the N- to the C-terminal end) the two apposing membranes by bringing closer the tail anchor of VAMP2 to that of Syn1. The energy generated during the formation of the four-helical bundle has been proposed to be the driving force for the fusion process [33]. Within the interior of the SNARE bundle, the four helices are connected by 16 layers of interacting surfaces mediated by the side chain of the residues which are mostly hydrophobic and are arranged perpendicular to the axis of the four-helical bundle. The middle of the bundle is usually characterized by a layer (defined as the 0 layer) of interaction mediated by hydrophilic residues of three Gln (Q) residues (contributed each by Syn1, S25N and S25C), and one Arg (R) residue (contributed by VAMP2) (Fig. 3C) [7,26]. The 7 layers of interaction preceding the 0 layer are defined as −7 to −1, while the 8 layers C-terminal to the 0 layer are defined as 1 to 8. As mentioned above, SNAREs are thus structurally classified as Q-SNAREs and R-SNAREs dependent on the presence of either Q or R at this position, with the Q-SNAREs further divided into Qa (for Syn subfamily), Qb (for S25N subfamily), and Qc (for S25C subfamily) SNAREs [26,28]. Solution of the crystal structure of an endosomal SNARE complex consisting of the SNARE domains of Syn7 (Qa), Vti1b (Qb), Syn8 (Qc), and VAMP8 (R) (Fig. 3B, lower panel) supports this stoichiometric principle derived from analysis of the neuronal SNARE complex [8]. It is now generally believed that one member of each subfamily contributes a single SNARE motif, resulting in the Qa:Qb:Qc:R configuration of a SNARE complex. Thus, in general, most v-SNAREs are R-SNAREs (such as VAMP1, 2, 3, 4, 5, 7, and 8) although some R-SNAREs (such as Sec22b and Ykt6) may function as one of the two light chains of t-SNARE. Qa SNAREs are always the heavy chain of a t-SNARE and Qb SNAREs usually participate as one of the light chain. Most Qc SNAREs act as a light chain of t-SNARE, but some Qc SNAREs (such as GS15, Bet1 and likely Slt1) may function as v-SNAREs [3,5,34,35].

3.4. Classification of the N-terminal region of SNAREs

Most SNAREs (except for VAMP1, 2, 3, 5, 8, GS15, Bet1, SNAP-23, SNAP-25, and SNAP-29) are also characterized by an extended N-terminal domain with coiled-coil regions (Figs. 2 and 4) [36]. The N-terminal region of these SNAREs can be divided into 5 major types. The first is represented by Qa/Syn SNAREs and has a three-helical (consisting of Ha, Hb, and Hc regions) bundle preceded by an N-terminal domain (Figs. 2, 5 and 6). The Habc 3-helical bundle in some Qa SNAREs such as Syn1 and Syn7 can fold back to interact with the C-terminal SNARE motif, generating a closed conformation [33,36–38], while the Habc bundle of other Qa SNAREs such as Syn5 and Syn16 does not interact with the C-terminal SNARE motif and has an open conformation [36,39–42]. The closed conformation of Qa SNAREs needs to be opened by some regulators before and/or during the assembly of t-SNARE. The N-terminal domain of some Qa SNAREs is involved in the interaction with such regulators. SLY1 binds with the N-terminus of Syn5 and Syn18 [40–42] and VPS45 with the N-terminus of Syn16 [39]. The N-terminal helical regions of Vti1b, Syn6, and Syn8 have been shown to form a three-helical bundle [38,43]. The sequence similarities of Vti1a, GS27 and GS28 with Vti1b (Fig. 6A) and bioinformatics analysis [43] predict that the N-terminal region of Vit1a, GS27 and GS28 also forms a three-helical bundle. Syn10 is highly homologous to Syn6 (Fig. 6B) and its N-terminal region may also adopt a structure similar to that of Syn6. The third type of N-terminal extension is characterized by those of Ykt6 and Sec22b and it has been referred to as

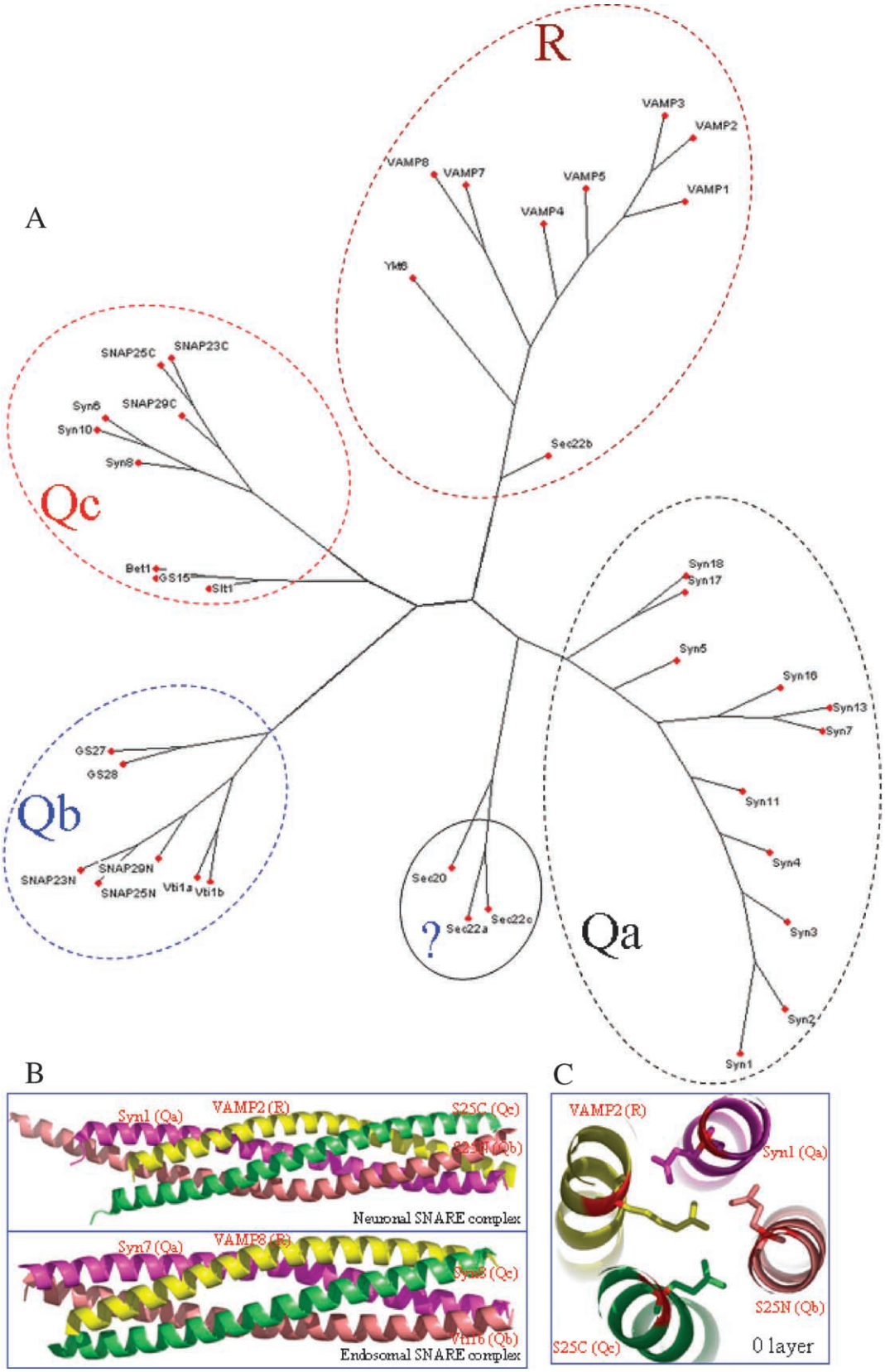


Fig. 3. Phylogenetic tree of SNAREs. (A) Segregation of human SNAREs into four major groups (Qa/Syn; Qb/S25N, Qc/S25C, and R/VAMP) according to their amino acid relatedness of their SNARE motifs. (B) Similar four-helical bundle of synaptic (upper) [7] and endosomal (lower) [8] SNARE complexes, as depicted using the PyMol program (http://pymol.sourceforge.net/). (C) The 0-layer of neuronal SNARE complex as depicted also using PyMol program.

