Syntaxin 18, a SNAP Receptor That Functions in the Endoplasmic Reticulum, Intermediate Compartment, and cis-Golgi Vesicle Trafficking*

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Members of the syntaxin family are target-soluble N-ethylmaleimide-sensitive factor-attachment protein receptors involved in vesicle docking and/or fusion within the exocytic and endocytic pathways. By using the yeast two-hybrid system, we have identified a novel member of the syntaxin family, syntaxin 18, that binds to α-soluble N-ethylmaleimide-sensitive factor-attachment protein. Subcellular fractionation and immunocytochemical analysis revealed that syntaxin 18 is principally located in the endoplasmic reticulum. We examined the effect of overexpression of FLAG-tagged syntaxin 18 and a mutant lacking the N-terminal 81 amino acid residues on protein transport and organelles in the early secretory pathway. Both expressed proteins localized to the endoplasmic reticulum, and the expressed FLAG-syntaxin 18 caused remarkable aggregation of endoplasmic reticulum membranes. Although expression of the FLAG-syntaxin 18 lacking the N-terminal region produced less effect on the morphology of the endoplasmic reticulum, dispersion of the endoplasmic reticulum-Golgi intermediate compartment and cis-Golgi was elicited. Moreover, overexpression of the FLAG-syntaxin 18 mutant inhibited protein export from the endoplasmic reticulum. These results taken together suggest that syntaxin 18 functions in transport between the endoplasmic reticulum and Golgi.

In eukaryotic cells protein transport within the secretory pathway is mediated by vesicles that transit between organelles. Transport vesicles are formed from donor compartments and are targeted to acceptor compartments, where they deliver cargo molecules through membrane fusion. The docking and/or fusion of transport vesicles with target membranes is mediated by the SNAP1 receptor (SNARE) machinery (1). The original SNARE hypothesis predicts that the docking of transport vesicles to target membranes is mediated by the specific pairing of v-SNAREs (members of the VAMP or synaptobrevin family) on transport vesicles with t-SNAREs (members of the syntaxin and SNAP-25 families) on target membranes (2–4). The presence of many isomers of t- and v-SNAREs that uniquely localize to different membrane compartments may provide a basis for the specificity of the docking step in different transport pathways (2). A recent study, however, suggests that a simple one to one pairing of SNAREs is not sufficient to account for the specificity of vesicle docking and fusion (5).

Protein transport from the ER to the Golgi occurs in both forward (anterograde) and backward (retrograde) directions. Proteins are transported via the anterograde pathway from the ER to the Golgi, where ER resident proteins are specifically captured in a receptor-dependent manner and retrieved via the retrograde pathway (6–11). At least two distinct transport vesicles, termed COPI vesicles and COPII vesicles, mediate transport between the ER and Golgi (for reviews see Refs. 12 and 13). COPII vesicles, which contain the small GTPase Sar1p and two heterodimers (Sec23p-Sec24p and Sec13p-Sec31p) (14), are formed exclusively from the ER membrane (15). The COPII coat is likely replaced with COPI in the transit to the intermediate compartment, an organelle between the ER and Golgi termed the ERGIC or vesicular tubular clusters (16–18). COPII vesicles, which contain the small GTPase Arf1p and coatomer (19–21), have been suggested to mediate both anterograde (from the ERGIC to cis-Golgi) and retrograde (from the ERGIC to the ER) transport (22).

Yeast COPI- and COPII-coated vesicles contain three types of SNAREs (Bos1p, Bet1p, and Sec22p) (15) that interact with t-SNARE Sed5p located in the Golgi (23) in a Ypt1p-dependent manner (24). A recent reconstitution study revealed that Bos1p is required for only anterograde transport, Bet1p for only retrograde transport, and Sec22p is involved in both anterograde and retrograde directions (25). Sec22p interacts both with Ufe1p, a potential retrograde t-SNARE located in the ER, and with Sed5p in the Golgi (26). Ufe1p also interacts with Cdc48p and likely plays a role in ER membrane fusion during karyogamy (27).

