

Transport-vesicle targeting: tethers before SNAREs

Suzanne R. Pfeffer*

*Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5307, USA

Protein secretion and the transport of proteins between membrane-bound compartments are mediated by small, membrane-bound vesicles. Here I review what is known about the process by which vesicles are targeted to the correct destination. A growing family of proteins, whose precise modes of action are far from established, is involved in targeting. Despite the wide diversity in the identities of the players, some common themes are emerging that may explain how vesicles can identify their targets and release their cargo at the correct time and place in eukaryotic cells.

The targeting of transport vesicles to the correct membrane destination involves a much larger set of proteins than anticipated and several layers of protein–protein interactions. The process of ‘vesicle targeting’ includes all of the steps involved in delivering a newly formed transport vesicle to its target. In the broadest sense, targeting requires molecular motors and the actin and/or microtubule-based cytoskeletons to bring a vesicle from one part of the cell to another. Then tethering proteins collect and restrain vesicles at or near their cognate target membranes. Finally, a core layer of proteins interact to bring vesicle membranes in exceedingly close apposition with their cognate target membranes, thereby driving membrane fusion. Each of these processes is regulated by other proteins, to enhance the spatial and temporal control of membrane-trafficking events.

SNAREs and company at the core

At the core of every pairing between transport vesicle and target membrane lies an interaction between so-called SNARE proteins. First discovered as proteins found on synaptic vesicles and at the presynaptic plasma membrane, SNAREs are members of a family of highly conserved proteins that reside on vesicles (v-SNAREs) or target membranes (t-SNAREs; refs 1–4). Almost every step in membrane trafficking is carried out by a distinct set of SNARE pairs, and the SNAREs that mediate a given transport step (from endoplasmic reticulum (ER) to Golgi, from Golgi to plasma membrane, and so on) are conserved from yeast to humans⁵.

Nearly incontrovertible proof of the importance of SNARE proteins in neurotransmitter release from vesicles at synapses comes from the discovery that the SNAREs that couple synaptic vesicles with the presynaptic plasma membrane (namely vesicle-associated membrane protein (VAMP; also called synaptobrevin), syntaxin and synaptosome-associated protein of relative molecular mass 25,000 (SNAP-25)) are the precise and select targets of the clostridial botulinum and tetanus toxins⁶. These toxins block exocytosis (the process of secretion, involving fusion of the transport vesicle with the plasma membrane) by proteolysis of one or another of the presynaptic SNARE molecules, leading to severe paralysis and, in some cases, a horrible death.

The neuronal v- and t-SNAREs form exceedingly stable, parallel coiled-coil complexes that are stable in 0.1% SDS^{7–10}. The stability of this complex has led to the proposal that the energy gained from complex formation may be harnessed to drive the membrane-fusion reaction. Rothman and co-workers have shown that purified neuronal v- and t-SNAREs, when reconstituted into distinct liposome vesicles, are themselves capable of driving liposome fusion, albeit at a rate that is significantly slower than the rate of exocytosis of synaptic vesicles¹¹. The slow rate observed could have been due, in part, to the presence of the syntaxin amino-terminal domain,

which slows the assembly of the homologous yeast SNARE complexes as much as 2,000-fold *in vitro*¹². Release of this regulatory domain would be expected to enhance significantly the rate of tight SNARE pairing and membrane fusion. Thus, independent of any other proteins, paired SNAREs seem to be capable of stabilizing vesicles and target membranes in a closely apposed orientation, sufficient to drive liposome fusion¹¹.

Although SNAREs are clearly important for membrane fusion, an increasing body of information, summarized below, indicates that they are not sufficient to ensure that the steps preceding membrane fusion — that is, the accurate targeting and docking of vesicles — are carried out. There is little doubt that additional proteins function to enable SNARE-complex formation. For example, a protein may catalyse the conformational rearrangement of syntaxin’s regulatory N-terminal domain to allow SNARE complexes to form. Indeed, GTPases called Rab proteins are known to regulate the formation of SNARE complexes *in vivo*^{13–15}. Moreover, the slow rate of reconstituted, SNARE-mediated vesicle fusion could also reflect a requirement for extra factors to drive fusion in conjunction with SNAREs. For example, a new set of proteins that function in vesicle docking (such as the early-endosomal autoantigen EEA-1; see below) bind to phosphorylated, phosphatidylinositol lipids. It remains to be determined whether this lipid-binding characteristic may also enable this class of ‘docking factors’ to contribute to the fusion event *per se*.

Regulating access to SNAREs

As membrane fusion involves the tight association of SNARE molecules with each other, access to SNAREs should be tightly regulated; if cognate v- and t-SNAREs could pair at all times, all of the organelles in the cytoplasm might become stuck together¹⁶. For example, a v-SNARE that is found on ER-derived transport vesicles and is therefore also present in the ER could bind tightly to a cognate Golgi t-SNARE and thereby sandwich together two entire organelles. Thus it is not surprising that there are proteins that block SNARE accessibility.

n-Sec1 is the prototypic t-SNARE protector (Fig. 1). This mammalian homologue of the yeast protein Sec1 binds directly and tightly to the t-SNARE on the presynaptic plasma membrane, syntaxin-1A; moreover, binding of n-Sec1 to syntaxin-1A blocks v-SNARE–t-SNARE association¹⁷. Sec1 homologues exist and could regulate other t-SNAREs.

t-SNARE protectors could block v-SNARE binding by steric hindrance. Alternatively, as SNAREs are likely to exist in distinct conformations, SNARE protectors may bind preferentially to a particular conformation of a t-SNARE that interacts only weakly with a v-SNARE. An important area for further investigation will be to understand how and when n-Sec1 is released from syntaxin to allow