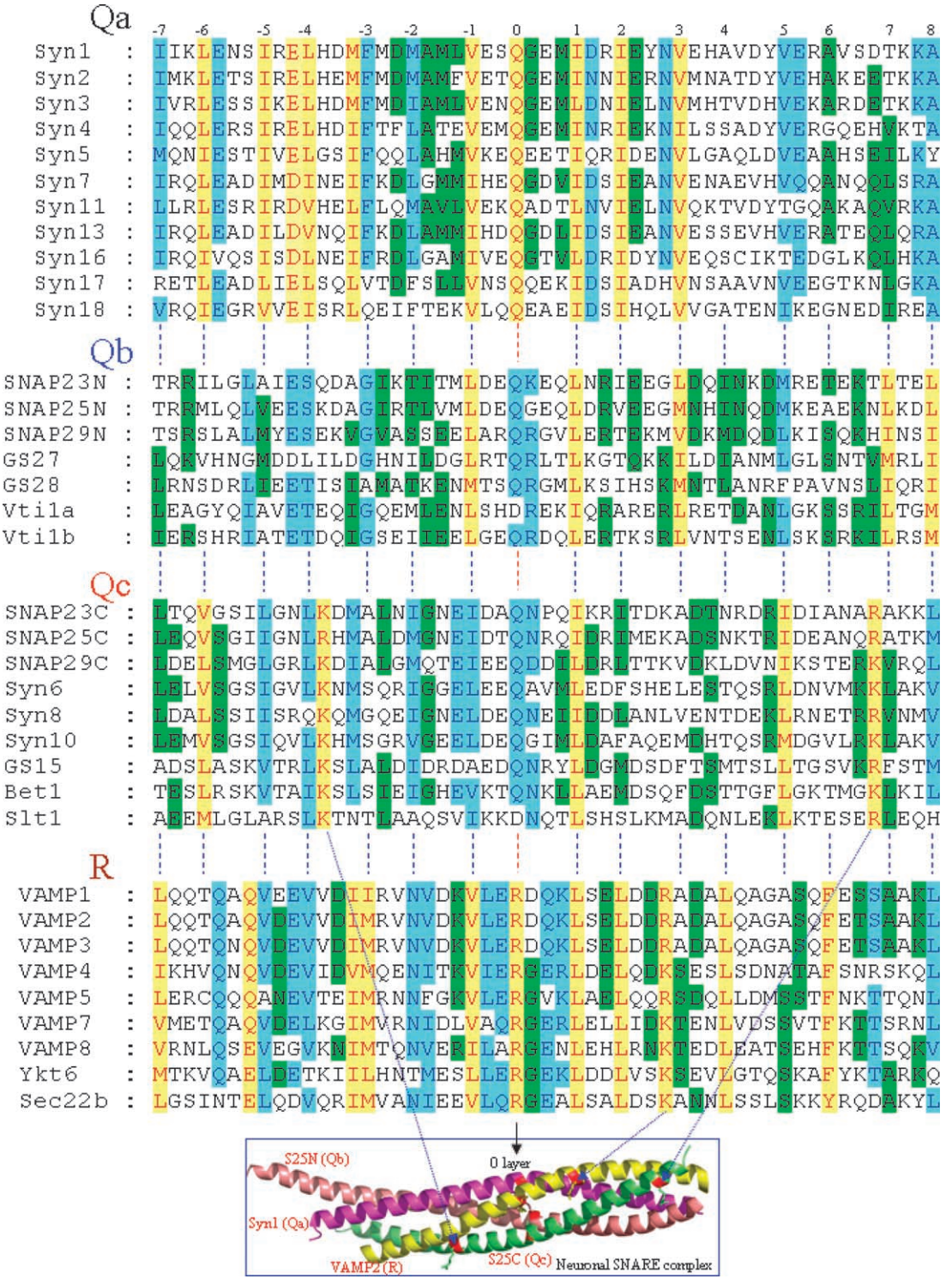
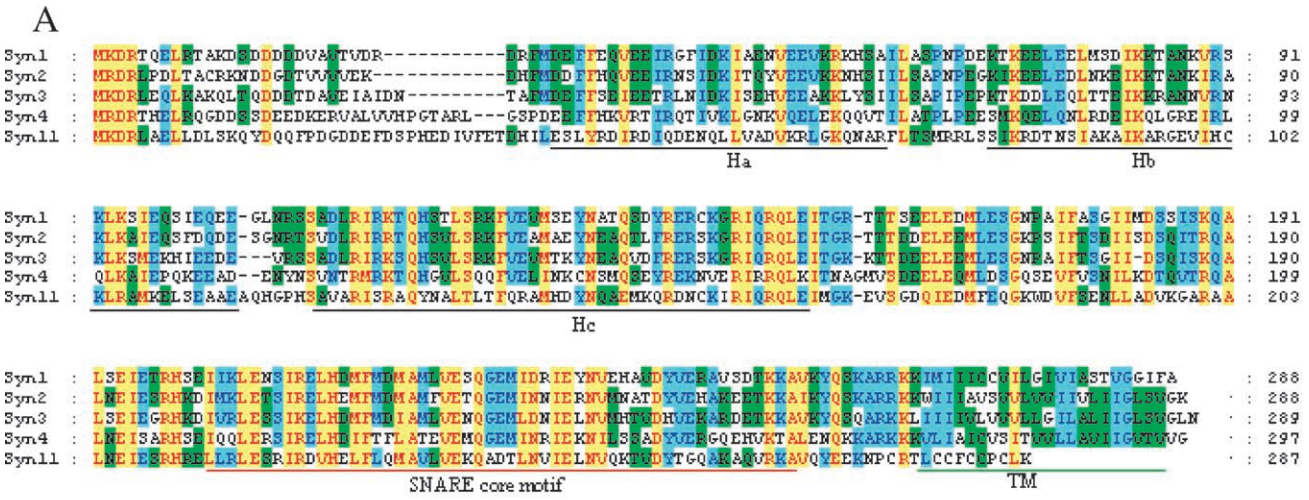
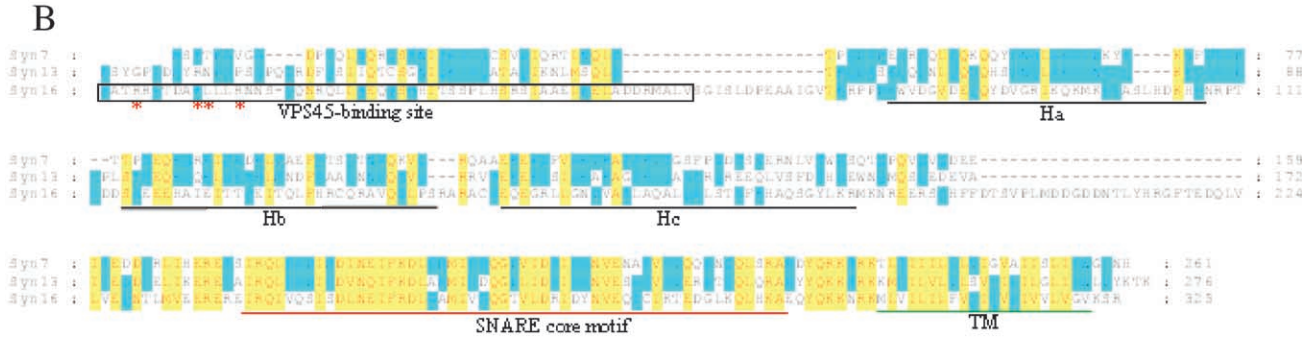


Fig. 4. Amino acid sequence alignment of the SNARE motifs of human Qa SNAREs, Qb SNAREs, Qc SNAREs, and R SNAREs as indicated. The positions in the four-helical SNARE bundle (bottom) of some conserved residues of Qc and R SNAREs (shown in sticks) are indicated by dashed lines.

Fig. 5. Qa syntaxins. (A) Amino acid sequence alignment of human Syn1, Syn2, Syn3, Syn4 and Syn11. The Habc regions according to those defined for Syn1 are indicated. The core SNARE motif and the C-terminal transmembrane (TM) hydrophobic tail anchor are also indicated. (B) Alignment of amino acid sequences of human Syn7, Syn13 and Syn16. The VPS45-binding region of Syn16 is boxed with key residues involved in interaction indicated by red asterisks. The core SNARE and TM domains are also indicated. (C) Alignment of amino acid sequences of human Syn5, Syn17, and Syn18. The SLY1-binding region of Syn5 and Syn18 is boxed with key residues involved in interaction indicated by red asterisks. The core SNARE and TM domains are similarly indicated. (D) Segregation of syntaxins into three major subgroups based on amino acid sequence homologies. (E) The three-helical bundle of N-terminal Habc region of rat Syn1 [147] as depicted using the PyMol program.


A
Syn1
Syn2
Syn3
Syn4
Syn11
Ha
Hb
Hc
SNARE core motif
TM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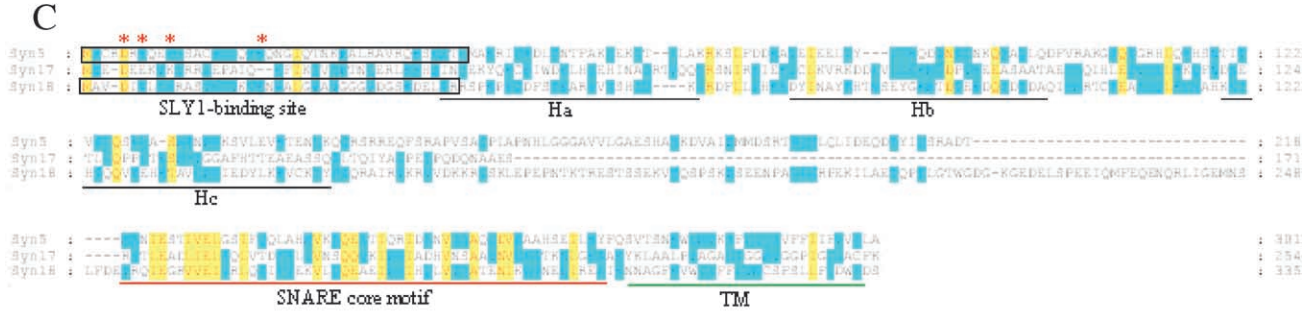
Syn13
Syn16
VPS45-binding site
Ha
Hb
Hc
SNARE core motif
TM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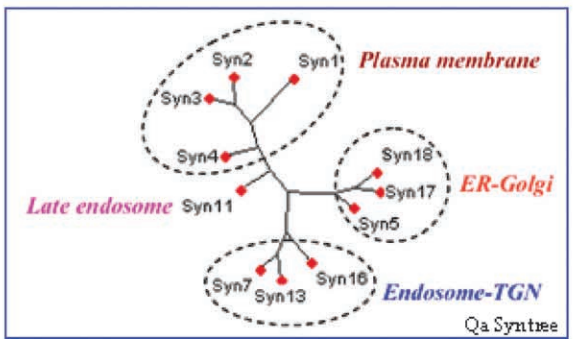
Syn17
Syn18
SLY1-binding site
Ha
Hb
Hc
SNARE core motif
TM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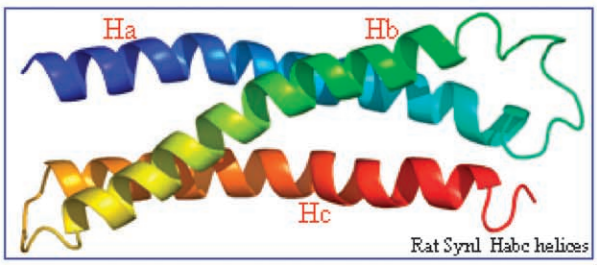
Syn1
Plasma membrane
Syn3
Syn4
Syn18
Syn17
ER-Golgi
Late endosome
Syn11
Syn5
Syn7
Syn13
Syn16
Endosome-TGN
Qa Syntree