Biochemical analyses and EST database analysis revealed...
the presence of many mammalian SNAREs that participate in transport between the ER and Golgi. Those includeembrin, rBet1, rSec22b, and syntaxin 5, which correspond to Bos1p, Bet1p, Sec22p, and Sed5p, respectively, in yeast (28–34). However, syntaxins located in the ER such as Ufe1p have not yet been identified. One possibility is that the Ufe1p homologue does not exist in mammalian cells; instead, syntaxin 5 plays a role as a t-SNARE in the ER as well as in the Golgi apparatus. Previous studies showed that the 41- and 34-kDa forms of syntaxin 5 are present in the ER and Golgi, respectively, (35, 36). In this paper we report a novel syntaxin species (designated syntaxin 18) that was identified as an a-SNAP-interacting protein in our yeast two-hybrid screening. Morphological and functional analyses revealed that syntaxin 18 is principally located in the ER and functions in transport between the ER and Golgi.

EXPERIMENTAL PROCEDURES

cDNA Cloning of Syntaxin 18—The full cDNA of bovine a-SNAP was subcloned into the yeast expression vector pGB79 using EcoRI and BamHI (pGB7-a-SNAP). The pGB7-a-SNAP plasmid was transformed into yeast strain HP7c, and a yeast two-hybrid screen was carried out essentially according to the manufacturer’s protocol using a GAL4 DNA activation domain fusion library in pGAD10 (MATCHMAKER human leukocyte cDNA library, CLONTECH). Positive clones were isolated by growth selection on plates lacking Trp, Leu, and His followed by β-galactosidase filter assays. The isolated clones were sequenced with an automated DNA sequence (ABI PRISMTM377; Applied Biosystems). One of the clones encoded a novel syntaxin family member. The insert of this clone was used to screen a human brain cDNA library. A full-length cDNA was isolated using standard plaque hybridization techniques.

Subcellular Fractionation—Subcellular fractionation was conducted essentially as described previously (37). Livers from Wistar rats that fasted overnight were removed, rinsed once in homogenization buffer (0.25 M sucrose, 1 mM EDTA), and then weighed. The livers were perfused with homogenization buffer, cut into small pieces, and then mashed by passing through a stainless steel sieve (150-μm mesh). To the mashed livers were added 3 volumes (g/ml) of homogenization buffer and then homogenized in a Dounce homogenizer (5 strokes). The homogenate was centrifuged at 600 × g for 10 min, and a yeast two-hybrid screen was carried out essentially according to the manufacturer’s protocol using a GAL4 DNA activation domain fusion library in pGAD10 (MATCHMAKER human leukocyte cDNA library, CLONTECH). Positive clones were isolated by growth selection on plates lacking Trp, Leu, and His followed by β-galactosidase filter assays. The isolated clones were sequenced with an automated DNA sequence (ABI PRISMTM377; Applied Biosystems). One of the clones encoded a novel syntaxin family member. The insert of this clone was used to screen a human brain cDNA library. A full-length cDNA was isolated using standard plaque hybridization techniques.

Monoclonal anti-Man II was obtained from Babco.

α-SNAP Binding Assays—The mammalian expression plasmids pEBG (kindly donated from Dr. B. Mayer of the Massachusetts Institute of Technology) and pFLAG-CMV-2 (Eastman Kodak Co.) were used to express proteins fused with the N-terminal GST for α-SNAP and with the N-terminalFLAG epitope for syntaxin 18A181, syntaxin 5, and p47. To perform in vitro α-SNAP binding assays, COS7 cells grown in Dulbecco’s modified Eagle’s medium supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, and 7.5% fetal calf serum were transfected with the expression plasmids using a LipofectAMINE PLUS reagent according to the manufacturer’s protocol. At 24 h after transfection, the cold were harvested and lysed in binding buffer (0.25 M sucrose in homogenization buffer and then overlaid on gradients of 8 ml of 1.15 M sucrose, 10 ml of 0.86 M sucrose, and 8 ml of 0.5 M sucrose (in mesh). To

