Seven Novel Mammalian SNARE Proteins Localize to Distinct Membrane Compartments*

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Raj J. Advani‡, Hae-Rahn Bae‡, Jason B. Bock‡, Daniel S. Chao‡, Yee-Cheen Doung‡, Rytis Prekeris‡, Jin-San Yoo‡, and Richard H. Scheller§

From the Howard Hughes Medical Institute, Department of Molecular and Cellular Physiology, Stanford University, Stanford, California 94305-5045

Soluble N-ethylmaleimide-sensitive factor-attachment protein receptor (SNARE) proteins of the vesicle-associated membrane protein (VAMP) and syntaxin families play a central role in vesicular trafficking through the formation of complexes between proteins present on vesicle and target membranes. Formation of these complexes is proposed to mediate aspects of the specificity of vesicle trafficking and to promote fusion of the lipid bilayers. In order to further understand the molecular mechanisms that organize membrane compartments, we have characterized seven new mammalian proteins of the VAMP and syntaxin families. The proteins are broadly expressed; however, syntaxin 13 is enriched in brain and VAMP 8 in kidney. The seven novel SNAREs localize in distinct patterns overlapping with Golgi, endosomal, or lysosomal markers. Our studies support the hypothesis that evolutionary radiation of these two gene families gave rise to sets of proteins whose differential expression and combinatorial associations define and organize the membrane compartments of cells.

The distribution and restriction of molecules to membrane compartments is an essential process of eukaryotic cells. Distinct organelles of the secretory pathway are synthesized and maintained by budding of transport vesicles from a donor compartment followed by fusion of these vesicles with an acceptor membrane (1). The molecular mechanisms responsible for vesicle biogenesis, protein sorting, and membrane fusion are not yet fully understood. While yeast genetics, in vitro biochemistry, and studies of synaptic vesicles have identified many of the components essential for these processes (2–4), the full repertoire of important proteins and their mechanisms of action are yet to be determined. One particularly interesting issue is how a vesicle loaded with specific cargo recognizes the appropriate target. It is becoming clear that several independent mechanisms contribute to the specificity of vesicle trafficking, and it is the sum of these multiple layers of specificity that results in a process with high fidelity (5).

A vesicle-target membrane recognition event mediated by interaction of integral membrane proteins of the vesicle (v-SNAREs) and target (t-SNAREs) membranes represents one layer of targeting specificity, acting at the final step of membrane fusion (6, 7). This process has been extensively studied in the mammalian presynaptic nerve terminal, where formation of a heterotrimeric complex between the v-SNARE, VAMPs 1 or 2, and the t-SNAREs syntaxin 1 and SNAP-25 is thought to serve as a membrane recognition mechanism and may drive fusion of the lipid bilayers (8, 9).

These proteins have subsequently been found to be prototypic members of gene families that span species as well as membrane compartments (6). For example, syntaxin homologs in yeast have been localized to the Golgi (Sed5p) (10), endosomes (Pep12p) (11), lysosomes (Vam3p) (12), and the plasma membrane (Sso1p and Sso2p) (13). In particular, the SNAREs present on yeast vacuoles have been extensively characterized, and genetic manipulations support the idea that a v-SNARE and a t-SNARE are required on opposite membranes for efficient target recognition and membrane fusion (12). Together these studies postulated that members of the syntaxin and VAMP families define membrane compartments and that specific pairing of these proteins represents a determinant of the specificity of vesicle trafficking.

Thus studies to date have led to speculation that most, if not all, vesicle trafficking steps require a distinct v-t-SNARE complex. With the entire sequence of the yeast genome now known, all the syntaxin and VAMP family members that can be detected by sequence homology searches have been identified. However, the more complex intracellular architecture and multiple differentiated and specialized tissues of mammals relative to yeast would seemingly require the evolution of additional SNAREs. For example, while six syntaxins are present in the yeast genome, a recent search of the data base of mammalian expressed sequence tags (ESTs) proposed the presence of at least 16 mammalian syntaxins (14). In order to fully understand the organization of membrane compartments in mammalian cells, it remains necessary to further characterize the spectrum of SNARE proteins. To this end, we have characterized seven new mammalian proteins that we propose to be SNAREs important in intracellular membrane trafficking. While some of the new SNAREs are widely expressed, others are enriched in specific tissues, suggesting an enhanced requirement for particular trafficking steps in those cells. The proteins are specifically localized to the Golgi region, endosomes, and lysosomes, suggesting a role in regulating membrane trafficking to and from these organelles.