E
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Rat Syn1 Habc helices

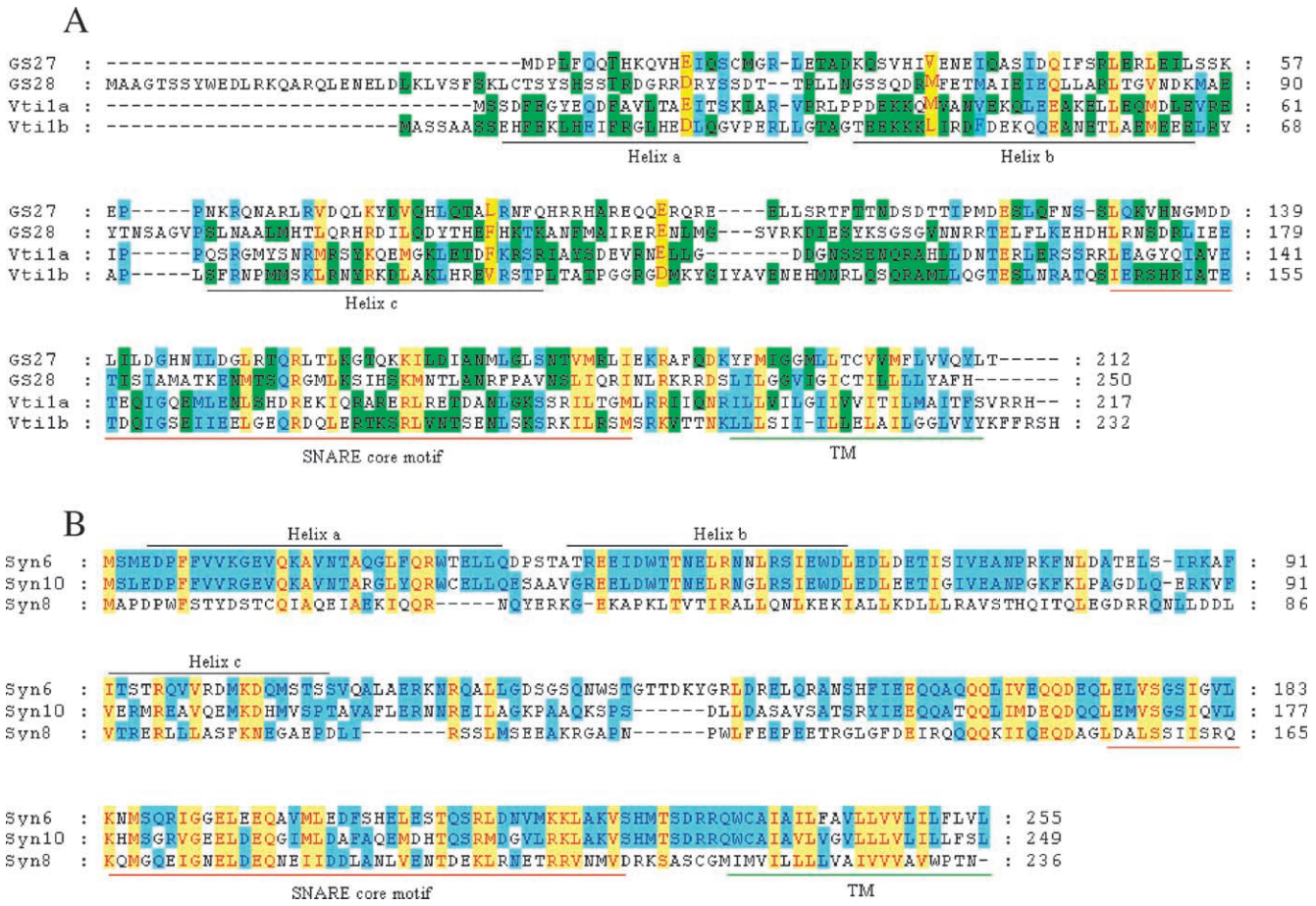

Fig. 6. Qb and Qc SNAREs with a single SNARE motif. (A) Alignment of amino acid sequences of human Qa SNAREs GS27, GS28, Vti1a, and Vti1b. The potential helical regions (based on that of Vti1b [38]), the core SNARE motif and TM are indicated. (B) Alignment of amino acid sequences of human Qb SNAREs Syn6, Syn10 and Syn8. The potential helical regions (based on that of Syn6 [43]), the SNARE motif and TM are indicated.

longin domain [44,45] (Fig. 7A). The resolved structure of the N-terminal region of Ykt6 and Sec22b shows that they adopt a profilin-like structure consisting of a β-sheet (formed by five antiparallel β-strands (β1–β5) with the first α-helix (α1) on one side and two C-terminal antiparallel α-helices (α2 and α3) on the other side of the beta-sheet (Fig. 7B) [46,47]. Like Syn1, Ykt6p adopts a folded back (closed) conformation in which the N-terminal profilin-like domain binds to its C-terminal SNARE domain [46]. Although the N-terminal region of Sec22b also has a profilin-like structure, Sec22b is likely to adopt an open conformation as the N-terminal domain does not affect the property of C-terminal SNARE motif [47]. Amino acid sequence similarities (Fig. 7A) suggest that the N-terminal regions of VAMP7, Sec22a and Sec22c are also likely to have a profilin-like structure. Interestingly, the Trs20/SEDL subunit of the TRAPP complex, which is involved in the tethering process in the early secretory pathway, also exhibits a profilin-like structure [48]. This raises the possibility that this tethering complex could modulate the function of SNAREs containing profilin-domain through competitive interaction with some common regulators yet to be identified. The longin domain of Ykt6 is shown to have palmitoylating activity towards itself and other proteins, such as yeast Vac8p during vacuolar fusion [49,50]. Whether other longin domains are able to mediate palmitoylation remains to be investigated. The forth type of N-terminal extension is exemplified by that of VAMP4, an R-SNARE enriched in the *trans*-Golgi network (TGN). VAMP4 is present only in vertebrates and the N-terminal extension is well conserved among various orthologues (Fig. 8). One of the functions of the N-terminal extension is to direct the targeting of VAMP4 to the TGN and it involves a signal harboring a di-leucine motif and an acidic cluster [51–53]. Finally, the N-terminal extensions of Sec20 and Slt1 have not been characterized [54–56]. Collectively, the N-terminal domain of SNAREs is involved in diverse regulations of SNAREs such as intramolecular regulation of the functionality of the SNARE domain (such as Syn1 and Ykt6), interaction with regulatory factors (such as Munc18-1 interaction with the closed Syn1, interaction of VPS45 with the N-terminus of Syn16, and binding of SLY1 to the N-terminus of Syn5 and Syn18), intracellular targeting of the SNARE (the N-terminal extension of VAMP4), enzy-

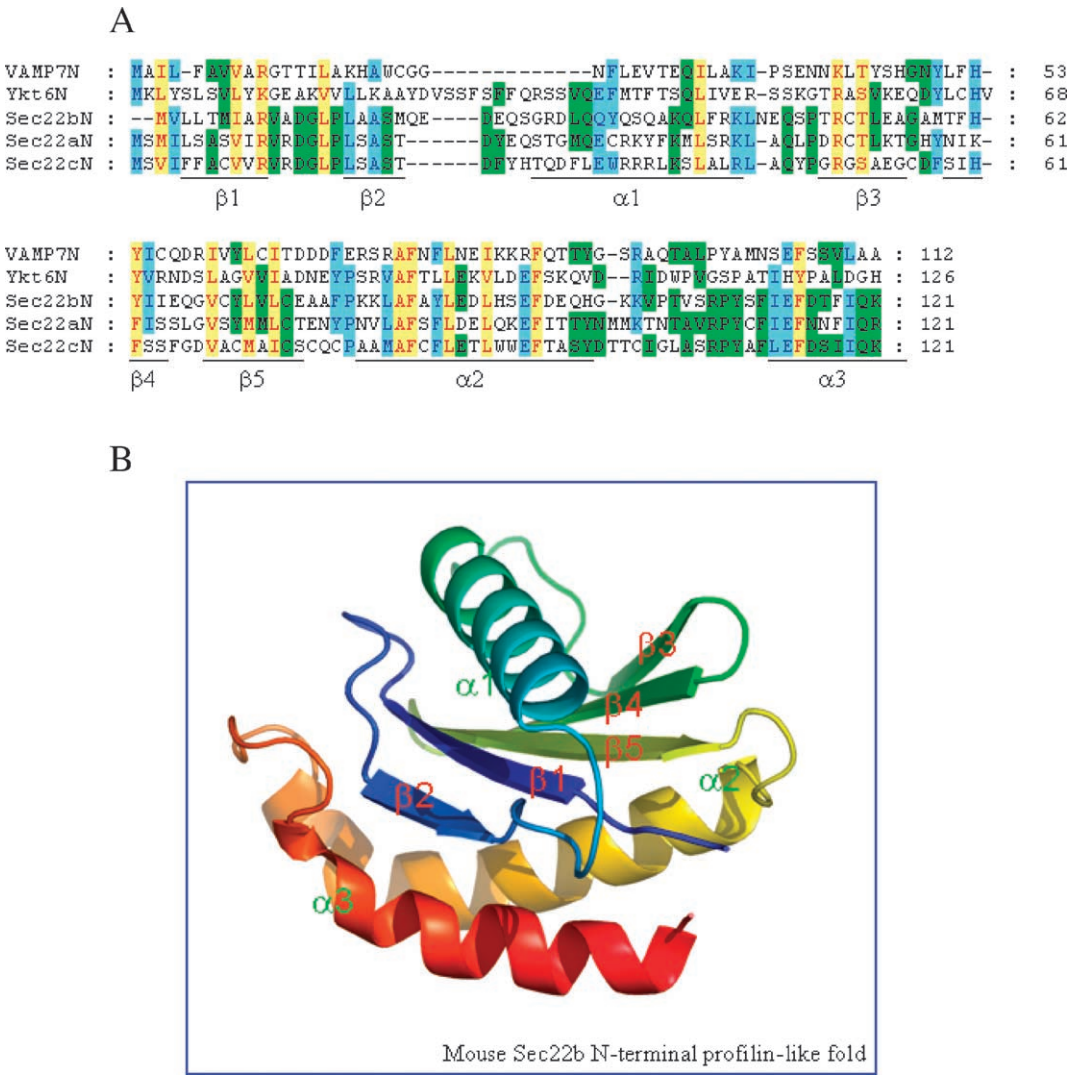

Fig. 7. Longin domains. (A) Amino acid sequence alignment of N-terminal Login domains of VAMP7, Ykt6, Sec22b, Sec22a, and Sec22c. The regions with defined secondary structures based on Sec22b [47] are indicated. (B) The profilin-like fold of the N-terminal login domain of Sec22b as depicted using the PyMol program with the secondary structures indicated.

matic activity (Ykt6), and/or other roles that remain to be defined.