RESULTS

 Syntaxin 18 Is a Novel Mammalian Syntaxin—We screened a human leukocyte expression library using a full-length α-SNAP cDNA as a bait in the yeast two-hybrid system. Forty-two clones positive for both His+ cell growth and β-galactosidase expression were isolated. Most of the positive clones encoded known syntaxin family members. However, one clone encoded a novel protein that displays homology to syntaxin family proteins. A complete cDNA was isolated by screening a human brain cDNA library. Sequence analysis showed an open reading frame encoding a novel syntaxin (termed syntaxin 18) that consists of 395 amino acids with a calculated molecular weight of 38,673 (Fig. 1A). Like other syntaxins, syntaxin 18 possesses a putative TMD near the C terminus and predicted coiled-coil regions. A BLAST search revealed that syntaxin 18 shares 27.8% identity (43.6% similarity) to the Caenorhabditis elegans T10H9 predicted gene product (Fig. 1B). Syntaxin 18 does not show conspicuous sequence similarities to known syntaxins and their yeast homologues. The most similar one is Ufe1p, a t-SNARE implicated in both retrograde transport (41) and ER
membrane fusion (27) in *Saccharomyces cerevisiae*, but the overall sequence identity is only 11.9% (22.7% similarity). The low sequence similarities between syntaxin 18 and other known syntaxin species may explain why syntaxin 18 has not been discovered by extensive EST database searches.

**Syntaxin 18 Binds to α-SNAP in Intact Cells**—To confirm the yeast two-hybrid interaction in mammalian cells, we performed immunoprecipitation experiments. Lysates of COS7 cells coexpressing syntaxin 18 tagged with the FLAG epitope at the N terminus (FLAG-syntaxin 18) and GST-tagged α-SNAP (GST-α-SNAP) were incubated with an anti-FLAG M2 monoclonal antibody, and the immunoprecipitates were analyzed by SDS-PAGE followed by immunoblotting with anti-α-SNAP and anti-FLAG antibodies. As positive and negative controls, we expressed FLAG-syntaxin 5 and FLAG-p47, respectively, and carried out similar experiments. Syntaxin 5 is a t-SNARE that resides mostly in the ERGIC and the cis-Golgi region (35, 36, 42). p47 competes with α-SNAP for binding to syntaxin 5 but does not bind to α-SNAP (43). As shown in Fig. 2, GST-α-SNAP was coimmunoprecipitated with FLAG-syntaxin 18 as well as with FLAG-syntaxin 5 but not with FLAG-p47. These results suggest that syntaxin 18 interacts specifically with α-SNAP.

**Syntaxin 18 Is Principally Located in the ER**—To characterize further syntaxin 18, we produced a rabbit polyclonal antibody against a bacterially expressed fusion protein. On immunoblot analysis of rat liver and several cell lines including
HeLa, COS7, and NRK, the affinity purified antibody specifically detected a protein of 42 kDa, which is in close agreement with the calculated mass of syntaxin 18 (data not shown). A ubiquitous expression of this protein was confirmed by Northern blotting. A transcript of 1.8 kilobase pairs was detected in tissues including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (data not shown).

We next sought to determine the subcellular localization of syntaxin 18. Total membranes were prepared from the post-nuclear supernatant of rat liver homogenates. They were further separated by sucrose gradient centrifugation into the rough ER membranes (rER), smooth ER membranes (sER), G2 membranes (a fraction at the 0.86 M, 1.15 M sucrose interface), and G1 membranes (a fraction at the 0.25 M, 0.86 M sucrose interface). Each fraction was analyzed by immunoblotting with antibodies against syntaxin 18 and markers of the ER (calnexin and the 41-kDa form of syntaxin 5) and Golgi (Man II and the 34-kDa form of syntaxin 5) (Fig. 3). The results of immunoblotting with marker proteins clearly demonstrate that adequate fractionation was achieved. Syntaxin 18 was found to be enriched in the sER fraction although it was detected to a lower extent in the rER and Golgi fractions.

Localization of syntaxin 18 was also investigated by indirect immunofluorescence confocal microscopy. Syntaxin 18 in HeLa cells displayed a tubular/reticular ER-like localization (Fig. 4A), which overlapped significantly with that of calnexin (Fig. 4B), but not with that of ERGIC-53/p58 (Fig. 4D), an ERGIC marker protein (44, 45), or GM-130 (Fig. 4F), a cis-Golgi protein involved in the tethering of COPI vesicles to Golgi membranes (46, 47). These subcellular fractionation and immunohistochemical analyses suggest that syntaxin 18 is principally located in the ER.

