Membrane fusion: **All done with SNAREpins?**

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SNARE proteins are sufficient to fuse artificial membranes together. In the cell, vesicle transport may rely on fusion mediated by interaction between vesicle (v) and target (t) SNAREs, whereas the homotypic fusion of organelle biogenesis may be mediated by t-SNARE–t-SNARE interaction.

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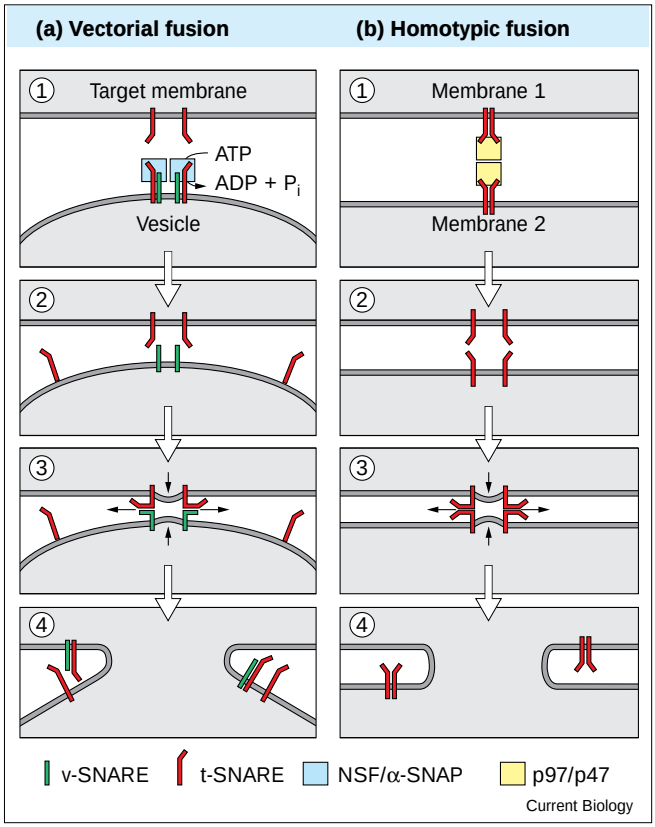
Membrane fusion is involved in the transport of proteins between intracellular organelles, and in organelle inheritance during cell division. For each organelle to retain its unique molecular composition despite extensive membrane flow, these membrane fusion events must be very strictly controlled. The first protein shown to be necessary for vectorial membrane fusion was the cytosolic ATPase *N*-ethylmaleimide-sensitive fusion protein (NSF). NSF associates with membranes via the 'soluble NSF-attachment protein', α-SNAP, which in turn binds to SNAP receptors, or SNAREs. The 'SNARE hypothesis' of Rothman and colleagues [1] proposed that the specific docking of transport vesicles onto their target membrane was mediated through the formation of complexes between cognate vesicle (v) and target (t) SNAREs. Recent *in vitro* evidence has confirmed this hypothesis, and the importance of SNAREs in many membrane fusion events.

Hydrolysis of ATP by bound NSF had been shown to disassemble the SNARE complex [2], and in the original hypothesis this disassembly was assumed to lead to membrane fusion. Recent evidence suggests, however, that NSF is not actually required at the fusion step itself. It is now clear that some vesicles contain not only v-SNAREs, but also t-SNAREs, and that these SNAREs form complexes within the same membrane, which can be disassembled by NSF [3]. Furthermore, in an *in vitro* system that reconstitutes fusion between yeast vacuoles, the requirement for Sec18p, the yeast homologue of NSF, can be fulfiled before the two populations of vacuoles are mixed [4]. It is more likely, therefore, that NSF acts to break apart unproductive SNARE complexes formed within the same membrane, so as to allow assembly of complexes between membranes. In addition to NSF, other molecules, such as the small GTPases of the Rab family and proteins of the Sec1p family, interact with the SNARE complex and appear to modulate the kinetics of complex assembly [5].

If the NSF step is upstream of fusion, are the SNAREs all that is needed for fusion? In the electron microscope, the complex between the v-SNARE synaptobrevin and the t-SNAREs syntaxin and SNAP-25 appears as a uniform rod, with a 'tail' contributed by the amino terminus of syntaxin [6]. Within the rod, the SNAREs adopt a parallel orientation, rather than the anti-parallel orientation depicted in early models [1]. This parallel orientation is consistent

Figure 1



Schematic representations of (a) vectorial and (b) homotypic membrane fusion. In vectorial fusion (a), complexes between v-SNAREs and t-SNAREs in the same membrane are disassembled by NSF/α-SNAP in an ATP-dependent manner (1). In homotypic fusion (b), p97/p47 is assumed to play a similar role in the disassembly of unproductive t-SNARE–t-SNARE complexes, although this remains to be shown experimentally. Disassembled SNAREs are free to form complexes between membranes (2). The 'zipping up' of these parallel complexes pulls the two membranes together (3), and eventually leads to fusion (4). Steps (2–4) in vectorial membrane fusion have now been reconstituted in a defined *in vitro* system [8].

