membrane fusion is advancing apace to an atomic level; however, the mechanism by which syntaxin is activated remains poorly understood.

Misura and colleagues[1] contribute to this debate by describing the structure of what may be a critical intermediate in the assembly of neuronal SNARE complexes — the complex of syntaxin 1a with another one of its partners, neuronal Sec1 (nSec1). Although Sec1 is essential for membrane fusion *in vivo*, its exact function is not yet clear. By following syntaxin as it steps through the cycle of SNARE-complex assembly and disassembly (Fig. 1), we can get some idea of where Sec1 may enter the dance.

The mechanism of syntaxin activation centres on the accessibility of its so-called H3 helix, a structural feature that must be exposed to allow syntaxin to assemble into SNARE complexes. Accessibility of the H3 helix is regulated by a three-helix bundle structure[3] (the inhibitory domain) at syntaxin's amino terminus. When the H3 helix is bound to the inhibitory domain in a putative four-helix bundle structure, SNARE-complex assembly is blocked[4,5]. In this conformation, syntaxin is 'closed'. Syntaxin is activated when the inhibitory domain is displaced from the H3 helix (step 1 in Fig. 1), resulting in an 'open' conformation to which other SNARE proteins can bind.

Open syntaxin then assembles with other SNAREs into a '*trans*'-SNARE complex that holds the two fusing membranes together (step 2). Most prominent among the multiple partners of syntaxin 1a are VAMP, a SNARE on the vesicle membrane, and SNAP-25, another target-membrane SNARE[6]. The helices from these proteins assemble with the syntaxin H3 helix into a four-helix bundle, forming the *trans*-SNARE complex required for membrane fusion (step 3).

Assembly of *trans*-SNARE complexes is sufficient for the fusion of synthetic membranes[7], but membrane fusion in the cell requires a number of other conserved proteins, including Sec1. The role of Sec1, which binds to syntaxin[8], might be to activate syntaxin, so regulating the assembly of SNARE complexes. The structure determined by Misura *et al.*[1] shows nSec1 bound to syntaxin 1a, with syntaxin's H3 helix distorted. In this structure, syntaxin 1a may have been shifted into an intermediate conformation, somewhere between closed and open. But, if nSec1 does indeed activate syntaxin 1a, other components must be required too, because nSec1 alone binds tightly to syntaxin and inhibits SNARE-complex assembly[9]. For this reason, Misura and colleagues speculate that SNARE-complex assembly is stimulated by other factors, which bind nSec1; nSec1 then changes conformation and releases syntaxin in an open conformation. A variety of Sec1-binding proteins have been identified and proposed to activate SNARE-complex assembly in this way. It will be important to see whether, as predicted by this model, SNARE-complex levels are altered in mutants in which Sec1 is non-functional.

Surprisingly, studies of yeast mutants place the essential function of Sec1 after SNARE-complex assembly (step 2) and before membrane fusion (step 3). But the situation in yeast and neurons may be different: unlike its neuronal counterpart, Sec1 from yeast lysates has no detectable affinity for its syntaxin homologue, and instead binds to assembled SNARE complexes[10]. In this case, Sec1 may be acting in conjunction with SNARE complexes to catalyse membrane fusion.

After the target and vesicle membranes have fused, '*cis*'-SNARE complexes remain assembled in the fused membrane until they are actively disassembled by a process that requires further proteins and energy supplied by ATP (step 4 in Fig. 1). At this point, the inhibitory domain of syntaxin binds the H3 helix, forming the closed conformation[5], and so prevents the reassembly of SNARE complexes (step 5). If the interaction between the inhibitory domain and the H3 helix is too weak, other factors may be required at this step to hold syntaxin closed. Misura *et al.* propose that nSec1 is needed here, to bind to the closed conformation of syntaxin and so prevent reassembly of *cis*-SNARE complexes. But, as Misura *et al.* point out, a strictly inhibitory role for Sec1 proteins is inconsistent with genetic data from several organisms indicating that Sec1 function is essential to activate vesicle fusion with membranes[11].

Isolation of two classes of Sec1 mutant from *Drosophila*[12] also shows that Sec1 may have a dual function — inhibition and activation — in neurotransmitter release. The inhibitory task of nSec1 in neurons may be to keep syntaxin in a closed conformation, if neuronal syntaxin alone prefers the open form. On binding other factors, nSec1 may

Cell biology

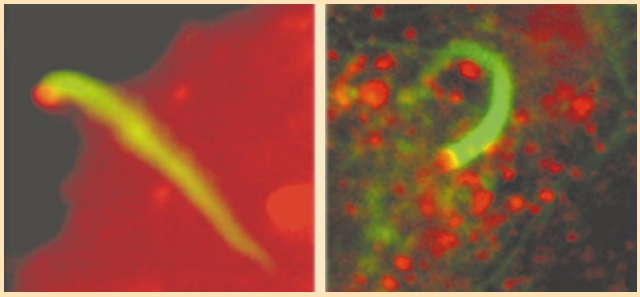
Rafting vesicles

Just as organisms move, so too do their cells and even the organelles within them. For example, vesicles — intracellular transport vehicles — move from organelle to organelle to reach the plasma membrane that encloses a cell, or in the reverse direction.

And just as animals need their skeletons for movement, there's a suspicion that the movement of vesicles relies in some way on the network of proteins that make up a cell's intracellular skeleton — the cytoskeleton. Indeed, some pathogens, such as *Listeria monocytogenes*, take over the cytoskeletal protein actin to propel themselves around the cell. So it seemed plausible that the cell itself makes use of its skeleton for vesicle movement. Now, however, A. L. Rozelle and colleagues offer evidence for a direct linkage between vesicle transport and actin (*Current Biology* **10,** 311–320; 2000).

Rozelle *et al.* started by looking at a lipid molecule called phosphatidylinositol-4,5-bisphosphate (or PIP_2 for short), which regulates both cytoskeletal and vesicle-trafficking proteins. An enzyme that results in the production of PIP_2 led to the formation of actin-containing 'comet tails' in mouse fibroblast cells. In the pictures reproduced here, green staining identifies these comets, and the red circular shapes at the ends of the comets are vesicles.

The authors also found that comet formation relies on the cell recruiting certain effector proteins to the vesicles found at the head of



comet tails. So, PIP_2 and these proteins may work together in some way to generate comets — probably, Rozelle *et al.* suggest, through two well-known actin-polymerizing proteins.

Rozelle *et al.* next showed that the vesicles at the heads of the comets were derived from an organelle called the Golgi complex or from the plasma membrane. These and other cellular membranes contain microregions, known as 'rafts' because cholesterol and sphingolipids 'float' in tight-knit groups in these areas. The vesicles at the comet heads mainly budded off from these rafts.

It seems certain that cells do use their cytoskeleton to move vesicles about. But what exactly does actin do? It might help the vesicle-budding process, or it could be a road along which motor proteins carry vesicles. Or, as suggested by Rozelle *et al.*, the comets themselves might push the vesicles along. **Amanda Tromans**