Overexpression of Syntaxin 18 Causes Aggregation of the ER—Overexpression of mouse Sec22b or membrin disrupts normal morphology of the ERGIC and Golgi (31). We examined the effect of overexpression of syntaxin 18 on the morphology of the organelles in the early secretory pathway. When FLAG-syntaxin 18 was expressed in HeLa cells, protein disulfide isomerase, an ER marker protein, displayed dot-like structures, in which the overexpressed syntaxin 18 was localized (Fig. 5). Electron microscopic analysis showed the presence of aggregated membrane structures in cells expressing syntaxin 18 (Fig. 6). In these cells Golgi stacks were not seen, instead...
small vesicles were observed at the perinuclear region. On the other hand, no significant morphological changes were observed in organelles such as mitochondria, lysosomes, and peroxisomes (Fig. 6 and data not shown), suggesting that overexpression of syntaxin 18 specifically affects the structures of organelles involved in the early secretory pathway. When a syntaxin 18 mutant lacking N-terminal 81-amino acid residues (syntaxin 18D81) was expressed, it was located in the ER (Fig. 5). ER aggregates were observed only when the syntaxin 18 mutant was highly expressed.

When FLAG-syntaxin 5 was overexpressed, no aggregated structure of ER membranes was observed (data not shown). This unequivocally excludes the possibility that the formation of the ERGIC and Golgi. HeLa cells were transfected with FLAG-syntaxin 18Δ81 (A–F). After 15 h, the cells were fixed and double-stained with antibodies against FLAG or syntaxin 18 (A, C, and E) and ERGIC-53/p58 (B), β-COP (D), or GM130 (F). Bar, 20 μm.

**Fig. 7.** Expression of FLAG-syntaxin 5 causes dispersion of the Golgi but not the ERGIC. HeLa cells were transfected with FLAG-syntaxin 5. After 15 h, the cells were fixed and double-stained with antibodies against FLAG or syntaxin 5 (A, C, and E), and ERGIC-53/p58 (B), β-COP (D), or GM130 (F). Bar, 20 μm.

**Fig. 8.** Expression of FLAG-syntaxin 18 causes aggregation of the ER. HeLa cells were transfected with FLAG-syntaxin 18 (A and B) or FLAG-syntaxin 18Δ81 (C and D). After 15 h, the cells were fixed and double-stained with anti-FLAG (A and C) and anti-protein disulfide isomerase (B and D). Several double-labeled dots were marked by arrows. Bar, 20 μm.
Cells were cotransfected with the FLAG-syntaxin 18 exported from the ER at permissive temperature (32 °C) (49). Syntaxin 18 resides in the ER at nonpermissive temperature (40 °C) and is VSVG-GFP from the ER to the Golgi. This mutant protein was expressed in Vero cells with FLAG-syntaxin 18 (A and D) or FLAG-syntaxin 5 (C and D). The cells were incubated at 40 °C for 15 h and then shifted to 32 °C. After 30 min, they were fixed and stained with anti-FLAG followed by a rhodamine-conjugated secondary antibody. Rhodamine fluorescence (A and C) and fluorescence derived from GFP (B and D) are shown. Bar, 20 μm.

Expression of FLAG-syntaxin 18Δ81 inhibits protein export from the ER to the Golgi. VSVG-GFP was expressed in Vero cells with FLAG-syntaxin 18Δ81 (A and B) or FLAG-syntaxin 5 (C and D). The cells were incubated at 40 °C for 15 h and then shifted to 32 °C. After 30 min, they were fixed and stained with anti-FLAG followed by a rhodamine-conjugated secondary antibody. Rhodamine fluorescence (A and C) and fluorescence derived from GFP (B and D) are shown. Bar, 20 μm.

Overexpression of Syntaxin 18Δ81 Causes Dispersion of the ERGIC and Golgi—We wanted to examine morphological changes of the ERGIC and Golgi without remarkable changes of the ER structure. For this purpose, we chose FLAG-syntaxin 18Δ81 because it produced less of an effect on the ER structure compared with the full-length construct.

In control HeLa cells, ERGIC-53/p58 was distributed in small vesicles and reticular structures mostly concentrated to the perinuclear Golgi region (Fig. 7). Overexpression of FLAG-syntaxin 18Δ81 caused a dispersion of the perinuclear staining of ERGIC-53/p58. A similar but more marked effect was observed in the distribution of β-COP, a component of COPI vesicles (20, 48). Staining for GM130 was also dispersed.

Overexpression of FLAG-syntaxin 5 caused similar changes in the distribution of β-COP and GM130, although the dispersion of GM130 was not remarkable compared with that of β-COP (Fig. 8). In contrast, no significant change was observed for the staining pattern of ERGIC-53/p58. Thus, the overexpression of syntaxin 18 and syntaxin 5 causes similar but different morphological changes of the compartments along the early secretory pathway, suggesting that the two syntaxins participate in the organization of the ER and Golgi in similar but distinct manners.