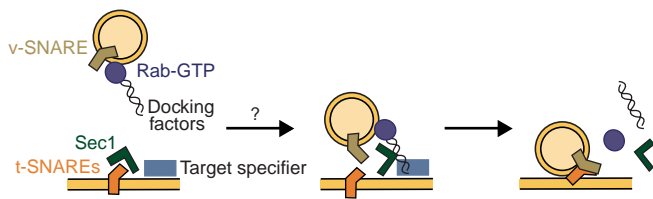


Figure 1 Heterotypic targeting and fusion. Left, to target a vesicle derived from one type of organelle, such as the Golgi, to another organelle (which would be the plasma membrane in this case), tethering and docking factors, such as the Exocyst complex, are recruited to nascent transport vesicles by an active (GTP-bound) Rab GTPase. On the target membrane, a target-specifying component (Sec3 is the target-specifying component of the Exocyst) recruits the vesicle by binding the vesicle-associated docking factor (Exocyst subunits other than Sec3). Centre, docking or other factors may then 'deprotect' or activate the t-SNARE to allow for t-SNARE–v-SNARE pairing (right). LMA-1, a soluble protein that seems to stabilize detangled SNAREs, may also bind the deprotected t-SNARE to help with the eventual formation of core SNARE complexes (not shown).

SNARE-complex formation, and how and when it rebinds. An unexpected link between n-Sec1 and proteins that connect presynaptic nerve terminals with their postsynaptic targets may hint at the spatial cues used for SNARE pairing at neurotransmitter-release sites in neurons¹⁸. Specifically, n-Sec1 binds to a complex that includes Mint-1 (LIN-10), CASK (LIN-2) and the transmembrane protein β-neurexin; the extracellular portion of β-neurexin links to the postsynaptic-cell protein neuroligin to stabilize a functional synapse¹⁸. The presynaptic components of this complex may act as a target specifier for vesicle release, and recruit SNAREs to this site through n-Sec1 (Fig. 1).

Even if t-SNAREs are shielded (or inhibited) from productive interaction with cognate v-SNAREs, the members of SNARE pairs must still find each other. Additional targeting factors could serve two purposes: they could provide a layer of recognition to bring vesicles in contact with protected SNAREs, and they could also catalyse SNARE 'deprotection' (or conformational activation). Much less is known about these extra factors and how they interact with and potentially activate SNAREs. Yet these essential gene products are likely to have key roles in the process of vesicle docking, before membrane fusion occurs.

SNAREs are not sufficient for vesicle targeting

Although clostridial neurotoxins cleave neuronal SNAREs and block their subsequent assembly, pre-assembled SNARE complexes resist toxin-mediated proteolysis¹⁹. Thus, a toxin-triggered block in exocytosis could be explained by a blockade in SNARE-complex formation. However, giant-squid nerve terminals injected with tetanus toxin actually contain about twice as many docked vesicles as do untreated nerve terminals²⁰. In addition, genetic or toxin-mediated removal of v- and t-SNAREs in *Drosophila* blocks neurotransmission but not synaptic-vesicle docking²¹. Thus, the

morphological association of synaptic vesicles with the presynaptic plasma membrane must require other interactions as well as v-SNARE–t-SNARE partnerships. The t-SNARE syntaxin on the presynaptic plasma membrane can interact with the calcium-sensing synaptic-vesicle protein synaptotagmin^{3,22}, and this interaction could in part explain persistent vesicle docking despite a blockade of v- and t-SNARE pairing. But other interactions probably regulate SNARE accessibility to ensure that fusion occurs only at the correct time and place²³.

Two other lines of work strongly indicate that SNAREs cannot be the sole determinants of vesicle targeting. The neuronal t-SNAREs, syntaxin and SNAP-25, are not restricted to sites of synaptic-vesicle fusion within the presynaptic membrane; they are also localized along the entire length of the axon, as well as in nerve terminals²⁴. Thus, other proteins must specify vesicle-release sites and also regulate the SNAREs located elsewhere. In addition, a single v-SNARE has been shown to drive two completely distinct transport events^{25,26}. If one v-SNARE pairs with t-SNAREs on two different target membranes, other proteins must direct the different vesicle types to the correct membrane destination.

Vesicle-docking and -tethering proteins

There are a number of recently discovered proteins that serve to link vesicles (or organelles) with their targets (Table 1 and see below). For the purposes of this review, it is useful to distinguish between proteins that lead to an initial, loose 'tethering' of vesicles with their targets from those responsible for tighter, more stable, 'docking' interactions (Fig. 1). Tethering could be considered to involve links that extend over distances of more than about half the diameter of a vesicle from a given membrane surface (>25 nm); I use the term 'docking' to refer to the holding of two membranes within a bilayer's distance of one another (<5–10 nm). Stable docking probably represents several distinct, molecular states: the molecular interactions underlying the close and tight association of a vesicle with its target may include the molecular rearrangements needed to trigger bilayer fusion.

Although studies of synaptic vesicles and presynaptic plasma membranes have provided a wealth of information about SNAREs, the investigation of other transport steps has provided the most clues to the components that act in vesicle tethering and docking before SNARE pairing. A common feature of many proteins that function in vesicle tethering and docking is their propensity to form highly extended, coiled-coil structures^{16, 27, 28}.