4. The emerging concept of SNARE action

The current concept of the action of SNAREs is that combinatory use of the various members of the Qa-, Qb-, Qc-, and R-SNAREs will give rise to a wide array of SNARE complexes, whose functions are determined in part by the subcellular targeting of newly-made SNAREs. A given SNARE such as Syn5 can be incorporated into (and is indeed found in) several different SNARE complexes (Syn5/GS27/Bet1/Sec22b, Syn5/GS28/Bet1/Ykt6, and Syn5/GS28/GS15/Ykt6) to mediate different transport events (Fig. 9) [16,57,58]. Similarly, the same t-SNARE such as Syn4/SNAP-23 interacts with different v-SNAREs such as VAMP2 [59], VAMP3 [60], VAMP7 [61], or VAMP8 [62] depending on the cell types. The same v-SNARE such as VAMP8 interacts with different t-SNAREs in different transport events of different cells: VAMP8 is present mainly in endocytic SNARE complexes (with Syn7/Vti1b/Syn8) in liver hepatocytes [63], but it interacts primarily with the surface t-SNARE (Syn4/SNAP-23) in pancreatic acinar cells [62]. The t-SNARE in the TGN assembled from Syn16, Vti1a, and Syn6 interact with either VAMP3 or VAMP4 as the v-SNARE [64]. Similarly, the endosomal t-SNARE assembled from Syn7, Vti1b, and Syn8 interacts with either VAMP7 or VAMP8 [62,65].

5. Known SNARE complexes (SNAREpins) in mammalian cells

Several SNARE complexes have been defined to function in various transport events in the secretory and/

Fig. 8. Alignment of amino acid sequences of N-terminal extension of VAMP4 from various species (h: human; r: rat; m: mouse; z: zebrafish; f: Fugu; x: *Xenopus*). The di-leucine and acidic cluster important for TGN accumulation of VAMP4 are indicated.

or endocytic pathways of mammalian cells (Fig. 9). The complex consisting of Syn5 (Qa), GS27 (Qb), Bet1 (Qc), and Sec22b (R) appears to function in mediating homotypic fusion of ER-derived COPII vesicles into larger transport intermediates referred to as EGTC (ER-Golgi transport container), ERGIC (*ER*-*G*olgi *i*ntermediate *c*ompartment), or VTC (*v*esicular *t*ubular *c*luster) [66,67]. Based on systematic analysis of yeast SNAREs [34], a similar SNARE complex is found in yeast. Since yeast Bet1p is the functional v-SNARE, Bet1 is likely the v-SNARE, while Syn5, GS27, and Sec22b may form the t-SNARE, although this remains to be experimentally investigated. EGTCs are dynamic structures that undergo maturation events (including recycling of proteins back to the ER) as they move towards the Golgi apparatus [68]. The SNARE complex consisting of Syn5 (Qa), GS28 (Qb), Bet1 (Qc), and Ykt6 (R) is suggested to act in the late stage of transport from the ER to the Golgi and is likely to mediate the fusion of matured EGTCs with the *cis*-face of the Golgi apparatus [57]. A likely possibility is that Bet1 stays on the EGTC and acts as the v-SNARE responsible for interaction with another t-SNARE assembled from the same heavy chain (Syn5) but two different light chains (GS28 and Ykt6) at the cis-Golgi. Retrograde transport vesicles are formed by COPI coat proteins in the maturing EGTCs and *cis*-Golgi for ER recycling and to maintain a dynamic balance of membrane traffic in the early part of the secretory pathway. The ER SNARE complex consisting of Syn18 (Qa), Sec20 (Qb-equivalent?), Slt1/Use1/p31 (Qc), and Sec22b (R) is likely to be responsible for

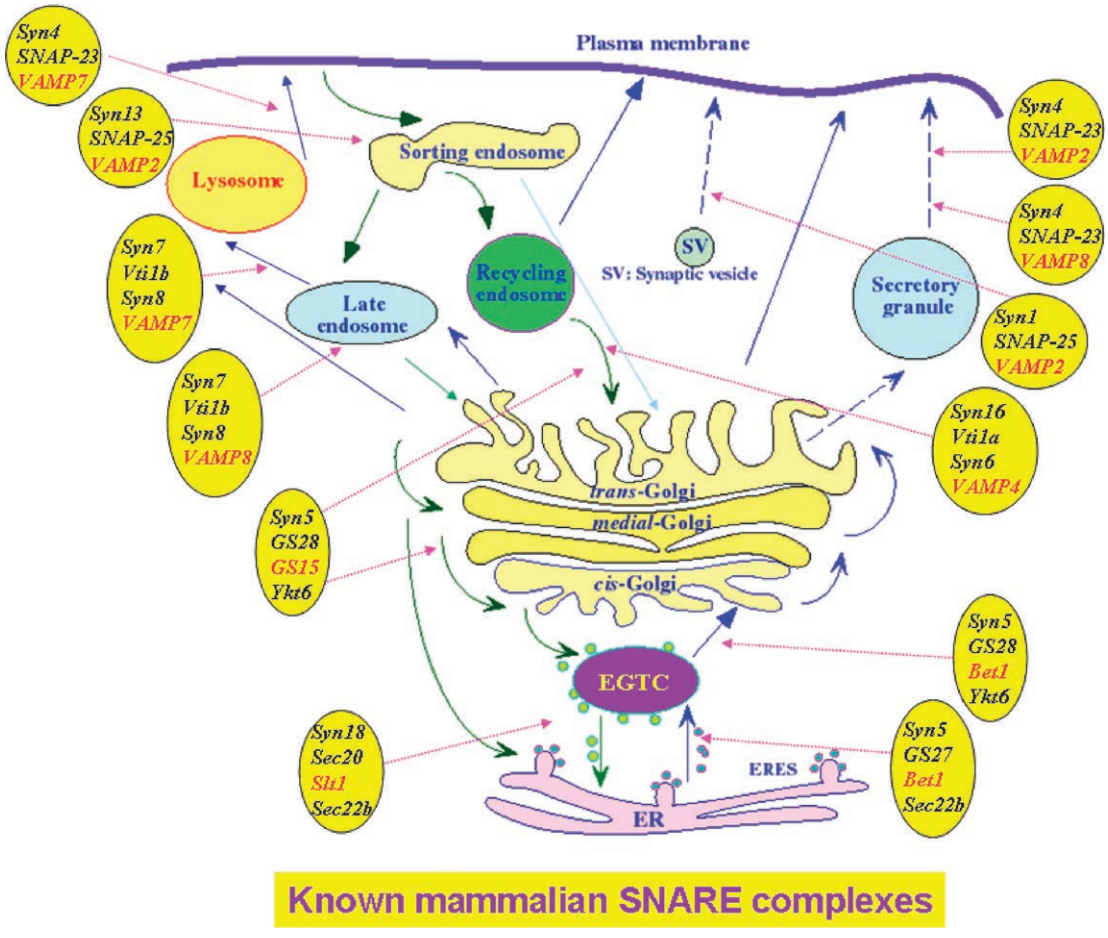

Fig. 9. Schematic summary of known mammalian SNARE complexes and their site(s) of action in the exocytic and/or endocytic pathways. The potential v-SNAREs are indicated in red.

receiving the recycling traffic [54–56,69]. In view of its segregation with Bet1 and GS15 (Fig. 3), Slt1 may act as the v-SNARE for the recycling vesicle to interact with t-SNARE assembled from Syn18, Sec20 and Sec22b in the ER. Like the interaction of yeast Sec20p with its regulator TIP20 [70], the mammalian Sec20 also interacts with RINT-1, a mammalian protein homologous to TIP20 (54, 69). The SNARE complex consisting of Syn5 (Qa), GS28 (Qb), GS15 (Qc), and Ykt6 (R) functions in intra-Golgi traffic and a similar complex is also found in yeast (16, 35, 58). Based on analysis in yeast, GS15 acts as the v-SNARE, interacting with t-SNARE assembled from Syn5, GS28, and Ykt6 [35]. In addition, a recent study suggests that this SNARE complex also mediates traffic from the endosomal compartments to the Golgi apparatus [71] and that GS15 is shifted to the endosomes when the endosomal function is perturbed. The endosomal compartments are known to be integrated with the secretory pathway by retrograde traffic from various endosomal compartments to the TGN. The major SNARE complex functioning in the retrograde traffic from early/recycling endosomes to the TGN consists of Syn16 (Qa), Vti1a (Qb), Syn6 (Qc), and VAMP4 (R) [64]. VAMP4 is most likely the v-SNARE for transport intermediates derived from early/recycling endosomes and it interacts with t-SNARE assembled from Syn16, Vti1a, and Syn6 at the TGN. The same TGN t-SNARE interacts also with VAMP3, which plays a minor role in this retrograde recycling pathway [64]. Although retrograde transport from the late endosome to the TGN has been well established, the exact nature of the SNARE complex involved in this event is currently unknown.

Several SNARE complexes are implicated in the endocytic pathway. Syn13 is likely the major Qa SNARE functioning in the early/sorting endosome and it interacts with SNAP-25 (Qb and Qc) and VAMP2 (R) to regulate fusion of early/sorting endosomes [72,73]. VAMP2 is the v-SNARE in this fusion event. Syn7 (Qa) is distributed throughout the entire endocytic pathway and is enriched in the later compartments of the endocytic pathway. Syn7 interacts with Vti1b (Qb) and Syn8 (Qc) to form the t-SNARE, which acts both in the late endosomes and lysosomes. By interacting with VAMP8, this t-SNARE may regulate fusion events in the late endosome [63]. Fusion of the late endosome with the lysosome or homotypic lysosome fusion might use the same t-SNARE but a different member of the VAMP subfamily (VAMP7) as the v-SNARE [65].

Exocytic traffic from the TGN to the cell surface is not only important for constitutive secretion and biogenesis of the plasma membrane but also for regulated traffic in diverse physiological processes. The neuronal SNARE complex consisting of Syn1, SNAP-25, and VAMP2 is the best studied and has served as a paradigm for other vesicular transport events [2,7,18,19]. The functionality of VAMP2 as the v-SNARE, the assembly of the t-SNARE from Syn1 and SNAP-25, as well as the activity of the assembled *trans*-SNARE complex in mediating synaptic vesicle fusion with the plasma membrane are subjected to diverse regulatory mechanisms. This SNARE complex is also the converging point for many cellular regulations on presynaptic events in the brain.

A major mode of insulin action is to mobilize glucose transporter 4 (GLUT4) by stimulating fusion of GLUT4-containing intracellular vesicles with the plasma membrane. In this case, VAMP2 functions as a v-SNARE for GLUT4-containing vesicles and it interacts with t-SNARE assembled from Syn4 and SNAP-23 [59]. VAMP8 was recently shown to be the premier v-SNARE of zymogen granules in pancreatic acinar cells and it mediates regulated fusion with the apical surface, interacting with t-SNARE assembled from Syn4 and SNAP-23 [62]. The Syn4/SNAP-23 t-SNARE may also mediate the fusion of secretory lysosomes with the plasma membrane by interacting with VAMP7 as the v-SNARE [61]. The exact SNARE complex involved in constitutive transport from the TGN to the surface remains to be defined, although ubiquitously-expressed Syn2, 3, and/or 4 are likely the heavy chains of the t-SNARE. These Syns have been shown to exhibit differential distributions in either the apical or the basolateral domain of polarized epithelial cells and may be a contributing factor to polarized traffic from the TGN to various surface domains [74,75]. The v-SNARE for general TGN-surface transport remains unknown, although VAMP7 is implicated in apical transport in polarized epithelial cells by acting as a v-SNARE [76].