The abbreviations used are: v-SNARE, SNARE on vesicle; ER, endoplasmic reticulum; EST, expressed sequence tag; Igπ120, lysosomal glycoprotein of 120 kDa; NRK, normal rat kidney; PCR, polymerase chain reaction; SNAP-25, synaptosome-associated protein of 25 kDa, SNARE, soluble N-ethylmaleimide-sensitive factor-attachment protein receptor; TGN, trans-Golgi network; t-SNARE, SNARE on target membrane; VAMP, vesicle-associated membrane protein; bp, base pair(s); kb, kilobase(s).
EXPERIMENTAL PROCEDURES

Isolation of SNAREs

All ESTs were identified from the GenBank™ EST Data Bank. All clones were obtained from Genome Systems, Inc. (St. Louis, MO). DNA sequencing was performed using Sequenase 2.0 (Amersham Pharmacia Biotech).

Mouse Vti1a and Vti1b—The GenBank™ EST Data Bank of ESTs was searched with the yeast Vti1 sequence (15). Two distinct sets of clones were found. The EST with accession number AA013839 contains the full-length sequence termed mVti1b. The EST with accession number AA016379 contains an open reading frame of 183 amino acids; however, it has an 11-base pair insert relative to accession number W13616 at bp 560, which causes a frameshift. W13616 is identical to AA016379 from 220–651 bp, but lacks 1–219 bp. Thus mVti1a base pairs 1–219 are derived from AA016379 and 220–651 are derived from W13616. This yields an open reading frame of 217 amino acids.

Syntaxin 13—As per our earlier search of the GenBank™ EST data base (14), we obtained a human brain cDNA clone (accession number AA167677). Two 19-bp oligonucleotides were designed and used to amplify a 460-bp sequence using the human brain cDNA clone as a template for the polymerase chain reaction (PCR). The PCR product was then 5'-labeled by a random hexamer priming reaction and used to screen (60 °C) 5 × 106 plaques from a rat brain stem and spinal cord ZAPII cDNA library (Stratagene Inc.). Screening yielded ten positive, overlapping clones, which were excised into pBluescript KS vector (Stratagene Inc.). The largest clone (2.9 kb) contained an 804-bp open reading frame. This clone was designated syntaxin 13. Tissue distribution and membrane extraction of syntaxin 13 was performed as described previously (16).

Syntaxin 11—As per our earlier search of the GenBank™ EST data base (14), we obtained a human germinal B cell clone (accession number AA215741). Sequencing of this clone demonstrated that it contains an 861-bp open reading frame coding for the 287-amino acid protein syntaxin 11.

VAMP 4—As per our earlier search of the GenBank™ EST data base (14), we obtained a human germinal center B cell clone (accession number AA049140). Sequencing of this clone demonstrated that it contains a 453-bp open reading frame coding for the 141 amino acid protein VAMP 4.

VAMP 8—As per our earlier search of the GenBank™ EST data base (14), we obtained an embryonic clone (accession number AA0090730). Sequencing of this clone demonstrated that it contains a 303-bp open reading frame coding for the 101-amino acid protein VAMP 8.

Expression Constructs

Epitope-tagged, full-length mVti1b, VAMPs 4 and 8, and syntaxins 11 and 13 were prepared by using PCR with custom designed oligonucleotide primers with appropriate restriction sites. The PCR product was subcloned into either the pcDNA3 (mVti1b, VAMP 4, and syntaxins 11 and 13) or pCMV4 (VAMP 8) mammalian expression vector containing the myc epitope. All constructs were verified by DNA sequencing.

Antibodies

Rabbit syntaxin 13 antiserum was generated against a synthetic peptide corresponding to amino acids 22–51 of syntaxin 13. Syntaxin 11 and 13 or pCMV4 (VAMP 8) mammalian expression vector containing the myc epitope. All constructs were verified by DNA sequencing.
13-specific antibodies then were affinity purified and used for immuno-blotting at 1:250 dilution. The specificity of the antibody was confirmed by the ability of synthetic peptide to block the signal in Western blotting (data not shown). For immunofluorescence, anti-mannosidase II monoclonal (Babco) was used at a 1:1000 dilution, anti-transferin receptor (PharMingen) at a dilution of 1:1000, anti-synapsin I at 1:1000 (Chemicon), and anti-myc at 1:1500 (Santa Cruz Biotechnology).