Vesicle tethering. Perhaps the best characterized tethering factor is p115, a peripheral-Golgi membrane protein in mammalian cells that was purified on the basis of its ability to stimulate transport between Golgi cisternae²⁹ (Table 1). At the ultrastructural level, p115 is myosin-shaped: its dimeric structure comprises two globular heads and an extended coiled-coil tail followed by an acidic carboxy terminus^{30,31}. p115 is homologous to the yeast protein Uso1 which is required for ER-to-Golgi transport^{30,32,33}. Uso1 is much larger than p115 (length 150 nm versus 45 nm); a larger coiled-coil portion in Uso1 accounts for the size differences between these proteins. The architecture of p115/Uso1 is well suited to a tethering fac-

Table 1 Recently described tethering and docking interactions

Event	GTPase	Tethering/docking factor	Comments
Endosome fusion (mammalian cells) ⁵⁹	Rab5	EEA-1/Rabaptin-5	Homotypic interaction
Golgi-to-prevacuole (yeast) ^{46,47}	Ypt21	Vac1	Vac1 links vesicle to Sec1 homologue Vps45 at the target
ER-to-Golgi (yeast) ^{35,38}	Ypt1	Uso1/TRAPP	TRAPP is at the target; not yet known if Uso1 interacts with TRAPP
Intra-Golgi (mammalian) ^{38,39}	Rab1 *	p115/giantin/GM130	Giantin is on the vesicle; GM130 and p115 are on the Golgi
Secretory vesicle to plasma membrane (yeast) ⁵¹	Sec4	Exocyst	Exocyst links vesicle with target; Sec3 marks release site; Sec15 is recruited by the GTPase Sec4

* Rab1 is important for intra-Golgi transport but it is not yet known to which docking factor it is linked.

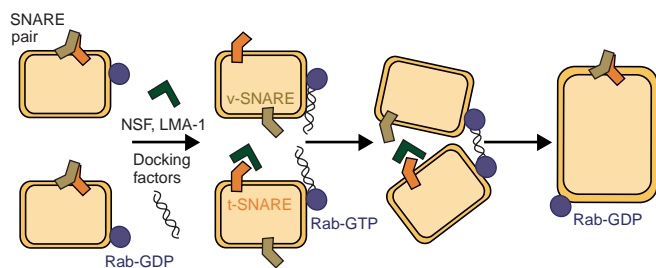


Figure 2 Homotypic endosomal targeting and fusion. The fusion of two biochemically identical, early-endosome compartments is an example of homotypic membrane fusion. In this case, *cis*-SNARE pairs (pairs of v-SNAREs and t-SNAREs found on a single organelle) are first disengaged by NSF and then stabilized by LMA-1 ('priming'). In parallel, organelles are brought together by Rab-regulated tethering/docking factors such as EEA-1 or Rabaptin-5, to enable the potential collision and association of LMA1-stabilized SNARE proteins and the subsequent fusion of the early-endosome compartments. GTP hydrolysis by Rab is involved in controlling the frequency of the docking process.

tor: a long coiled-coil domain provides a flexible arm with which to 'catch' or trap incoming transport vesicles and to link them loosely with their targets.

Genetic and biochemical studies support a function in vesicle tethering for p115/Uso1. Uso1 is needed to allow the formation of SNARE complexes involved in ER-to-Golgi transport³⁴, consistent with the idea that it acts before membrane fusion. In addition, Uso1 seems to lead to vesicle tethering in cell extracts³⁵. Barlowe and colleagues found that exogenous Uso1 decreased the fraction of freely diffusible ER-derived transport vesicles generated in an *in vitro* ER-to-Golgi transport reaction. Tethering required both functional Ypt1, the GTPase of the Rab family that is needed for this transport step³⁶, and a new peripheral-membrane protein, Sec35 (ref. 37), but not functional v- or t-SNAREs³⁵. A striking aspect of these experiments was the finding that vesicles remained associated with the target membrane even when SNARE pairing was blocked by an anti-t-SNARE antibody. Moreover, after tethering was complete, the Rab GTPase was no longer required. Thus vesicle tethering involves a series of molecular interactions that precede SNARE pairing. In ER-to-Golgi transport, p115/Uso1 is required, and tethering is helped by the Rab GTPase Ypt1.

A large macromolecular complex named TRAPP (for transport protein particle) is also required for ER-to-Golgi transport in yeast³⁸ (Table 1). TRAPP is a large complex, of approximate relative molecular mass 800,000, that contains about ten polypeptides. TRAPP co-localizes with the relevant t-SNARE on the *cis*-Golgi and is needed for vesicle docking *in vitro*; thus, TRAPP may 'catch' vesicles derived from the ER. Of obvious interest is whether p115/Uso1 links ER-derived transport vesicles to TRAPP, before SNARE complexes form. How p115/Uso1 associates with ER-derived vesicles is also not yet known; however, this process seems to require that transport vesicles contain active Ypt1 (ref. 35 and see below).

As mentioned above, p115 also functions in intra-Golgi transport. Warren and colleagues have shown that p115 binds the N terminus of a Golgi protein, GM130 (ref. 39 and Table 1). p115 also binds a protein named giantin, which is present on Golgi-derived transport vesicles, and can promote *in vitro* Golgi-derived vesicle docking directly⁴⁰. Like p115, GM130 and giantin also contain large regions of extended coiled-coils that could function to tether and 'restrain' transport vesicles^{16,27,28}. Thus, giantin could drive transport-vesicle capture by latching onto p115 molecules protruding outwards from the Golgi membrane surface.