6. Regulation of SNARE function

The function of SNAREs is subjected to diverse regulation at various stages of their generation and action. These include transcriptional regulation of gene expression, targeting of SNAREs to the correct compartment (s), the functionality of v-SNARE in the vesicle, the assembly and functional status of t-SNARE in the target compartment, long-range interaction of vesicles with the target compartment during tethering, and the assembly and activity of the *trans*-SNARE complex. Many regulators are being uncovered and they play either positive and/or negative roles in the functionality of SNAREs or SNARE complexes. In addition, post-translational modifications such as phosphorylation [53,77–79], palmitoylation [29–31], and prenylation [29,80] are also likely to regulate the function of SNAREs. The major regulators are summarized in Figs. 10–12, and/or Table 3. Examples of these regulators are briefly described here.

6.1. NSF and α-SNAP

NSF and α-SNAP represent the most essential regulators of SNAREs as they enable the disassembly of cis-SNARE complexes. The concerted action of α-SNAP and NSF

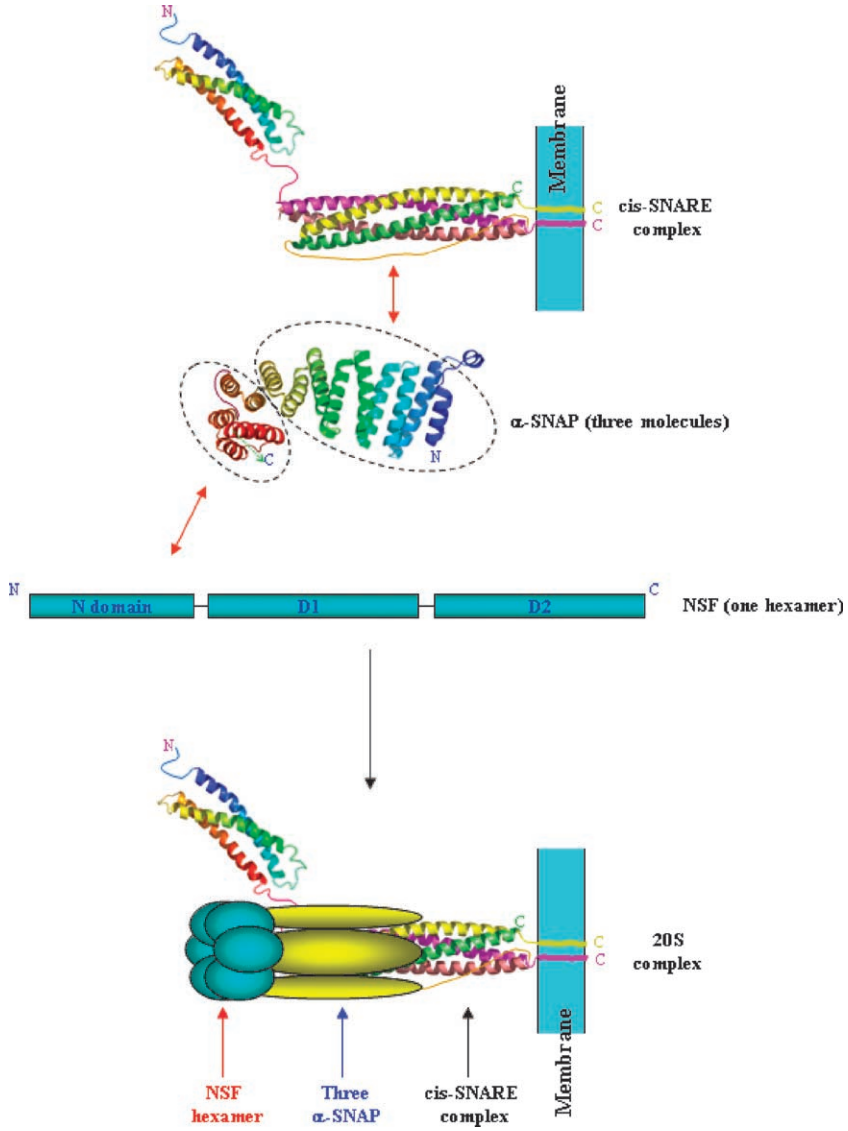

Fig. 10. The schematic illustration of the 20 S complex formed by the cis-SNARE complex, three molecules of α-SNAP and an NSF hexamer. The structure of yeast α-SNAP (1QQE) depicted by PyMol is used to illustrate that the N-terminal sheet and C-terminal globular domain interact with the cis-SNARE complex and the N-terminal domain of NSF, respectively. Hydrolysis of ATP by NSF (D1 domain) leads to the disassembly of the complex into free SNAREs. The D2 domain of NSF mediates the hexamer formation.

causes dissociation of cis-SNARE complexes formed after SNARE-mediated fusion events, releasing free SNAREs for repeated use. Three molecules of α-SNAP act as bridges to link up the *cis*-SNARE complex with a hexameric NSF to form a transient 20 S complex (Fig. 10) [21–24]. NSF contains an N-terminal (N) domain of about 200 residues followed by two ATPase domains of about 280 residues each (designated D1 and D2 for the first and C-terminal domains, respectively). The D2 ATPase domain mediates the hexamerization of NSF, while the N domain interacts with a C-terminal globular domain (consisting of C-terminal 5 α-helices) of α-SNAP. The D1 ATPase domain effects the dissociation of the SNARE complex through hydrolysis of ATP and conformational changes of the hexamer. The first 9 α-helices of α-SNAP arrange in antiparallel to form an N-terminal sheet whose positively charged residues interact with the acidic surfaces of the SNARE complex [81]. Association of NSF with α-SNAP into the 20 S complex stimulates the ATPase activity of the former. The functional importance of α-SNAP is underscored by genetic analysis of *hyh* (hydrocephalus with hop gait) mouse and the discovery that the *hyh* phenotype is due to a mutation of α-SNAP gene [82]. A role for α-SNAP in apical protein localization and control of neural cell fate has been suggested. Besides α-SNAP, two homologous proteins referred to as β-SNAP and γ-SNAP are known [83]. β-SNAP is highly homologous to α-SNAP and is expressed selectively in neurons. β-SNAP can act together with or

A

Name	Yeast homolog	Locations	AA	GenBank acc #	Interacting SNAREs	Syn target structure
SLY1	Sly1p	Golgi	642	NP_057190	Syn5, Syn18	N-terminus
VPS45	Vps45p	TGN	570	AAC50931	Syn16	N-terminus
VPS33A	Vps33p	Endosome	596	NP_075067	Syn7?	?
VPS33B	Vps33p	Endosome	617	NP_061138	Syn7?	?
Munc18-1	Sec1p	PM	603	NP_003156	Syn1	Closed Syn
Munc18-2	Sec1p	PM	593	NP_008880	Syn2, Syn3	Closed Syn
Munc18-3	Sec1p	PM	592	NP_009200	Syn4	Closed Syn

B

	Mun18-1	Mun18-2	Mun18-3	VPS45	VPS33A	VPS33B	SLY1
Mun18-1	100	63	49	19	15	10	13
Mun18-2		100	44	21	13	13	18
Mun18-3			100	18	13	10	15
VPS45				100	18	15	18
VPS33A					100	32	14
VPS33B						100	10
SLY1							100

C

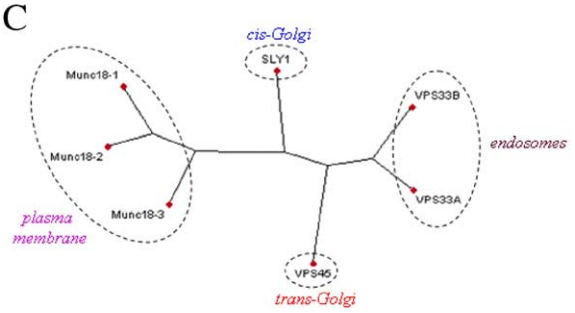

Fig. 11. Mammalian SM proteins. (A) List of 7 known mammalian SM proteins and their major features. (B) Amino acid sequence identities among the 7 mammalian SM proteins. (C) Phylogenetic tree showing the segregation of the 7 mammalian SM proteins into four major subtypes.

regulate α-SNAP function during regulated exocytosis [84,85]. Unlike α-SNAP, γ-SNAP (which is as widely expressed as α-SNAP) does not interact with SNAREs, although it interacts with NSF [86], suggesting that it has a different role. Consistent with this, α-SNAP but not γ-SNAP is essential for ER-Golgi transport [87].

6.2. Sec1/Munc18-like (SM) proteins

There are at least seven mammalian members of the SM protein family: Munc18-1, Munc18-2, and Munc18-3, VPS33A, VPS33B, VPS45, and SLY1 (Fig. 10). Munc18-1, Munc18-2, and Munc18-3 are functionally homologous to yeast Sec1p and function at the plasma membrane (PM). VPS33A and VPS33B correspond to yeast Vps33p and act in the endocytic pathway. VPS45 and SLY1 correspond to yeast Vps45p and Sly1p, respectively, and are involved in traffic at the *trans*- and *cis*-faces of the Golgi apparatus [88,89]. Interactions with Syns occur through the approximately 140 residue N-terminal region of SM proteins [42], leaving the rest of the molecule for other functions. Munc18-1, Munc18-2, and Munc18-3 bind to the closed conformation of Syn1-4. This interaction is dependent on both the N-terminal Habc region and the SNARE motif of Syns [37]. SLY1 and VPS45 interact with a short N-terminal region of Syn5/18 and Syn16, respectively, without the involvement of the Habc region or the SNARE motif [39–41]. This differential binding properties of Munc18s vs. SLY1 and VPS45 result from the Syn-binding site of Munc18s being present on the opposite side of the folded surface in comparison to SLY1 and VPS45 [42]. Although Munc18-1, Munc18-2, and Munc18-3 are all related to Sec1p and act at the PM, they are likely to have distinct functions paralleling the more sophisticated cellular physiology of the vertebrate. Munc18-1 is most abundantly expressed in the neuron and is involved in synaptic vesicle fusion [19,88], while Munc18-2 is more widely expressed and acts to regulate Syn2 and 3 [90,91]. Munc18-3 is believed to interact preferentially with Syn4 and has been implicated in GLUT4 translocation to the PM in response to insulin [59,92]. VPS33A and VPS33B are likely to serve unique functions as mutations of VPS33A in mice lead to a buff phenotype [93], while mutation of VPS33B in human is associated with the arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome [94].