**RNA Blotting**

Human, rat, and mouse multiple tissue Northern blots were purchased from either Origene Technologies (blots with six lanes) or CLONTECH (blots with eight lanes). A human blot was used for syntaxin 11; mouse blots were used for mVti1a, mVti1b, VAMP 4, and VAMP 8; and a rat blot was used for VAMP 7. A random-primed 32P probe was generated using full-length coding region for each gene. Blots were prehybridized in ExpressHyb Solution (CLONTECH) for 1 h at 60 °C and hybridized for 2 h at 60 °C in prehybridization solution containing 32P-labeled probe (2 × 10^6 cpm/ml). Blots were washed at high stringency in 0.1× SSC, 0.1% SDS at 65 °C. 32P-labeled bands were visualized using a PhosphorImager (Molecular Dynamics).

**Immunofluorescence Microscopy**

Primary hippocampal CA3/CA1 cultures were obtained and maintained as described previously (17). Normal rat kidney (NRK) cells were transiently transfected using the LipofectAMINE Plus system (Life Technologies, Inc.). They were fixed with 4% paraformaldehyde and immunostained as described previously (18).

**Bioinformatics**

Most sequence and protein analyses were performed using the GCG software, Version 9.1, package of tools (19). Coiled-coil predictions were made using the program Coils (20).

**RESULTS**

**Mammalian VAMP Homologs**—The ever growing data base of ESTs has provided a wealth of partially sequenced cDNAs. A recent search of this data base identified a host of potential new SNARE proteins (14). We have investigated three new members of the VAMP family (Fig. 1). All three proteins have a C-terminal hydrophobic domain that is predicted to serve as a membrane anchor. The region of all three proteins before this hydrophobic stretch is predicted to form an amphipathic helix and may participate in coiled-coil interactions (20). It is this C-terminal region that is conserved among the three family members. Interestingly, N-terminal to the conserved core, the proteins diverge both in sequence and in length. VAMP 8 is similar in size to the founding family members, VAMPs 1 and 2, yet lacks their proline-rich head domain. In contrast, both VAMPs 4 and 7 are longer than the original VAMPs. No significant homology to other proteins are found in these domains.

Tissue distribution studies of SNAREs can yield important insights into their function. A broad distribution is indicative of a role in a ubiquitous trafficking pathway while a more specific distribution suggests a specialized pathway. Northern blot analysis shows both VAMP 4 and VAMP 7 to be broadly expressed (Fig. 2). While the VAMP 4 transcript is abundant in heart, VAMP 7 transcripts are not detected in this tissue. VAMP 8 is abundantly expressed in kidney, and the transcript is found at lower levels in other tissues. This expression pattern suggests that VAMP 8 may be important in a trafficking step specialized in polarized epithelial cells, such as delivery of vesicles to either apical or basolateral aspects of the cell.

Defining the subcellular localization of SNARE proteins is essential to understanding their function. To this end, we transfected NRK cells with epitope-tagged SNARE constructs. We have previously shown this procedure accurately reflects

![Fig. 3. VAMP 4 localization.](image_url) NRK cells were transfected with myc-tagged VAMP 4 and then fixed, permeabilized, and stained with the following antibodies: anti-myc (A, C, and E), anti-syntaxin 6 (TGN) (B), anti-transferrin receptor (endosomes) (D), and anti-lgp120 (lysosomes) (F).

![Fig. 4. VAMP 7 localization.](image_url) NRK cells were transfected with myc-tagged VAMP 7 and then fixed, permeabilized, and stained with the following antibodies: anti-myc (A, C, and E), anti-mannosidase II (cis/medial Golgi) (B), anti-transferrin receptor (endosomes) (D), and anti-lgp120 (lysosomes) (F).
the localization of other SNARE proteins (see “Discussion”). In transfected cells, VAMP 4 appears punctate in a juxtanuclear position (Fig. 3). These puncta do not overlap with those of either transferrin receptor (endosomes) or lgp120 (lysosomes), but they may partially overlap with the syntaxin 6 trans-Golgi network (TGN) pattern. Much but not all of the staining appears close to the nuclear envelope, suggesting a Golgi localization.