Tethering would enhance the efficiency of vesicle transport by holding a vesicle close to its site of budding and adjacent to its cognate target. In the transfer of proteins between Golgi cisternae, such

tethering would be extremely advantageous²⁷. Similarly, tethering interactions are likely to be involved in concentrating synaptic vesicles at the synapse⁴¹.

Vesicle docking. Four other components have been identified that are likely to serve as tethering and/or docking factors (Table 1): the Exocyst (or Sec6–Sec8 complex)^{42,43}, the Rabaptin-5 complex⁴⁴, EEA-1 (ref. 45), and EEA-1's yeast orthologue, Vac1 (refs 46,47). The Exocyst is a large, 19.5S complex that contains Sec3, Sec5, Sec6, Sec8, Sec10, Sec15 and Exo70 and is required for exocytosis in yeast⁴². The Exocyst is a highly extended structure that is about 30 nm in length, and it is localized to the tips of yeast buds, the site of exocytosis in *Saccharomyces cerevisiae*⁴⁸. Sec3 appears to localize the Exocyst complex to the plasma-membrane target⁴⁹. In contrast, the t-SNAREs required for exocytosis in *S. cerevisiae* are distributed uniformly over the plasma membrane⁵⁰. It seems likely, then, that the Exocyst functions to tether transport vesicles at the docking site on the plasma membrane to enable the final reaction, SNARE assembly, to occur. Guo *et al.*⁵¹ showed that the Sec15 component of the Exocyst interacts directly with the GTPase Sec4, allowing the docking factor, Exocyst, to link a transport vesicle with its target.

In polarized epithelial cells, the mammalian Exocyst, the Sec6–Sec8 complex, localizes to sites of cell–cell interactions, at the apex of the basolateral domain⁵². Available data are also consistent with a vesicle-docking role for the mammalian complex⁴³.

Another targeting factor in mammalian cells is the Rabaptin-5 complex, which interacts preferentially with endosome-associated GTP-bound (active) Rab5 (refs 44,53,54). Rabaptin-5 occurs as a complex with a nucleotide-exchange factor (Rabex-5) that can activate Rab5 (ref. 55). Rabaptin-5 has two distinct Rab-GTPase-binding sites that could in principle, tether two separate endosomes together⁵³. Rab5-GTP also interacts with EEA-1 (ref. 45), an early-endosome-associated protein that contains long stretches predicted to form coiled coils⁵⁶. The C terminus of EEA-1 contains a so-called 'FYVE'-finger domain that binds phosphatidylinositol-3-phosphate in endosome membranes^{57,58}. Zerial and co-workers have now shown that EEA-1 tethers endosomes together and acts upstream of SNAREs to trigger endosome fusion⁵⁹.

The yeast relative of EEA-1, Vac1, interacts with the Rab5-related GTPase Vps21 (refs 46,47). Vac1 also interacts with the Sec1 homologue involved in this transport step, Vps45 (refs 46,47). These interactions have the potential to link a Rab-GTP-bearing vesicle with a Vac1-containing target, bringing the vesicle in direct contact with SNARE constituents.

Rab GTPases recruit tethering and docking proteins

Rabaptin-5/EEA-1, p115 and the Exocyst act in conjunction with a Rab GTPase to carry out their functions in vesicle tethering and docking (Fig. 1). Rab GTPases interconvert between inactive, GDP-bound forms and active, GTP-bound forms. GTP hydrolysis is not coupled to fusion; rather, Rab conformation, which depends on the guanine nucleotide to which a Rab is bound, regulates the recruitment of docking factors from the cytosol onto membranes. In this way, Rab GTPases regulate vesicle docking. If it is mainly nascent transport vesicles that contain active Rab GTPases, then only functional transport vesicles will bind to the docking factors. Thus docking will take place only between transport vesicles and their targets, rather than between entire organelles.

In contrast, when homotypic (like-to-like) fusion between two organelles is the goal, the overall level of Rab-GTP could modulate fusion rates⁶⁰. An example of homotypic fusion is the fusion of two biochemically identical early-endosome compartments (Fig. 2). In endosome–endosome fusion, cells rely on the dynamic activation and inactivation of Rab5 to maintain the size of the endosome compartment and to prevent the formation of large vacuoles^{61,62}.

The aim of a homotypic fusion process is rather different from that of a heterotypic vesicle–fusion event in which a transport vesicle buds from one compartment and delivers its cargo to the next.

In heterotypic processes, it makes little sense to inactivate a Rab before vesicle docking and fusion: once a vesicle forms, it should find its target and fuse. In this case, it should be vesicle formation that is rate limiting.

Rab5 catalyses both homotypic endosome fusion and the fusion of newly formed, clathrin-coated endocytic vesicles with early endosomes. It would appear, then, that Rab5 catalyses both heterotypic and homotypic fusion processes. Perhaps Rab5-GTP on clathrin-coated vesicles is longer lived than Rab5-GTP on endosomes, ensuring efficient heterotypic vesicle delivery while restraining homotypic endosome fusion. This type of regulation could be accomplished by differential localization of the nucleotide exchanger for Rab5, Rabex-5.

To ensure that Rab proteins remain active on transport vesicles, the transport machinery may make use of a set of Rab-interacting proteins that lock the Rabs in their active conformations^{36,63}. But regardless of whether or not Rabs cycle rapidly between active and inactive forms (as in endosome fusion) or are clamped in their GTP-bound conformations, we have proposed that their primary function is to recruit cytosolic docking factors that are needed for vesicle translocation (see below), tethering/docking and fusion⁶³.

