How do transport vesicles recognize the appropriate acceptor membrane? Recognition is thought to occur, at least in part, by the specific binding of proteins on the vesicle (v-SNAREs) to distinct partners on target membranes (t-SNAREs). This has been termed the SNARE hypothesis and, on page 199 of this issue, Nichols *et al.*[1] examine some of the key tenets of the model.

The SNARE hypothesis predicts that pairing of v- and t-SNAREs is regulated by low-molecular-weight proteins known as Rabs, which hydrolyse GTP. After the v- and t-SNAREs have paired, the soluble ATPase, *N*-ethylmaleimide-sensitive factor (NSF), binds the SNARE complex through the soluble NSF-attachment protein (α-SNAP) and hydrolyses ATP. This results in reorganization of the complex and membrane fusion[2,3] (Fig. 1a).

Using an elegant combination of genetics and biochemistry, Nichols *et al.* have manipulated the SNARE composition of the donor and acceptor membranes. Consistent with earlier hypotheses, they show that a prerequisite for fusion of two membranes is a t-SNARE on one membrane and a v-SNARE on the other. But, in conflict with the SNARE hypothesis, their experiments reveal a role for NSF prior to docking — before the acceptor and donor membranes ever see each other[1,4].

The seed of the SNARE hypothesis was planted in the late 1980s and early 1990s, with a molecular dissection of the proteins involved in synaptic vesicle transport in neurons (reviewed in ref. 5). Studies of neurotransmitter-filled vesicles from the brain uncovered a prototype v-SNARE, known as VAMP (ref. 6) or synaptobrevin. The prototype t-SNARE, called syntaxin1, was characterized as a nerve-terminal protein that associates with proteins on the synaptic vesicle. This interaction between proteins on opposing membranes was proposed to mediate docking of the vesicle at the plasma membrane[7].

It soon became clear that such pairing between v- and t-SNAREs defined a model that encompassed many different vesicle-trafficking steps throughout the secretory pathway, in cells as evolutionarily distant as yeast and neurons[8]. Studies in yeast identified a set of v- and t-SNAREs that mediate transport between the endoplasmic reticulum and the Golgi apparatus[9,10]. A different set of SNAREs was found to underlie shuttling between the Golgi apparatus and vacuole (lysosome)[11]. The emerging concept was that specific pairs of vesicle and target membrane proteins — in general, of the VAMP and syntaxin families, respectively — mediate the fidelity of vesicle trafficking. Moreover, the cytosolic proteins α-SNAP and NSF, which promiscuously interact with SNARE pairs, were thought to mediate this membrane fusion throughout the secretory pathway[3] (Fig. 1a).

No sooner was this hypothesis put forth, than potentially conflicting data were generated. First, although vesicle fusion occurs at specialized regions of the plasma membrane, the t-SNAREs are not localized to specific regions — they are distributed over most of the cell membrane. Perhaps even more troubling, a fraction of the synaptic t-SNAREs were found on vesicles, and these were suggested to be the physiologically active component (Fig. 1b). Second, neurotoxin cleavage of SNARE proteins, which renders them nonfunctional, does not deplete the pool of vesicles that seem to be docked and ready for membrane fusion. Finally, physiological studies indicated that the last requirement for ATP hydrolysis occurred considerably before the fusion of vesicles, making it difficult to understand how NSF could directly drive the membrane-fusion event.

To address these discrepancies, Nichols *et al.*[1] used a biochemical *in vitro* assay[12]. Vacuolar membranes are isolated from two different yeast strains, and the rate of fusion is monitored through a biochemical reaction that only occurs when the internal vacuolar contents of the two strains are intermixed. Vacuole fusion is considered to be 'homotypic', because the donor and acceptor membranes have identical protein constituents, including their v- and t-SNAREs. The power of the assay lies in the fact that the constituents of the vacuolar membranes of each yeast strain can be altered independently.

The authors identified the v- and t-SNARE proteins that are required for homotypic vacuole fusion by searching the recently completed yeast genome sequence for VAMP- and syntaxin-related sequences. They then created strains that were deficient in either of these newly found vacuolar v- and t-SNAREs. When vacuoles containing either v-SNAREs or t-SNAREs alone were mixed, fusion was reduced to near background levels. Fusion was also greatly reduced when one set of vacuoles contained both v- and t-SNAREs, and the other set lacked both proteins. But when one set of vacuoles contained only the v-SNARE, and the other set contained only the t-SNARE, efficient fusion occurred. So, consistent with the SNARE hypothesis, Nichols *et al.* concluded that v- and t-SNAREs are required, on opposing membranes, for

Mammalian SNAREs

Protein	Accession number		Protein	Accession number	
Syntaxin 1a	M95734		VAMP 1	M24104	
Syntaxin 1b	M95735		VAMP 2	M24105	
Syntaxin 2	L20823		VAMP 3	S63830	(Cellubrevin)
Syntaxin 3	L20820		VAMP 4	D86817	AA197391
Syntaxin 4	L20821		VAMP 5	AA222692	
Syntaxin 5	L20822		VAMP 6	W69164	
Syntaxin 6	U56815		VAMP 7	X96737	
Syntaxin 7	D60600	AA081523	VAMP 8	AA049140	
Syntaxin 8	AA111025	W41301	Rsec22a	U42209	
Syntaxin 9	AA150357		Msec22b	U91538	
Syntaxin 10	N35629	W24393	Hsec22c	H59647	
Syntaxin 11	AA227632		Rbet1a	U42755	
Syntaxin 12	R29508		Mbet1b	W75334	W83047
Syntaxin 13	AA167677		Membrin	U91539	
Syntaxin 14	T08774		GOS-28	U49099	
Syntaxin 15	AA244750		Hykt6a	H18270	H23796
Syntaxin 16	AA100145		Hykt6b	AA016381	
SNAP-25	M22012				
SNAP-23	U55936				

Differential expression patterns, subcellular localization and protein–protein interactions between v- and t-SNAREs may determine the organization of membrane compartments in cells, by controlling the specificity of vesicle trafficking between these compartments. If SNAREs work in this way, then there must be more of them than have been identified to account for the known anterograde and retrograde trafficking steps. As a first step towards understanding the complete complement of SNAREs in mammalian species, we searched the NCBI EST database with the sequences of known SNAREs. Relevant sequences were determined through alignment of prospective, with known, SNAREs using the BESTFIT and PILEUP programs. Prospective sequences were randomized and again aligned with known SNAREs. In all cases the original quality score was at least ten standard deviations higher than that obtained after randomization. Bold type indicates newly identified SNAREs. All sequences can be found at NCBI Entrez Web site: http://www3.ncbi.nlm.nih.gov/Entrez/ **J.B.B. & R.H.S.**