VAMP 7 in transfected NRK cells appears more peripherally localized than mannosidase II and only partially overlaps with an endosomal marker (Fig. 4). Interestingly, the staining of VAMP 7 and the lysosomal marker lgp120 coincide almost precisely (see panels E and F, arrows). This localization strongly suggests that VAMP 7 is important for membrane trafficking events involving lysosomes.

VAMP 8 appears on broadly distributed puncta throughout the cell with a juxtanuclear enrichment. (Fig. 5). It is unlikely these structures are individual transport vesicles, which are typically below the resolution of light microscopy. This pattern is typical of the distribution of certain classes of endosomes or lysosomes and yet does not colocalize with transferrin receptor or lgp120. We hypothesize that these structures are a distinct class of endosomes.

Mammalian Syntaxin Homologs—Two new syntaxin homologs were characterized in this study (Fig. 6). Both are the typical syntaxin length and contain a predicted coiled-coil domain near the C terminus. Interestingly, while syntaxin 13 has

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**Fig. 5. VAMP 8 localization.** NRK cells were transfected with myc-tagged VAMP 8 and then fixed, permeabilized, and stained with the following antibodies: anti-myc (A, C, and E), anti-mannosidase II (cis/medial Golgi) (B), anti-transferrin receptor (endosomes) (D), and anti-lgp120 (lysosomes) (F).

**Fig. 6. Sequence comparison of syntaxins.** Sequences were aligned using the Pileup program. Identical amino acids were darkly shaded and conserved amino acids lightly shaded by the Boxshade program. The dark bar indicates the C-terminal hydrophobic region predicted to act as a membrane anchor. Pairwise comparisons of syntaxins were performed with the Bestfit program and yielded the following results (identical, similar, and quality scores, respectively). Syntaxin 11 versus syntaxin 1a: 29%, 44%, 375; syntaxin 11 versus Pep12p: 23%, 37%, 122; syntaxin 11 versus syntaxin 13: 23%, 33%, 120; syntaxin 13 versus syntaxin 1a: 26%, 40%, 177; and syntaxin 13 versus Pep12p: 27%, 43%, 181.

**Fig. 7. Syntaxin 11 localization.** NRK cells were transfected with myc-tagged syntaxin 11 and then fixed, permeabilized, and stained with the following antibodies: anti-myc (A, C, and E), anti-syntaxin 6 (TGN) (B), anti-transferrin receptor (endosome) (D), and anti-lgp120 (lysosomes) (F).
a C-terminal hydrophobic domain, in syntaxin 11, 6 of the 13 C-terminal residues are cysteines. These cysteine residues are predicted to be palmitoylated, providing a means of membrane attachment similar to SNAP-25 (21). A similar C-terminal cysteine rich domain is found on one of the alternatively spliced forms of syntaxin 2 (6). Interestingly syntaxin 13 is quite similar (53% identical) to another recently cloned SNARE, syntaxin 7 (22).

Northern blotting reveals two syntaxin 11 transcripts that are most abundantly expressed in lung, placenta, and heart with almost no mRNA observed in brain (Fig. 2). When NRK cells are transfected with epitope-tagged syntaxin 11, the pattern appears membrane bound in a juxtanuclear region (Fig. 7). Epitope-tagged syntaxin 11 is not extracted from the insoluble pellet by NaCl, high pH, or Triton X-100 (TX) and sediments at 100,000 × g. Supernatants (S) and pellets (P) then were analyzed for the presence of syntaxin 13 using immunoblotting.