Homotypic and heterotypic docking and priming

So far I have mainly summarized events involved in heterotypic vesicle targeting and fusion. Heterotypic fusion events probably require Rab-type GTPases, motor proteins (in some cases), large coiled-coil tethering proteins, release-site identifiers and SNAREs (Fig. 1 and see above). Tethering and docking of a transport vesicle at the target membrane probably precede the formation of a tight core SNARE complex.

In neuronal exocytosis, the term 'priming' has been used to include all of the molecular rearrangements and ATP-dependent protein and lipid modifications that take place after initial docking of a synaptic vesicle but before exocytosis, such that the influx of calcium ions is all that is needed to trigger nearly instantaneous neurotransmitter release⁹⁴¹.

However, during heterotypic targeting events, complexes of v- and t-SNAREs that formed at the target membrane during a previous release event also need to be unpaired. The ATPase NSF (for N-ethylmaleimide-sensitive factor) can disassemble SNARE complexes in the presence of a soluble co-factor named SNAP (soluble NSF-attachment protein)^{64,65}. Such unpairing would make available free t-SNAREs which could then engage in a subsequent round of membrane fusion. This process might take place relatively late during an *in vitro* heterotypic fusion reaction, as translocation and initial tethering events could take place first. Thus, NSF action may also be part of the overall 'priming' process.

The term priming has been used differently in the study of homotypic fusion events (Fig. 2). Homotypic fusion is often measured *in vitro* by loading two vesicle populations (of a similar type) with different molecules, and then monitoring content mixing. This type of assay provides sensitive detection of a single event that resulted from fusion between two representatives of the different populations of membranes. However, it is not possible to detect any of the multiple fusion events that probably took place within either population before the event that led to content mixing. This is important because the molecular products of previous, unscored fusion events (complexes of SNARE proteins and other docking factors) would need to be 'untangled' before another round of homotypic fusion would be possible. Thus, all *in vitro* homotypic reactions are likely to require the early action of 'untangling factors' such as NSF before mixing of the two populations can be detected^{66,67}. In homotypic fusion, untangling could appear to be the first step because of the experimental protocol used; Wickner has called this process 'priming'^{66,67}.

Wickner and colleagues have also discovered a soluble complex that seems to stabilize untangled SNAREs. This factor, termed

LMA-1 (ref. 68), stabilizes t-SNAREs on yeast endosomes after their NSF-mediated disengagement from v-SNAREs⁶⁹. LMA-1 is recruited to membranes by NSF, and can be found in complexes containing the t-SNARE after NSF has acted and been released. In addition to its function in homotypic prevacuolar fusion, LMA-1 also facilitates heterotypic yeast ER-to-Golgi transport⁷⁰.

Homotypic fusion events could, in principle, be simpler than heterotypic fusion events as they require coalescence of two similar structures. Whereas heterotypic membrane recognition may invoke several layers of specific interactions to ensure accurate target recognition, homotypic fusion could rely on self-association of identical proteins found on two separate vesicles of a similar type (such as two endosomes). In homotypic events, SNARE untangling and stabilization may take place either before or in parallel with organelle-tethering events: concomitant with NSF and LMA-1 action, EEA-1, in conjunction with an active Rab GTPase, might drive endosome coalescence. Available (and stabilized) SNAREs could then pair, leading to fusion.

It is not yet known how many individual SNARE complexes are needed to drive efficient fusion. Given what is known about cell-fusion reactions mediated by viral glycoproteins, membrane fusion is likely to require more than a single SNARE complex. However, it is highly unlikely that all SNAREs present on a single endosome are needed for each round of membrane fusion.

Finally, t-SNARE protection may be more important in heterotypic than in homotypic fusion events, because of the need to maintain the identity of distinct membrane-bound organelles. Thus, LMA-1 may not enter into play in a heterotypic event until the t-SNARE has first been deprotected in the context of a newly arrived, tethered transport vesicle. In homotypic events, the prevalence of so-called *cis*-SNARE complexes (v- and t-SNAREs paired on a single organelle as a result of a previous fusion event)⁷¹ may have a similar function to the protection of t-SNAREs on a heterotypic target-membrane compartment; the NSF-mediated uncoupling of the *cis*-SNARE complex could be a rate-influencing factor for the overall fusion process, but might not need to be as carefully orchestrated as deprotection of the t-SNARE in relation to vesicle docking.

Diversity in homotypic fusion events

Two homotypic fusion events occur by a mechanism distinct from that described above for endosome fusion. Whereas yeast vacuole fusion requires a v-SNARE on one membrane and a t-SNARE on another⁷², homotypic fusion of mammalian postmitotic Golgi cisternae and of yeast ER membranes appears to be mediated by the pairing of two t-SNAREs^{73,74}. In addition, the ATPases p97/Cdc48, rather than NSF, are required here. Regardless of the formal possibility that an unknown v-SNARE protein participates in these homotypic fusion events, it will surely be important to characterize these processes further. Of particular interest are the potential cell-cycle-coordinated control of p97/Cdc48 and the regulation of t-SNARE-t-SNARE pairing.

Translocation of vesicles to docking sites

Certain vesicle-trafficking steps require the translocation of a vesicle over a significant distance. For example, vesicles that carry proteins from the Golgi to the cell surface are likely to use motor proteins and a cytoskeletal track to get close to their target before tethering would be appropriate. Both the actin- and the microtubule-based cytoskeletons have been implicated in these processes, along with an increasing number of new motor proteins. Once the vesicles reach their targets, they come into contact with tethering factors that can restrain them there.