Overexpression of Syntaxin 18Δ81 Inhibits Transport from the ER to the Golgi—We wondered whether disorganization of the ERGIC and Golgi caused by overexpression of syntaxin 18Δ81 may be due to a defect in transport from the ER to the Golgi. To test this possibility, we measured the transport of VSVG-GFP from the ER to the Golgi. This mutant protein resides in the ER at nonpermissive temperature (40 °C) and is exported from the ER at permissive temperature (32 °C) (49). Cells were cotransfected with the FLAG-syntaxin 18Δ81 and VSVG-GFP constructs and then incubated at 40 °C for 15 h. The two proteins were colocalized in the ER at 40 °C (data not shown). When temperature was shifted to 32 °C, and the cells were incubated for 30 min, VSVG-GFP was still localized in the ER in cells expressing FLAG-syntaxin 18Δ81 (Fig. 9), whereas VSVG-GFP was transported to the perinuclear Golgi region in control cells. A similar result was obtained when full-length syntaxin 18 was expressed (data not shown). This defect in transport was closely related to the dispersion of the β-COP-containing compartment. Dispersed β-COP staining was observed for cells in which the export of VSVG-GFP from the ER was prevented (data not shown). When FLAG-syntaxin 5 was overexpressed, the export of VSVG-GFP from the ER was not affected (Fig. 9). The exported protein was observed in structures at the perinuclear region, where it was colocalized with the expressed syntaxin 5.

Syntaxin 18 Interacts with rSec22b but Not with rBet1—The results described above strongly suggest that syntaxin 18 functions in protein transport between the ER and Golgi. Therefore, we carefully compared the structure of syntaxin 18 with that of Ufe1p and found several similar structural features between them. First, the length of the putative TMD of syntaxin 18 is 17 amino acids, which is similar to that of Ufe1p (16-amino acids). Second, syntaxin 18 and Ufe1p contain 4 and 2 phenylalanyl residues, respectively, in the TMD. Syntaxins located in the early secretory pathway have two or more phenylalanyl residues in the TMD, whereas plasma membrane syntaxins have one or no phenylalanyl residues (50, 51). Third, syntaxin 18 and Ufe1p contain negatively charged residue(s) on the C-terminal side of the TMD. Among syntaxins so far discovered, only Ufe1p has a negatively charged residue (Asp-343) in this region (50, 51).

This finding prompted us to examine whether syntaxin 18 interacts with rSec22b. Ufe1p interacts with Sec22p, a v-SNARE implicated in transport between the ER and Golgi but not with other ER-Golgi v-SNAREs such as Bet1p and Bos1p (26). Triton X-100 extracts of rat liver total membranes were incubated with an affinity purified anti-syntaxin 18 antibody, and the immunoprecipitates were analyzed by immunoblotting. For comparison, immunoprecipitation was performed using an antibody against syntaxin 5. Syntaxin 5 associates with rSec22b, membrin (Bos1p homologue), and rBet1 in mammalian cells (31). As shown in Fig. 10, rSec22b was coprecipitated with syntaxin 18, whereas no coprecipitation was observed for rBet1. Membrin was precipitated, but the amount was trivial. On the other hand, consistent with a previous result (31), the three SNAREs were coprecipitated with syntaxin 5 (Fig. 10). These results suggest that syntaxin 18 and syntaxin 5 exist in distinct complexes containing overlapping component(s) such as rSec22b, and syntaxin 18 has SNARE-interacting properties similar to Ufe1p.

DISCUSSION

By using the yeast two-hybrid system we identified a novel syntaxin (syntaxin 18). Syntaxin 18, which possesses a relatively short TMD (17-amino acid residues), is principally located in the ER. This localization is consistent with the idea that proteins located in the ER and Golgi possess shorter TMDs than plasma membrane proteins (52, 53). Immunoprecipitation analysis showed that syntaxin 18 interacts with rSec22b, a v-SNARE involved in protein transport between the ER and Golgi (31).