6.3. Munc13s

Munc13-1, Munc13-2, Munc13-3, and Munc13-4 are homologous proteins containing several Ca^{2+}-binding C2 domains implicated in interactions with diacylglycerol, Ca^{2+}, and phospholipids [95–97]. Munc13-1, Munc13-2, and Munc13-3 are expressed in different cells/regions in the brain. Knockout of both Munc13-1 and Munc13-2 abolishes spontaneous and evoked synaptic transmissions [98]. Munc13-1/2 is proposed to be essential for the priming process for synaptic vesicles tethered onto the presynaptic plasma membrane [19]. The priming process empowers the tethered/docked vesicles with the competence of evoked fusion. Mechanistically, Munc13-1/2 might facilitate the dissociation of Munc18-1 from Syn1, to open-up Syn1 so that it can interact with SNAP-25 to form t-SNARE, and/or to facilitate the formation of a partial complex of Syn1/SNAP-25 on the presynaptic membrane with VAMP2 on the synaptic vesicle [19,99]. A small region (residues 1181-1345) in the C-terminal part of Munc13-1 has been shown to interact with N-terminal region (residues 53–79) of Syn1 [99]. The tethering of synaptic vesicles is regulated by Rab3 and its effector RIM1 [19,100]. Since Munc13-1 interacts with RIM1 [101], the progression from the tethering to the priming event is therefore regulated by the Rab3–RIM1–Munc13-1 interaction cascade, meaning that Munc13-1/2 effectively facilitates the formation of the partial *trans*-SNARE complex implicated in the early priming event [19]. An important role for Munc13-4 in the regulated secretion of cytolytic granules at the immunological synapse of cytotoxic lymphocytes has been revealed. Familial hemophagocytic lymphohistiocytosis (FHL) is associated with defective cytotoxic lymphocytes. A subtype of FHL (FHL3) is caused by mutations of Munc13-4, resulting in defective exocytosis of cytolytic granules. Munc13-4 is suggested to prime tethered/docked cytolytic granules, rendering them

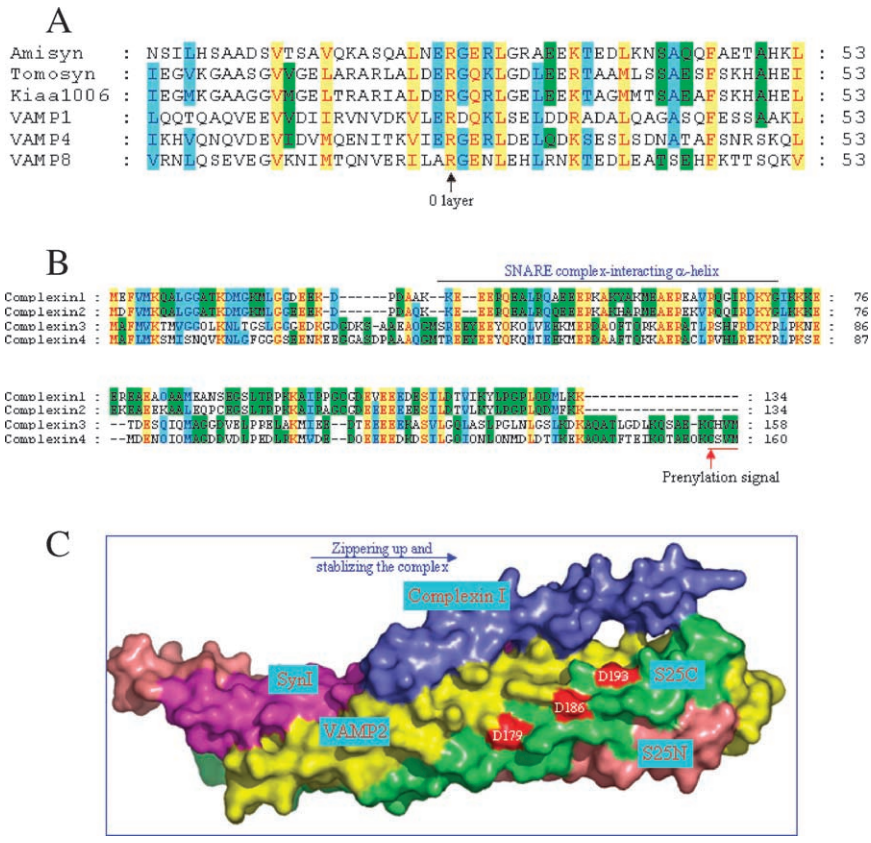

Fig. 12. Examples of regulators of the neuronal SNARE complex. (A) Alignment of amino acid sequences of the SNARE domains of human amisyn, tomosyn, tomosyn-like KIAA1006 with those of human VAMP1, VAMP4, and VAMP8. The position of the 0 layer residue is indicated. (B) Alignment of amino acid sequences of complexin I, complexin II, and two candidate members (complexin III and complexin IV) that are yet to be characterized. The region of α-helix involved in interacting with the SNARE complex is indicated. The potential prenylation sites of complexin III and complexin IV are indicated. (C) Schematic illustration of the helix region of complexin I sealing the groove between Syn1 and VAMP2 in the neuronal SNARE complex. The D179, D186, and D193 residues of SNAP25 important for Ca^{2+}-dependent interaction with synaptotagmin I [114] are indicated in red.

competent for regulated exocytosis, via undefined interactions with SNAREs [102].

6.4. Complexins

Complexins I and II are small proteins (134 residues) (Fig. 11B, Table 3) regulating synaptic transmission [103,104]. They are enriched in neurons where they colocalize with Syn1 and SNAP-25, although complexin II is also expressed ubiquitously at lower levels. Analysis of neurons from knockout mice lacking both complexins I and II suggests that complexins act at, or following, the Ca^{2+}-triggered step of fast synchronous transmitter release [105]. Recent structural studies suggest that complexins might stabilize the *trans*-SNARE complex formed between the synaptic vesicle and the presynaptic surface membrane, thus regulating a late step in Ca^{2+}-triggered neurotransmitter release [104,106,107]. A complexin I α-helix (residues 32–72 as indicated in Fig. 11B) binds in an antiparallel orientation to the groove formed between the Syn1 and VAMP2 helices in the second half of the SNARE complex (starting from −3 layer onwards), thus stabilizing the zippered of the complex (Fig. 11C). Complexins thus act like "adhesive tape" to seal the groove formed by Syn1 and VAMP2, ensuring that synaptic vesicles are in the fully-primed state. Accordingly, complexins are proposed to complete the priming process of synaptic vesicles by facilitating the transition the Munc 13-1/2-mediated partially assembled SNARE complex to the fully assembled state [19]. In addition, complexins seemingly facilitate interaction of the transmembrane domains of VAMP2 and Syn1 [108]. Two proteins homologous to complexins I and II, tentatively named complexins III and IV (Fig. 11B), have been identified, but their biochemical properties and cellular roles in traffic have not been investigated. Interestingly, complexins III and IV contain C-terminal consensus motifs for prenylation, suggesting that, like Ykt6, they might be anchored to the cytoplasmic side of the membrane via a prenyl group.

6.5. Synaptotagmin I

Synaptotagmin I is the premier member of the synaptotagmin protein family consisting of at least 13 members [109]. It is preferentially expressed in neurons and is associated with the synaptic vesicle as a type I membrane

Table 3
A list of some other human regulators of SNAREs (in addition to SM proteins)

Name of human protein	AA #	GenBank acc #	Interacting SNAREs	Reference
α-SNAP	295	NP_003818	SNARE complex	[81,83]
β-SNAP	298	NP_071363	SNARE complex	[83,84]
γ-SNAP	312	NP_003817	SNARE complex	[83,86]
NSF	744	NP_006169	α-SNAP-SNAREs	[21–24]
Amisyn	210	Q8NFX7	Syn1-SNAP25	[118]
Tomosyn	1115	NP_640337	Syn1-SNAP25	[116,117]
KIAA1006	1186	BAA76850	Syn1-SNAP25	[117]
Pallidin	172	NP_036520	Syn13	[148]
Snapin	136	NP_036569	SNAP-25	[149]
Synip	245	NP_848604	Syn4	[150]
Complexin I	134	NP_006642	SNARE complex	[103,107]
Complexin II	134	Q6PUV4	SNARE complex	[103,107]
Complexin III	158	AAP41127	?	
Complexin IV	160	NP_857637	?	
Munc13-1	1665	XP_038604	Syn1	[95,99]
Munc13-2	1591	O14795	Syn1	[95]
Munc13-3	1954	XP_085234	Syn1	[95]
Munc13-4	1090	NP_954712	?	[96,97,102]
Synaptophysin	313	NP_003170	VAMP2	[119]
Synaptotagmin I	422	NP_005630	SNARE complex	[19,112]
GATE-16	117	NP_009209	GS28	[132,133]
EpsinR	625	Q14677	Vti1b	[12]
p115	962	NP_003706	GS28, Syn5	[16]
VCIP135	1222	NP_079330	Syn5/p97/47	[151]
Septin5	369	AAC39779	Syn1	[152]
Granuphilin	671	NP_542775	Syn1	[153]
Hrs	777	NP_004703	SNAP-25	[73,154]
FIG	462	NP_065132	Syn6	[130]
EEA1	1411	NP_003557	Syn6	[129]
Syntaphilin	494	NP_055538	Syn1	[155]
Syntabulin	663	AAU93914	Syn1	[156]
CDC42	191	NP_001782	VAMP2	[136]
SIP30	266	Q8VIL3	SNAP-25	[157]
RINT-1/TIP20	821	NP_068749	Sec20/BNIP1	[54,69]
Taxilin	546	NP_787048	Syn1, Syn3, Syn4	[158]

protein with its short N-terminal region oriented towards the lumen of the synaptic vesicle. Its larger C-terminal cytoplasmic domain contains two tandem Ca^{2+}-binding C2 (C2A and C2B) domains. Exocytosis of synaptic vesicles in the synapse is strictly regulated by Ca^{2+} concentrations and synaptotagmin I is probably the Ca^{2+} sensor that couples this ion flux to the exocytosis of synaptic vesicles that occurs on account of an action potential [110–112]. Synaptotagmin I binds directly to Syn1 and SNAP-25 of the t-SNARE. The tandem C2 (C2A and C2B) domains cooperate to enhance the penetration of some hydrophobic residues into the lipid bilayer of the target compartment in response to Ca^{2+}. The C2B domain apparently also interacts with phosphatidyl inositol 4,5-biphosphate (PtdIns4,5P) in response to Ca^{2+} binding, thus steering the penetration of hydrophobic residues of C2A and C2B into the target membrane [112,113]. Several acidic residues in the C-terminal region of SNAP-25 (red-colored surfaces indicated in Fig. 12) are known to be important for its Ca^{2+}-triggered interaction with synaptotagmin-1 [114]. Through the simultaneously enhanced interactions with t-SNARE (such as through the acidic residues of SNAP-25), PtdIns4,5P, and the lipid bilayer, synaptotagmin I triggers the complexin-stabilized *trans*-SNARE complex to catalyze the fusion process in the presynaptic membrane in response to a rise in Ca^{2+} levels [112,115]. Complexins also appear to act with synaptotagmin I at this fusion stage by facilitating the interaction of the transmembrane domains of the vesicle-located VAMP2 and Syn1 on the pre-synaptic membrane [108].