In yeast, the Exocyst complex and the GTPase Sec4 interact genetically with actin, profilin, Myo2 (a type-V myosin motor) and Smy1 (a kinesin-like protein) (reviewed in ref. 43). Sec4, a Rab GTPase, could first recruit a motor to the vesicle, allowing translo-

cation along actin filaments that extend towards release sites. Translocation would bring a vesicle into contact with the Exocyst complex to permit tethering and subsequent 'functional docking' (that is, SNARE pairing). More likely, perhaps, is a scenario in which a vesicle contains more than one Sec4 molecule. Multiple Rab proteins on a single vesicle could interact with distinct effectors, leading to steady-state translocation and also to targeted vesicle delivery. In this case, a vesicle might be linked by Rab GTPases to a motor protein as well as to tethering factors.

The Golgi-associated GTPase Rab6, in its active conformation, interacts with a new, kinesin-like motor protein named Rabkinesin-6 (ref. 75). Rabkinesin-6 is also localized to the Golgi complex, and may be part of a larger docking complex that uses the microtubule-based cytoskeleton to direct vesicle trafficking. Rab5 also appears to be involved in the regulation of endosome motility (M. Zerial, personal communication). Rabphilin, a Rab3A effector, interacts with the actin-bundling protein α -actinin in the absence of Rab3A-GTP76. This may provide a means for Rab3A-GTP to modulate the organization of the local actin-based cytoskeleton in relation to events preceding synaptic-vesicle exocytosis. Finally, Rab8 also influences the organization of the actin-based cytoskeleton in some way⁷⁷.

The emerging view is that, when necessary, Rab GTPases can link vesicles to motor proteins. Rab proteins can also enable vesicle tethering by recruiting the tethering factors to the vesicle. In some cases, vesicle translocation is not required, in which case the tethering process can be initiated more rapidly. This almost entirely unexplored area will surely receive a great deal of attention in the coming months.

Conclusions

The past five years have witnessed a virtual explosion in our knowledge of membrane targeting and fusion. The diversity of proteins involved at each step in membrane traffic could not have been predicted. In addition to the diversity of SNARE molecules, individual transport events appear to use entirely distinct, multisubunit tethering complexes and unique Rab GTPases. Thus, vesicle targeting requires many more molecules and protein-protein interactions than those provided by just SNAREs. This extra complexity is likely to help to ensure the exquisite selectivity and spatial and temporal regulation of membrane targeting in eukaryotic cells. A fuller understanding will surely result from the stepwise reconstitution of each of the underlying molecular interactions, using purified proteins as well as proteins reconstituted into liposomes^{11,78}. An understanding of the connection between molecular motors and the targeting machinery also awaits both functional reconstitution and molecular dissection. □