![Figure 8](image_url) Syntaxin 13 is an integral membrane protein, highly enriched in brain tissue. A, the major mRNA species recognized by syntaxin 13 cDNA probe has a predicted size of 2.9 kb and is expressed predominantly in brain. The longer exposures also revealed the presence of a weak signal in other tissues. The abbreviations are the same as in Fig. 2. B, postnuclear supernatants were analyzed by immunoblotting with affinity purified anti-syntaxin 13 rat serum. A band of 33 kDa was detected in brain and a band of 39 kDa in pancreas. C, the rat brain was homogenized and fractionated into postnuclear (PNS), cytosolic (Cyt), and crude membrane (Mem) fractions (lanes 1–3). The membrane fraction then was extracted with either 50 mM Tris (C), 1 M NaCl (NaCl), 0.2 M sodium carbonate at pH 11 (pH), or 1% Triton X-100 (TX) and sedimented at 100,000 × g. Supernatants (S) and pellets (P) then were analyzed for the presence of syntaxin 13 using immunoblotting.

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Syntaxin 13 is of particular interest because of its expression pattern. Highest levels of the syntaxin mRNA are found in brain while only very low levels are found in other tissues (Fig. 8A). For further studies of syntaxin 13, a peptide antibody was raised corresponding to residues 22–51. In protein blotting studies, a 33-kDa band is detected in brain, a much fainter signal is detected in pancreas, and lower levels of reactivity are found in other tissues upon longer exposure (Fig. 8B). Cell fractionation studies show that the protein is present in the pellet of a 100,000 × g spin, that it is not extracted by either high salt or high pH, but it becomes soluble when the membranes are solubilized in Triton X-100 (Fig. 8C). This is consistent with syntaxin 13 being an integral membrane protein. To confirm that the C-terminal hydrophobic region acts as a membrane anchor, we deleted this region and transfected it into NRK cells. As predicted, the protein appeared cytosolic (Fig. 9G). While the full-length syntaxin 13 immunostaining pattern in transfected cells overlaps with the Golgi marker mannose-6-phosphate II (cis/medial Golgi) (B), anti-transferrin receptor (endosomes) (D), and anti-lgp120 (lysosomes) (F). NRK cells were also transfected with myc-tagged syntaxin 13 with the C-terminal hydrophobic region deleted and then fixed, permeabilized, and stained with anti-myc (G) and anti-lgp120 (H).
that the syntaxin 13 staining is largely localized to the cell body.

**Mammalian Vti1 Homologs—** A recent screen for yeast proteins interacting with the sorting receptor VPS10 uncovered Vti1p (yVti1p), a putative v-SNARE (15). In a sucrose density gradient, Vti1p co-migrates with both Golgi and prevacuolar markers. Different temperature-sensitive mutants of Vti1 independently disrupt ER to Golgi or Golgi to vacuolar trafficking. Vti1p is also proposed to interact with two t-SNAREs, Pep12p on the prevacuolar compartment and Sed5p on the Golgi. This raises the possibility that a single v-SNARE may mediate two trafficking steps through independent interactions with multiple t-SNAREs.

To gain insight into this protein in mammalian systems, we identified two classes of mouse ESTs (mVti1a and mVti1b) that are similar to yVti1 in several ways, including sequence homology, overall size, and position of a predicted membrane anchor (Fig. 11). In contrast to the initial report of yVti1p, we are not able to identify any significant sequence homology between the yeast or mammalian Vti1 proteins and the other v-SNAREs, VAMP 1 or ySft1. The membrane anchor of mVti1b is somewhat unusual in that it contains a negatively charged glutamic acid residue midway along the hydrophobic domain.

Both mVti1a and mVti1b are broadly expressed in mammalian tissues consistent with a role in a ubiquitous intracellular vesicle transport process. mVti1b is expressed as a single transcript of about 1.2 kb while mVti1a is expressed in a series of five transcripts that vary in size from 1.2 to 4.5 kb. It is not yet known if the mVti1a transcripts encode different proteins; however, the relative distribution of the various transcripts varies between tissues (Fig. 2).

To determine the subcellular localization of mVti1b and test if the C-terminal hydrophobic region serves as a membrane anchor while containing a glutamic acid residue, we transfected NRK cells with an epitope-tagged-mVti1b construct (Fig. 12). The epitope-tagged protein is not extracted by NaCl or urea, and is partially extracted by Triton X-100, consistent with a mem-

**Fig. 10. Syntaxin 13 localization in neurons.** 9-Day old cultures of primary hippocampal neurons were fixed, permeabilized, and stained with the following antibodies: anti-syntaxin 13 (A and C), anti-transferrin receptor (endosomes) (B), and anti-synapsin I (synaptic terminals) (D).