6.6. Amisyn, tomosyn, and KIAA1006

The C-terminal region of tomosyn, its closely-related protein KIAA1006 and amisyn contain a 60-residue region characteristic of R-SNAREs (Fig. 11A). It has been shown that the R-SNARE domain of tomosyn and amisyn can replace VAMP2 and interact with Syn1 and SNAP-25 to form a non-fusogenic SNARE complex [116–118]. As tomosyn, KIAA1006 and amisyn do not contain a hydrophobic transmembrane domain [116–118], they act as competitive inhibitors of synaptic vesicle VAMP2 by vying for the same t-SNARE. In addition, the SNARE complex formed with tomosyn cannot interact with complexins

[117]. The potential modulation of the R-SNARE domains of tomosyn, KIAA1006 and amisyn by factors that may bind to their respective N-terminal regions provides a possible avenue for coupling various regulatory processes to synaptic transmission.

6.7. Synaptophysin

Synaptophysin is one of the major proteins of synaptic vesicles and is preferentially expressed in neurons. It spans the membrane four times with both N- and C-termini facing the cytoplasm [119]. Association of synaptophysin with VAMP2 inhibits the ability of VAMP2 to interact with t-SNARE [19,119,120]. A role for synaptophysin in directing the targeting of newly-made VAMP2 to the synaptic vesicle has also been proposed [121]. Synaptophysin may therefore regulate the targeting and functionality of VAMP2. No major defect in synaptic transmission is observed when synaptophysin is absent in its knockout mice [122], suggesting that its role in regulating the functional status of VAMP2 is minor or very subtle. Alternatively, the lack of a discernible phenotype might reflect the functional replacement of the absent synaptophysin by its homolog, synaptoporin, or the more distantly-related proteins such as synaptogyrin (neuron-enriched) or cellugyrin (ubiquitously-expressed form of synaptogyrin) [123].

6.8. p115

p115 is a tethering factor that functions by simultaneous interaction with giantin on COPI-generated vesicles and with GM130 on the cis-Golgi [124]. A SNARE-related coiled-coil region of p115 interacts with many SNAREs (Syn5, GS28, GS27, Ykt6, GS15, Bet1, Sec22b) of the Golgi apparatus, and its direct interaction with Syn5 and GS28 has also been demonstrated. Accordingly, p115 stimulates the formation of at least two Syn5-containing SNARE complexes (Syn5-GS28-GS15-Ykt6 and Syn5-GS27-Bet1-Sec22b), suggesting that p115 modulates the formation of *trans*-SNARE complexes involved in several transport events in the Golgi apparatus [16]. Mutational analysis of p115 suggests that the SNARE-modulating activity of p115 is more important than its tethering activity in maintaining the structure and function of the Golgi apparatus [125].

6.9. Hrs

Hrs interacts with SNAP-25 and functions in regulating the sorting event at the interface between early and late endosomes [126,127]. Like p115, a SNARE-like coiled coil region of Hrs is shown to inhibit the incorporation of an R-SNARE into the SNARE complex through competitive binding with a t-SNARE complex. Through this activity, Hrs inhibits early endosomal fusion mediated by Syn13, SNAP-25, and VAMP2 [73].

6.10. EEA1

Rab5 is a major player regulating the sorting endosome and it acts via several downstream effectors [128]. EEA1 is one such Rab5 effector and it functions as a tethering factor regulating fusion of the early endosome [72]. EEA1 has been shown to interact with Syn6 via its C-terminal region which is also involved in interaction with Rab5 [129]. Although the majority of Syn6 is detected at the TGN, a fraction of Syn6 is also found in EEA1-containing sorting endosomes. The biological consequence of EEA1–Syn6 interaction remains to be investigated.

6.11. FIG

Another protein interacting with Syn6 is the TGN-localized FIG (also called CAL, PIST, GOPC1) [130]. FIG contains two coiled-coil regions followed by a single PDZ domain. The second coiled-coil region and its C-terminal flanking region interact with Syn6. Again, the biological consequences of this interaction remain to be investigated. Knockout of the FIG gene in mice results in selective ablation of acrosome formation during spermatogenesis [131]. The acrosome is believed to form from the Golgi apparatus and the absence of FIG leads to fragmented acrosomal vesicles, suggesting a role for FIG in the fusion of these vesicles into the acrosome.

6.12. GATE-16

GATE-16 is a small Golgi protein that interacts with both NSF and GS28 [132]. In addition to disassembling cis-SNARE complexes through ATP hydrolysis, NSF also exhibits an ATPase-independent activity during in vitro Golgi formation. NSF/α-SNAP facilitates the interaction of GATE-16 with GS28 in a manner that requires ATP-binding but not ATP hydrolysis. Interestingly, GATE-16 binding prevents GS28 from interacting with Syn5 [133]. Since Syn5 is the heavy chain of t-SNARE and GS28 is a Qb-SNARE that serves as one of the light chains, GATE-16 has the property of preventing the assembly of a functional t-SNARE. Interestingly, GATE-16 is a member of a family of autophagy-related ubiquitin-like proteins (GATE-16, MAP1-LC3, GABARAP, and Apg8L) that are substrates of Apg4B protease [133a]. How this property is related to its role in Golgi function remains elusive.

7. Physiological studies of SNAREs

Recent studies on mammalian SNAREs have advanced from cellular studies to systematic functions in mice, including the generation of gene knockouts and genetic analysis of mutant mice. In addition, genetic analyses of human diseases resulting from mutations in SNAREs and regulators have also provided additional understanding. The

physiological roles of some SNAREs and their regulators in the context of the whole organism, as deduced from targeted gene knockout analysis in mice, genetic analysis of mutant mice, and/or genetic analysis of human diseases, are listed in Table 4. Some of these studies are briefly described below.

7.1. VAMP2

As the v-SNARE of synaptic vesicles in the neuron, VAMP2 has been extensively studied through various approaches. More recently, the VAMP2 gene has been knocked out in mice [134]. Homozygous knockout mice die immediately after birth. Consistent with the conclusion drawn from previous studies, neurophysiological analysis of hippocampal neurons prepared from embryos showed that the fast Ca^{2+}-triggered fusion of synaptic vesicles with the presynaptic membrane is decreased more than 100-fold, thus confirming a key role for VAMP2 in synaptic transmission mediated by primed vesicles. In addition, spontaneous synaptic vesicle fusion induced by hypertonic sucrose (which measures exocytosis of the entire pool of readily releasable vesicles as compared to assessing only primed vesicles measured by Ca^{2+}-triggered exocytosis) is reduced by 10-fold. Consistently, spontaneous miniature excitatory currents occur at about 15% of the frequency of control neurons. These results suggest that VAMP2 may not be absolutely essential for vesicle fusion but is a key player that controls the rate of fusion, particularly for the fast Ca^{2+}-triggered fusion of primed vesicles. A recent study also suggests a crucial role for VAMP2 in fast recycling of synaptic vesicles [13]. After depletion of the readily releasable vesicle pool, replenishment of the pool is delayed in VAMP2-null mice. Although the total pre-synaptic vesicles, docked vesicles, and actively recycling vesicles are unaffected, the shape and size of vesicles are altered in the absence of VAMP2. Furthermore, stimulus-dependent endocytosis of horseradish peroxidase and fluorescent FM1-

Table 4
A list of SNAREs and regulators that are investigated by gene knockout experiments, genetic analysis of mutant mice, or genetic analysis of human diseases

Name	Reference	Major phenotypes
VAMP2	[134]	Die after birth; 100-fold decrease of evoked synaptic exocytosis
VAMP3	[137]	No major defects
VAMP8	[62]	Defect in regulated exocytosis of zymogen granules in pancreatic acinar cells
Syn4	[141]	Die before E7.5; 50% decrease in GLUT4 translocation and glucose uptake by the skeletal muscle of Syn4+/− mice
Syn11	[165]	Mutations caused type 4 Familial hemophagocytic lymphohistiocytosis (FHL-4) syndrome
SNAP-25	[144]	Embryonic lethality; evoked synaptic exocytosis is abolished
Vti1b	[143]	No major defects but Syn8 is unstable; 20% mice has reduced activity of lysosomal delivery of proteins in hepatocytes
α-SNAP	[82]	Mutations caused hyh (hydrocephalus with hop gait) phenotype of mice due to defects in apical protein localization and control of neural cell fate
Synaptotagmin I	[110]	Die after birth; evoked synaptic exocytosis is reduced
Munc18-1	[145]	Die after birth; complete loss of synaptic exocytosis; neurodegeneration due to extensive apoptosis
Munc13-1	[159]	90% reduction of readily releasable vesicles and evoked transmitter release due to defect in synaptic vesicle maturation (priming)
Munc13-2	[98]	No major defects but with sporadic seizures in older animals (above 12 months of age)
Munc13-1 and Munc13-2	[98]	Mice were often born dead or die after birth; total arrest of synaptic (both evoked and spontaneous) exocytosis
Munc13-3	[160]	Increased paired-pulse facilitation at parallel fiber-Purkinje cell synapses; impaired ability to learn complex motor tasks
Munc13-4	[102]	Mutations caused type 3 Familial hemophagocytic lymphohistiocytosis syndrome due to defects in exocytosis of lytic granules of lymphocytes
Complexin I	[105]	No major overt defects; severe neurological symptoms characterized by ataxia
Complexin II	[105,161,162]	No major overt defects; reduced hippocampal long-term potentiation (LTP); deficits of motor and cognitive function caused behavioral altercations.
Complexin I and Complexin II	[105]	Die after birth; defect in a late stage of Ca^{2+}-triggered (evoked) synaptic exocytosis
VPS33a	[93]	Mutations caused buff phenotype in mice due to defect in biogenesis of lysosome-related organelles
VPS33b	[94]	Mutations caused arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome (a multisystem disorder) likely due to defect in traffic of lysosomes and/or lysosome-related organelles.
Synaptophysin	[122]	No major defects
Synaptogyrin	[123]	No major defects
Synaptophysin and synaptogyrin	[123]	No major overt defects; short-term and long-term synaptic plasticity were severely reduced; reduced LTP
Hrs	[163]	Die around E11; defect in ventral folding morphogenesis associated with increased apoptosis; Abnormally enlarged early endosomes
Septin 5	[164]	No major defects
FIG	[131]	Defective in acrosome formation during spermatogenesis

43 is delayed. VAMP2 acts thus as a nucleating factor for prompt endocytosis leading to quick reuse of synaptic vesicles following rapid exocytosis triggered by Ca^{2+}.