Previous studies showed that syntaxin 5 is located not only in cis-Golgi (34-kDa form) but also in the ER (41-kDa form) (35, 36) and interacts with rSec22b, membrin, and rBet1 (31). Given that the localization and interaction partner of syntaxin 5 partly overlaps with that of syntaxin 18, it was of interest to investigate the phenotype of cells in which the function of syntaxin 18 or syntaxin 5 is inhibited. Since overexpression of syntaxins causes inhibition of protein transport in several pathways (54–56), we overexpressed syntaxin 18 or syntaxin 5 in HeLa cells, and we examined transport from the ER to the Golgi and organelle morphology.

When syntaxin 18Δ81 was overexpressed in HeLa cells, the
structures of the ERGIC represented by ERGIC-53/p58 (44, 45), ERGIC-cis-Golgi represented by β-COP (48, 57), and cis-Golgi represented by GM-130 (46, 47) became dispersed. The medial-Golgi and trans-Golgi network represented by Man II and γ-adaptin, respectively, were also scattered (data not shown). In addition, the export of VSVG-GFP from the ER was blocked in cells expressing syntaxin 18A81. On the other hand, no significant changes were observed in the localization of EEA1, an early endosome maker protein (58), and in the uptake of transferrin (data not shown). These results suggest that overexpression of syntaxin 18 specifically causes disassembly of the compartments along the early secretory pathway by inhibiting protein export from or into the ER. Perhaps inhibition of retrograde transport causes a shortage of recycling membrane proteins such as v-SNAREs, which results in inhibition of the formation of COPII vesicles. Indeed, v-SNAREs such as Bet1p play a structural role in the assembly of the COPII coat (59). On the other hand, overexpression of syntaxin 5 affected the structures of the ERGIC-cis-Golgi and cis-Golgi but not the ERGIC structure. In agreement with the observation by Dascher et al. (54), VSVG-GFP was transported from the ER to a perinuclear region where expressed syntaxin 5 was located, suggesting that overexpression of syntaxin 5 does not affect the formation of COPII vesicles. Disassembly of the ERGIC-cis-Golgi and cis-Golgi structures may be due to the inhibition of the replacement of COPII with COPI or the blockage of the fusion of COPI with cis-Golgi. When fusion is inhibited in the anterograde pathway, vesicle components may return from the ERGIC to the ER via the retrograde pathway. This idea comes from the observation that an inactive Sar1p mutant, which inhibits anterograde transport, does not interfere with the transport of ERGIC-53/p58 from the ERGIC to the ER (16). Recycling of ERGIC components may supply proteins such as v-SNAREs required for the formation of COPII vesicles from the ER.

When full-length syntaxin 18 was overexpressed, aggregated ER structures emerged. Less aggregation occurred when syntaxin 18A81 was overexpressed, suggesting that the N-terminal region of syntaxin 18 is important for the formation of ER aggregates. Similar aggregated ER structures were observed upon overexpression of ER membrane proteins such as microsomal aldehyde dehydrogenase (60) and inositol 1,4,5-trisphosphate receptor (61). In cells overexpressing microsomal aldehyde dehydrogenase, however, the transport of VSVG from the ER was not impaired (60). In contrast, the transport of VSVG was blocked when syntaxin 18 was overexpressed.

It is obvious that the formation of the ER aggregates is due to a specific effect by the overexpression of syntaxin 18 because overexpression of syntaxin 5 had no effect on ER morphology. It is known that Ufe1p forms higher order multimers, and this interaction may facilitate the homotypic ER membrane fusion (27). Similarly, syntaxin 18 molecules may interact with each other via the cytoplasmic domain, leading to ER aggregation. In some circumstances, membrane fusion is inhibited when syntaxins are overexpressed (54–56). Why does the overexpression of syntaxins cause different effects on membrane fusion events? One possibility is that different effects may reflect the different SNARE requirements for heterotypic and homotypic fusion reactions. In heterotypic fusion, a more quantitative balance between v-SNAREs and t-SNAREs may be important. In homotypic fusion, on the other hand, an abundance of syntaxin molecules may simply increase the probability for the proper SNARE complex formation, which promotes membrane fusion. However, further studies are required to address this question.

In conclusion, our results suggest that syntaxin 18 functions in the ER, intermediate compartment, and cis-Golgi vesicle trafficking. Several structural and functional features of syntaxin 18 are similar to those of Ufe1p. Functional studies of syntaxin 18 and other SNAREs in mammalian cells should lead to an understanding of the organization of membrane compartments and the regulation of organelle assembly.

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