**Fig. 12. mVti1b localization.** NRK cells were transfected with myc-tagged mVti1b and then fixed, permeabilized, and stained with the following antibodies: anti-myc (A, C, E, and G), anti-mannosidase II (cis/medial Golgi) (B), anti-syntaxin 6 (TGN) (D), anti-transferrin receptor (endosomes) (F); and anti-lgp120 (lysosomes) (H).
brane association. In immunofluorescent studies, the protein appears membrane bound and is found in a juxtanuclear crescent domain overlapping with the cis/medial Golgi marker mannosidase II and the trans-Golgi network marker syntaxin 6. The most intensely immunoreactive regions appear closer to the nuclear membrane than either the transferrin receptor marker of endosomes or the lgp120 marker of lysosomes suggesting that at least some of the Vti1b staining may be in the Golgi apparatus itself. A localization to the Golgi region is consistent with a role for mVti1b in mediating both anterograde and retrograde post-Golgi vesicle trafficking. Interestingly, cells transfected with mVti1b appeared to mislocalize syntaxin 5 (data not shown). This is consistent with an interaction between mVti1b and syntaxin 5 since other proteins present in complexes with syntaxin 5 can also disrupt its localization when overexpressed (23).

**DISCUSSION**

Current hypotheses suggest that VAMPs 1 or 2 and syntaxin 1 associate with SNAP-25 to form a heterotrimeric structure that brings opposing membranes into very close or even direct apposition resulting in the fusion of membranes (9, 24). Analogous complexes of syntaxin and VAMP family members have been isolated for both ER to Golgi and TGN to endosome trafficking (23, 25). The formation of complexes between members of these families is specific since different syntaxins coimmunoprecipitate unique, although sometimes overlapping, sets of proteins (23). Thus while it is clear that other cellular mechanisms play a role in the vectorial transport of vesicles to target membranes, specific SNARE pairing is required for appropriate membrane fusion. These ideas give rise to the view that the localization of SNARE proteins of the VAMP and syntaxin
families define sets of membranes that have the capacity to fuse with each other based on the capacity of the proteins to form stable complexes.

If SNARE protein pairing is a critical event mediating membrane fusion events, defining the localization of particular VAMP and syntaxin family members to the correct vesicle or target membranes becomes essential. In this report, we define the subcellular localization of six novel mammalian SNARE proteins through the transfection of epitope-tagged constructs. We have previously used stable and transient transfection techniques as a means of localizing SNARE proteins (18). Expression levels vary greatly in the transfected cells making it possible for us to base our conclusions on cells that express relatively low levels of the proteins, thus reducing the possibility of mislocalization due to overexpression. As markers, we have used mannosidase II to identify the intermediate stacks of the Golgi, syntaxin 6 to identify the trans Golgi network, transferrin receptor to identify endosomes, and lgp120 to identify lysosomes. While much of the immunoreactivity revealed by these markers is localized in the Golgi region concentrated to one side of the nucleus, each of the specific staining patterns has a unique fine structure. Based on our analysis, the proteins characterized in this report are localized in the Golgi region where a diversity of organelles are localized. More detailed electron microscopic studies will be needed to precisely define the membrane compartments occupied by each of the SNAREs.

An additional complication arises when localizing SNAREs, especially v-SNAREs, in that they are trafficking proteins and thus cycle between donor and acceptor membranes. While our studies only evaluate the steady-state levels of the SNAREs, this information at least narrows the possible pathways these SNAREs are involved with. An interesting example is VAMP 7. Recall that this protein colocalizes almost precisely with the lysosomal protein lgp120. This observation likely narrows the possible trafficking pathways VAMP 7 is involved with from dozens down to three. VAMP 7 could be an anterograde v-SNARE present at low levels on transport vesicles targeted to the lysosome, with a high steady-state level on the lysosome. VAMP 7 could be a v-SNARE present at low levels on lysosome-derived vesicles targeted to other membrane compartments. It is even possible that VAMP 7 functions as a vesicle receptor for lysosome-targeted vesicles; there are examples of where the traditional roles of v- and t-SNAREs may be reversed (25).