- Rothman, J. E. Mechanisms of intracellular protein transport. *Nature* **372**, 55–63 (1994).
- Scheller, R. H. Membrane trafficking in the presynaptic nerve terminal. *Neuron* **14**, 893–897 (1995).
- Südhof, T. C. The synaptic vesicle cycle: a cascade of protein-protein interactions. *Nature* **375**, 645–653 (1995).
- Nichols, B. J. & Pelham, H. R. SNAREs and membrane fusion in the Golgi apparatus. *Biochim. Biophys. Acta* **1404**, 9–31 (1998).
- Ferro-Novick, S. & Jahn, R. Vesicle fusion from yeast to man. *Nature* **370**, 191–193 (1994).
- Montecucco, C. & Schiavo, G. Structure and function of tetanus and botulinum neurotoxins. *Quart. Rev. Biophys.* **28**, 423–472 (1995).
- Hayashi, T. et al. Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. *EMBO J.* **13**, 5051–5061 (1994).
- Weis, W. I. & Scheller, R. H. SNARE the rod, coil the complex. *Nature* **395**, 328–329 (1998).
- Rizo, J. & Südhof, T. C. Mechanics of membrane fusion. *Nature Struct. Biol.* **5**, 839–842 (1998).
- Hughson, F. M. Membrane fusion: structure snared at last. *Curr. Biol.* **9**, R49–R52 (1999).
- Weber, T. et al. SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759–772 (1998).
- Nicholson, K. L. et al. Regulation of SNARE complex assembly by an N-terminal domain of the t-SNARE, Sso1p. *Nature Struct. Biol.* **5**, 793–802 (1998).
- Lian, J. P., Stone, S., Jiang, Y., Lyons, P. & Ferro-Novick, S. Ypt1p implicated in v-SNARE activation. *Nature* **372**, 698–701 (1994).
- Sogaard, M. et al. A rab protein is required for the assembly of SNARE complexes in the docking of transport vesicles. *Cell* **78**, 937–948 (1994).
- Lupashin, V. V. & Waters, M. G. t-SNARE activation through transient interaction with a rab-like guanosine triphosphatase. *Science* **276**, 1255–1258 (1997).
- Pfeffer, S. R. Transport vesicle docking: SNAREs and associates. *Annu. Rev. Cell Dev. Biol.* **12**, 441–461 (1996).
- Pevsner, J. et al. Specificity and regulation of a synaptic vesicle docking complex. *Neuron* **13**, 353–361 (1994).
- Butz, S., Okamoto, M. & Südhof, T. C. A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell* **94**, 773–782 (1998).
- Hayashi, T., Yamasaki, S., Nauenburg, S., Binz, T. & Niemann, H. Disassembly of the reconstituted synaptic vesicle membrane fusion complex *in vitro*. *EMBO J.* **14**, 2317–2325 (1995).
- Hunt, J. M. et al. A post-docking role for synaptobrevin in synaptic vesicle fusion. *Neuron* **12**, 1269–1279 (1994).
- Broadie, K. et al. Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*. *Neuron* **15**, 663–673 (1995).
- Schiavo, G., Stenbeck, G., Rothman, J. E. & Sollner, T. H. Binding of the synaptic vesicle v-SNARE, synaptotagmin, to the plasma membrane t-SNARE, SNAP-25, can explain docked vesicles at neurotoxin-treated synapses. *Proc. Natl Acad. Sci. USA* **94**, 997–1001 (1997).
- Hanson, P. I., Heuser, J. E. & Jahn, R. Neurotransmitter release — four years of SNARE complexes. *Curr. Opin. Neurobiol.* **7**, 310–315 (1997).
- Garcia, E. P., McPherson, P. S., Chilcote, T. J., Takei, K. & DeCamilli, P. rbSec1A and B colocalize with syntaxin 1 and SNAP-25 throughout the axon, but are not in a stable complex with syntaxin. *J. Cell Biol.* **129**, 105–120 (1995).
- von Mollard, G. F., Nothwehr, S. F. & Stevens, T. H. The yeast v-SNARE Vti1p mediates two vesicle transport pathways through interactions with the t-SNAREs Sed5p and Pep12p. *J. Cell Biol.* **137**, 1511–1524 (1997).
- Lupashin, V. V., Pokrovskaya, I. D., McNew, J. A. & Waters, M. G. Characterization of a novel yeast SNARE protein implicated in Golgi retrograde traffic. *Mol. Biol. Cell* **8**, 2659–2676 (1997).
- Warren, G. & Malhotra, V. The organization of the Golgi complex. *Curr. Opin. Cell Biol.* **10**, 493–498 (1998).
- Orci, L., Perrelet, A. & Rothman, J. E. Vesicles on strings: morphological evidence for processive transport within the Golgi stack. *Proc. Natl Acad. Sci. USA* **95**, 2279–2283 (1998).
- Waters, M. G., Clary, D. O. & Rothman, J. E. A novel 115-kD peripheral membrane protein is required for intercisternal transport in the Golgi stack. *J. Cell Biol.* **118**, 1015–1026 (1992).
- Sapperstein, S. K., Walter, D. M., Grosvenor, A. R., Heuser, J. E. & Waters, M. G. p115 is a general vesicular transport factor related to the yeast ER-Golgi transport factor Uso1p. *Proc. Natl Acad. Sci. USA* **92**, 522–526 (1995).
- Yamakawa, H., Seog, D.-H., Yoda, K., Yamasaki, M. & Wakabayashi, T. Uso1p is a dimer with two globular heads and a long coiled coil tail. *J. Struct. Biol.* **116**, 356–365 (1996).
- Nakajima, H. et al. A cytoskeletal-related gene, USO1, is required for intracellular protein transport in *Saccharomyces cerevisiae*. *J. Cell Biol.* **113**, 245–260 (1991).
- Barroso, M., Nelson, D. S. & Sztul, E. Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes. *Proc. Natl Acad. Sci. USA* **92**, 527–531 (1995).
- Sapperstein, S. K., Lupashin, V. V., Schmitt, H. D. & Waters, M. G. Assembly of the ER to Golgi SNARE complex requires Uso1p. *J. Cell Biol.* **132**, 755–767 (1996).
- Cao, X., Ballew, N. & Barlowe, C. Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. *EMBO J.* **17**, 2156–2165 (1998).
- Novick, P. & Zerial, M. The diversity of Rab proteins in vesicle transport. *Curr. Opin. Cell Biol.* **9**, 496–504 (1997).
- Van Rheenen, S. M., Cao, X., Lupashin, V. V., Barlowe, C. & Waters, M. G. Sec35p, a novel peripheral membrane protein, is required for ER to Golgi vesicle docking. *J. Cell Biol.* **141**, 1107–1119 (1998).
- Sacher, M. et al. TRAPP: a highly conserved novel complex on the *cis* Golgi that mediates vesicle docking and fusion. *EMBO J.* **17**, 2494–2503 (1998).
- Nakamura, N., Lowe, M., Levine, T. P., Rabouille, C. & Warren, G. The vesicle docking protein p115 binds GM130, a *cis*-Golgi matrix protein, in a mitotically regulated manner. *Cell* **89**, 445–455 (1997).
- Sönnichsen, B., Lowe, M., Levine, T., Jamsa, E., Dirac-Svestrup, B. & Warren, G. A role for giantin in docking COPI vesicles to Golgi membranes. *J. Cell Biol.* **140**, 1013–1021 (1998).
- Robinson, L. J. & Martin, T. F. J. Docking and fusion in neurosecretion. *Curr. Opin. Cell Biol.* **10**, 483–492 (1998).
- TerBush, D. R., Maurice, T., Roth, D. & Novick, P. The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J.* **15**, 6483–6494 (1996).
- Hsu, S.-C., Hazuka, C. D., Foletti, D. L. & Scheller, R. H. Targeting vesicles to specific sites on the plasma membrane: role of the Sec6/8 complex. *Trends Cell Biol.* (in the press).
- Stenmark, H., Vitale, G., Ullrich, O. & Zerial, M. Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. *Cell* **83**, 423–432 (1995).
- Simonsen, A. et al. EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. *Nature* **394**, 494–498 (1998).
- Peterson, M. R., Burd, C. G., & Emr, S. D. Vac1p coordinates Rab and phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome. *Curr. Biol.* **9**, 159–162 (1999).
- Tall, G. G., Hama, H., DeWald, D. & Horazdovsky, B. F. The phosphatidylinositol 3-phosphate binding protein, Vac1p, interacts with a Rab GTPase and a Sec1p homolog to facilitate vesicle-mediated vacuolar protein sorting. *Mol. Biol. Cell* (in the press).
- TerBush, D. R. & Novick, P. Sec6, Sec8, and Sec15 are components of a multisubunit complex which localizes to small bud tips in *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**, 299–312 (1995).
- Finger, F. P., Hughes, T. E. & Novick, P. Sec3p is a spatial landmark for polarized secretion. *Cell* **92**, 559–571 (1998).
- Brennwald, P. et al. Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec4 function in exocytosis. *Cell* **79**, 245–258 (1994).
- Guo, W., Roth, D., Walch-Solimena, C. & Novick, P. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* **18**, 1071–1080 (1999).
- Grindstaff, K. K. et al. Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* **93**, 731–740 (1998).
- Vitale, G. et al. Distinct Rab-binding domains mediate the interaction of Rabaptin-5 with GTP-bound Rab4 and Rab5. *EMBO J.* **17**, 1941–1951 (1998).