VAMP2 participates also in regulated secretion of other cells such as fat and endocrine cells. VAMP2 acts as a v-SNARE of storage vesicles containing GLUT4 and interacts with t-SNARE assembled from Syn4 and SNAP-23, mediating fusion with the plasma membrane in response to insulin [59,92]. In addition, VAMP2 is implicated in vasopressin-regulated translocation of aquaporin 2-containing vesicles [135]. VAMP2 might also regulate secretion of endocrine cells, such as insulin secretion by pancreatic β-cells. Interaction of VAMP2 with CDC42 is recently shown to be involved in coordinating secretion through actin cytoskeleton arrangement in β-cells [136].

7.2. VAMP3

The amino acid sequence of ubiquitously expressed VAMP3 is 75% identical to that of VAMP2. It is preferentially associated with sorting/early and recycling endosomes. Remarkably, deletion of the VAMP3 gene in mice exhibits little effects on development or various physiological processes (such as GLUT4 translocation or endocytic traffic) [137]. VAMP3 is not necessary for either regulated GLUT4 translocation or general constitutive membrane recycling, suggesting that one or more proteins may provide functional redundancy. VAMP8, present in the early and late endosomes [63,65], is a prime candidate for functional overlap with VAMP3, a possibility that awaits experimental verification.

7.3. VAMP8

The amino acid sequence of VAMP8 is only 32% identical to that of VAMP2 [138]. The intracellular distribution of VAMP8 in the endocytic pathway is similar to VAMP3. Although VAMP8 is ubiquitously expressed, it is enriched in tissues with epithelial cells such as the kidney, intestine, pancreas, and lung [139]. VAMP8 mediates homotypic fusion of early and late endosomes by functioning as a v-SNARE to interact with a t-SNARE assembled from Syn7, Vti1b, and Syn8 [140]. Deletion of VAMP8 gene in mice does not significantly affect development [62]. Analysis of the endocytic pathway using VAMP8-null embryonic fibroblasts suggests that the endocytic pathway is not grossly altered in the absence of VAMP8. However, one striking observation is that VAMP-null pancreatic acinar cells are filled with an excess number of zymogen granules (the total number is increased about 3-fold). VAMP8 is enriched in the membrane of zymogen granules and is necessary for regulated secretion of zymogen granules, suggesting that VAMP8 is the major v-SNARE of zymogen granules in pancreatic acinar cells [62]. The role of VAMP8 in other regulated secretions remains to be investigated and the hypothesis that it has overlapping role with VAMP3 in the endocytic pathway needs to be tested by experiments.

7.4. Syn4

Syn4 is a widely expressed t-SNARE heavy chain on the plasma membrane and is enriched in the basolateral surface of polarized epithelial cells, whereas Syn2 and Syn3 are targeted preferentially to the apical surface [74,75]. Syn4 may also function at the apical surface as it is detectable there and has been implicated in apical secretion of zymogen granules of pancreatic acinar cells [62,75]. Syn4 is apparently essential for early embryonic development as Syn4-null embryos die before E7.5 [141]. Heterozygous knockout mice (Syn4+/−) developed normally. Interestingly, the Syn4+/− mice manifest impaired glucose tolerance with a 50% reduction in whole-body glucose uptake due to a 50% reduction in glucose transport in the skeletal muscle. Mechanistically, insulin-stimulated GLUT4 translocation in skeletal muscle is also significantly reduced. However, GLUT4 translocation and glucose uptake are not obviously affected in the adipose tissue and liver, suggesting a critical and selective role of Syn4 in insulin-stimulated GLUT4 deployment and glucose uptake in skeletal muscle [141]. Syn4 has also been implicated in lipopolysaccharide (LPS)-induced secretion of tumor necrosis factor (TNF) in macrophages [142]. Protein levels of Syn4, together with its interacting proteins (SNAP-23 and Munc18-3), are significantly increased by LPS in a temporal pattern coinciding with TNF secretion. This suggests that Syn4 acts at a rate-limiting step during TNFα secretion.

7.5. Vti1b

As a Qb light chain, Vit1b interacts with Syn7 (the Qa heavy chain) and Syn8 (the Qc light chain) to form the endosomal t-SNARE; thus regulating homotypic fusion of late endosome through interaction with VAMP8 as the v-SNARE [63,65]. It is also implicated in the fusion of late endosomes with lysosomes, and the homotypic fusion of lysosomes [65]. Knockout of the Vti1b gene does not affect embryo development and the majority of Vti1b-null mice behave normally [143]. Interestingly, Syn8 levels are selectively reduced in Vti1b-null cells, suggesting that interaction with Vti1b can stabilize Syn8. About 20% of Vti1b-null mice are smaller and lysosomal degradation of an endocytosed protein is slightly delayed in hepatocytes of these mice. Multivesicular bodies and autophagic vacuoles accumulate in hepatocytes of these smaller Vti1b-null mice. Whether Vti1a compensates for the loss of Vti1b in these mice remains to be investigated.

7.6. SNAP-25

SNAP-25 contributes both Qb and Qc SNARE motifs to the synaptic SNARE complex and thus plays a key role in

mediating synaptic vesicle fusion [7,19]. Ca^{2+}-regulated interaction of SNAP-25 with synaptotagmin I is important for Ca^{2+}-triggered fast exocytosis [114]. Complete ablation of the SNAP-25 gene results in embryonic lethality of the mutant mice [144]. The SNAP-25-null embryos are clearly morphologically abnormal compared to wild type around E17.5–E18.5, although major brain structures appear unaltered. Electrophysiological analysis suggests that SNAP-25 is essential for Ca^{2+}-evoked synaptic transmission at neuromuscular junctions and central synapses, although stimulus-independent spontaneous neurotransmitter release is not dramatically affected. This phenotype is similar to that of VAMP2-null mice with regards to evoked exocytosis in the neuron [134].

7.7. Munc18-1

Munc18-1 is believed to bind to Syn1 in its closed conformation involving both the N-terminal Habc region and the SNARE motif, thus modulating its interaction with SNAP-25 and t-SNARE assembly [19,37]. Mice lacking Munc18-1 develop normally but die immediately after birth [145]. Brain development and synaptogenesis occur normally. However, synaptic exocytosis (both Ca^{2+}-evoked and spontaneous events) is completely lost in the absence of Munc18-1. This abolition of synaptic transmission leads to neurodegeneration as a result of extensive apoptosis. The severe effect of Munc18-1 ablation contrasts with the partial spontaneous synaptic exocytosis that is observed in the absence of SNAP-25 or VAMP2. The complete dependence of synaptic transmission on Munc18-1 cannot be explained solely by its role in binding Syn1. Munc18-1 must perform other crucial functions, such as proper folding and intracellular targeting of Syn1, the assembly of the presynaptic t-SNARE, formation of *trans*-SNARE complex, and/or interaction with other regulatory factors.

7.8. Synaptotagmin I

Mice lacking synaptotagmin I develop normally, but the newborns die within 48 h after birth [110]. Electrophysiological analysis of hippocampal neurons derived from synaptotagmin I-null mice reveals severe impairment of synaptic transmission. Ca^{2+}-triggered synchronized synaptic exocytosis is specifically decreased, whereas asynchronous slow release processes, such as spontaneous synaptic activity (miniature excitatory postsynaptic current frequency) and release triggered by hypertonic solution or alpha-latrotoxin, are unaffected. This demonstrates a physiological role for synaptotagmin I in Ca^{2+}-evoked synchronous neurotransmitter release. Subsequent studies using the genetic approach in mice [111] and biochemical reconstitution in vitro [115] establish that synaptotagmin I is a Ca^{2+}-sensor that couples action potential to fast exocytosis, a function dependent on its Ca^{2+}-regulated interaction with phospholipids, PtdIns4,5P_2, and the *trans*-SNARE complex [19,112].

8. Future perspectives

Although the majority of SNAREs have presumably been identified and their general principles of action resolved, much more work is required for a precise understanding of their various physiological roles, molecular mechanisms of action, and regulation. A higher level of understanding will likely be achieved through biophysical approaches that yield quantitative descriptions of SNARE formation and action in terms of time, energy, space, and geometry. Formation of the *trans*-SNARE complex is presumably the core event underlying diverse fusion events and serves as the converging point for various regulatory processes. More regulators of SNAREs are expected to be identified and understanding their precise roles and mechanisms of action are essential to link up vesicle fusion with the rest of the cellular regulatory networks. The role of many other SNAREs at the organism level, explored through gene ablation approach with comprehensive assessment of diverse developmental and physiological processes, will provide more precise understanding of SNARE functions under physiological settings. Small chemicals that selectively modulate the interaction of SNAREs and their regulators, or even SNARE complex formation, should prove useful as tools to reversibly perturb the action and regulation of SNAREs in intact cells. This approach will not only allow us to gain further insights into the SNARE function but also provide a foundation for regulating cellular and physiological processes that are governed by SNAREs. The emerging interface (Chemical Biology) between biology and chemistry will definitely facilitate this process. More genetic analysis of human diseases is likely to uncover new associations of loss or partial loss of function of SNAREs and their regulators in connection with diseases. More exciting new discoveries in structural, molecular, mechanistic, biophysical, regulatory, and conceptual aspects of SNAREs and regulators will advance our understanding of their cellular, developmental, and physiological roles.

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