A blurring of the distinctions between v- and t-SNAREs also becomes apparent on the sequence level. There are now at least 15 members of the mammalian syntaxin gene family (Fig. 13). This family falls into two groups which are only distantly related to each other. The first group contains the “classical” syntaxin proteins, including syntaxin 1 localized to the plasma membrane and syntaxin 5 localized to the Golgi. Syntaxins 11 and 13 belong to this group. The second group contains syntaxin 6 localized to the TGN, as well as SNAP-25 and SNAP-23 (25). Surprisingly, the mammalian Vti1 clones also fit into this group, even though they are currently thought to be v-SNAREs. These data suggest that not only are the v-SNAREs part of one large family but that in the distant past both v- and t-SNARE proteins may have been one large gene family that served to mediate the events of membrane fusion. Early in evolution, these events were likely to have been homotypic fusion processes where the notion of “v” and “t” is irrelevant. Interestingly in recent homotypic fusion studies, while v-t-SNARE pairing was most efficient, a surprising amount of fusion occurred through presumed t-t-SNARE pairing (12).

The v-SNAREs as a group appear more divergent than the syntaxins or t-SNAREs. Thus only a subset of proposed v-SNAREs fits into a single evolutionary tree. The family of seven members has as its core the three original members VAMPS 1 and 2 and cellubrevin (26) as well as the distantly related msec22b (23), a likely v-SNARE for ER to Golgi trafficking (Fig. 14).

When both of these families of sequences are compared along the length of the proteins, the most highly conserved region is found to be the predicted coiled-coil region closest to the membrane (Figs. 13 and 14). This is consistent with this part of the protein being critical for complex formation and for driving membrane fusion. Differences between the sequences in this region are likely to be responsible for the specificity of pairing observed between SNAREs.

Overall, we have shown that several novel VAMP and syntaxin homologs are found in mammalian cells, displaying an intricate network of expression and localization patterns. Further work is needed to understand the full extent of these families and the specificity of the pairing between family members. Since many more SNARE homologs are available in the data base, it should soon be possible to understand the full repertoire of these molecules in mammalian genomes. By further characterization of their localization and pairing specificities, it should be possible to understand the logic which underlies the organization of membrane compartments in mammalian cells.

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REFERENCES

1. Palade, G. (1975) Science 189, 347–358
2. Rothman, J. E. (1994) Nature 372, 55–63
3. Bennett, M. K., and Scheller, R. H. (1994) Annu. Rev. Biochem. 63, 63–100
4. Jahn, R., and Sudhof, T. C. (1994) Annu. Rev. Neurosci. 17, 219–246
5. Scheller, R. H. (1995) Neuron 14, 893–897
6. Bennett, M. K., Garcia-Arraras, J. E., Efferink, L. A., Peterson, K., Flemming, A. M., Hazuka, C. D., and Scheller, R. H. (1995) Cell 74, 863–873
7. Sollner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Gerothanou, S., Tempst, P., and Rothman, J. E. (1995) Nature 362, 318–324
8. Sudhof, T. C. (1995) Nature 375, 645–653
9. Lin, R. C., and Scheller, R. H. (1997) Neuron 19, 1087–1094
10. Banfield, D. K., Lewis, M. U., Rabouille, C., Warren, G., and Pelham, H. R. B. (1994) J. Cell Biol. 127, 357–371
11. Beecher, K. A., Rieder, S. E., Enar, S. D., and Jones, E. W. (1996) Mol. Biol. Cell 7, 579–84
12. Nichols, B. J., Ungermann, C., Pelham, H. R., Wickner, W. T., and Haas, A. (1997) Nature 387, 199–202
13. Aalto, M. K., Ronne, H., and Keranen, S. (1993) EMBO J. 12, 4095–4104
14. Bock, J. B., and Scheller, R. H. (1997) Nature 387, 133–135
15. Mollard, G. F., Nothwehr, S. F., and Stevens, T. H. (1997) J. Cell Biol. 137, 1511–1524
16. Bock, J. B., Lin, R. C., and Scheller, R. H. (1996) J. Biol. Chem. 271, 17961–17965
17. Banker, G. A., and Cowan, M. W. (1977) Science 198, 5671–5679
18. Hay, J. C., Hirling, H., and Sudhoff, T. C. (1993) Nature 364, 346–349