54. Gournier, H., Stenmark, H., Rybin, V., Lippé, R. & Zerial, M. Two distinct effectors of the small GTPase Rab5 cooperate in endocytic membrane fusion. *EMBO J.* **17**, 1930–1940 (1998).
55. Horiuchi, H. *et al.* A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* **90**, 1149–1159 (1997).
56. Corvera, S. & Czech, M. P. Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. *Trends Cell Biol.* **8**, 442–447 (1998).
57. Gaullier, J. M. *et al.* FYVE fingers bind PtdIns(3)P. *Nature* **394**, 432–433 (1998).
58. Patki, V., Lawe, D. C., Corvera, S., Virbasius, J. V. & Chawla, A. A functional PtdIns(3)P-binding motif. *Nature* **394**, 433–434 (1998).
59. Christoforidis, S., McBride, H. M., Burgoyne, R. D. & Zerial, M. The Rab5 effector EEA1 is a core component of endosome docking. *Nature* **397**, 621–625 (1999).
60. Rybin, V. *et al.* GTPase activity of Rab5 acts as a timer for endocytic membrane fusion. *Nature* **383**, 266–269 (1997).
61. Bucci, C. *et al.* The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715–728 (1992).
62. Stenmark, H. *et al.* Inhibition of Rab5 GTPase activity stimulates membrane fusion in endocytosis. *EMBO J.* **13**, 1287–1296 (1994).
63. Schimmoller, F., Simon, I. & Pfeffer, S. R. Rab GTPases, directors of vesicle docking. *J. Biol. Chem.* **273**, 22161–22164 (1998).
64. Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H. & Rothman, J. E. A protein assembly-disassembly pathway *in vitro* that may correspond to sequential steps of synaptic vesicle docking, activation and fusion. *Cell* **75**, 409–418 (1993).
65. Söllner, T. *et al.* SNAP receptors implicated in vesicle targeting and fusion. *Nature* **362**, 318–324 (1993).
66. Mayer, A. & Wickner, W. Docking of yeast vacuoles is catalyzed by the Ras-like GTPase Ypt7p after symmetric priming by Sec18p (NSF). *J. Cell Biol.* **136**, 307–317 (1997).
67. Ungermann, C., Nichols, B. J., Pelham, H. R. B. & Wickner, W. A vacuolar v-t-SNARE complex, the predominant form *in vivo* and on isolated vacuoles, is disassembled and activated for docking and fusion. *J. Cell Biol.* **140**, 61–69 (1998).
68. Xu, Z., Mayer, A., Muller, E. & Wickner, W. A heterodimer of thioredoxin and I(B)2 cooperates with Sec18p (NSF) to promote yeast vacuole inheritance. *J. Cell Biol.* **136**, 299–306 (1997).
69. Xu, Z., Sato, K. & Wickner, W. LMA1 binds to vacuoles at Sec18p (NSF), transfers upon ATP hydrolysis to a t-SNARE (Vam3p) complex, and is released during fusion. *Cell* **93**, 1125–1134 (1998).
70. Barlowe, C. Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. *J. Cell Biol.* **139**, 1097–1108 (1997).
71. Ungermann, C., Sato, K. & Wickner, W. Defining the functions of trans-SNARE pairs. *Nature* **396**, 543–548 (1998).
72. Nichols, B. J. *et al.* Homotypic vacuolar fusion mediated by t- and v-SNAREs. *Nature* **387**, 199–202 (1997).
73. Rabouille, C. *et al.* Syntaxin 5 is a common component of NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments *in vitro*. *Cell* **92**, 603–610 (1998).
74. Patel, S. K., Indig, F. E., Olivieri, N., Levine, N. D. & Latterich, M. Organelle membrane fusion: a novel function for the syntaxin homolog Ufe1p in ER membrane fusion. *Cell* **92**, 611–620 (1998).
75. Echar, A. *et al.* Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science* **279**, 580–585 (1998).
76. Kato, M. *et al.* Physical and functional interaction of rabphilin-3A with alpha-actinin. *J. Biol. Chem.* **271**, 31775–31778 (1996).
77. Peranen, J., Auvinen, P., Virta, H., Wepf, R. & Simons, K. Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts. *J. Cell Biol.* **135**, 153–167 (1996).
78. Sato, K. & Wickner, W. Functional reconstitution of Ypt7p GTPase and a purified vacuole SNARE complex. *Science* **281**, 700–702 (1998).