Table 1

Fusion can be mediated by a v-SNARE–t-SNARE complex or a t-SNARE–t-SNARE complex.

Location	Type of fusion	NSF-type protein	Attachment protein	t-SNARE	Other SNARE partner	Reference
Artificial liposomes	Heterotypic	–	–	Syntaxin 1 and SNAP-25	Synaptobrevin (a v-SNARE)	[8]
Golgi–Golgi	Homotypic	NSF	α-SNAP	Syntaxin 5	GOS-28 (a v-SNARE)	[11]
Golgi–Golgi	Homotypic	p97	p47	Syntaxin 5	?	[11]
Golgi–ER	Heterotypic	Sec18p	Sec17p	Ufe1p	Sec22p (a v-SNARE)	[13]
ER–ER	Homotypic	Cdc48p	?	Ufe1p	Ufe1p (a t-SNARE)	[12]

with the formation of SNARE complexes within the same membrane [3], but it also suggests a way in which the 'zipping up' of the SNAREs might physically pull interacting membranes together, with the energy released during complex assembly overcoming the repulsive forces between the membranes (Figure 1). Interestingly, some viral fusion proteins are known to operate in a similar way. For example, influenza haemagglutinin inserts a fusion peptide into the target membrane, so that the protein spans the two membranes. It then adopts a hairpin configuration and pulls the membranes together [7].

In a remarkable set of experiments, Rothman and colleagues [8] have now shown that, when present in separate liposomes, the v-SNARE synaptobrevin and the t-SNAREs syntaxin and SNAP-25 can indeed form complexes, or 'SNAREpins', that link the two types of liposome. The linked liposomes then go on to fuse together when incubated at physiological temperatures. Fusion requires a v-SNARE in one liposome and t-SNAREs in the other, and does not occur when both liposomes contain either v-SNAREs or t-SNAREs, or when either liposome is protein free. As expected from current ideas about its role, NSF is not required for fusion in this stripped-down system (see Table 1 for a summary of the proteins involved in this and other fusion events).

Another important form of membrane fusion, homotypic fusion, occurs during cell division. Organelles such as the Golgi complex and the endoplasmic reticulum (ER) are fragmented and divided equally between the two daughter cells, and then reassembled in the daughter cells by homotypic fusion. In the Golgi, this involves both NSF and p97, an ATPase with sequence similarity to NSF [9]. The *in vitro* reassembly of Golgi cisternae from mitotic Golgi fragments can be catalyzed either by NSF or by p97, and the morphology of the resulting cisternae differs depending on whether NSF or p97 is used. Specifically, NSF generates fenestrated cisternae with attached vesicles, whereas p97 generates unfenestrated, blunt-ended cisternae with no vesicles. Homotypic fusion of ER membranes in yeast, on the other hand, does not require the yeast homologues of either NSF (Sec18p) or α-SNAP (Sec17p), but does require the yeast homologue of p97 (Cdc48p) [10].

Two recent papers [11,12] present evidence that the mechanisms underlying NSF-dependent and p97-dependent membrane fusion might be fundamentally different. Rabouille *et al.* [11] studied the NSF- and p97-mediated pathways of Golgi reassembly *in vitro*. The common element appears to be the t-SNARE syntaxin 5: both pathways were inhibited by addition of either anti-syntaxin 5 antibodies or the cytosolic region of syntaxin 5. The difference comes in the other components. Whereas α-SNAP is required for NSF to bind syntaxin 5, it appears that p47 mediates the binding of p97 to syntaxin 5 (Table 1). Consistent with this model, α-SNAP and p47 competed with each other for binding to syntaxin 5, and α-SNAP inhibited the p97 pathway and p47 inhibited the NSF pathway. Finally, an antibody to GOS-28, a Golgi v-SNARE, inhibited the NSF pathway, but not the p97 pathway. It was proposed that the p97 pathway might proceed either through the interaction of syntaxin 5 with another unidentified v-SNARE or, more likely, through the pairing of syntaxin 5 molecules on different membranes.

Experiments in a similar system in yeast show clear evidence for t-SNARE–t-SNARE-mediated fusion. Patel *et al.* [12] investigated homotypic ER fusion, also *in vitro*, but in yeast extracts. They looked at Ufe1p, which in ER membranes in yeast is known to act as a t-SNARE for retrograde traffic, interacting with the v-SNARE Sec22p to mediate a membrane fusion event that probably also requires Sec18p and Sec17p [13]. But inactivation or removal of the known v-SNAREs had no effect on homotypic ER fusion. Inactivation of an *in vitro* temperature-sensitive Ufe1p did, however, inhibit fusion. Interestingly, this effect was unilateral — inactivation of the protein in only one of the two interacting membranes was sufficient to inhibit fusion. Fusion of wild-type membranes could be inhibited, again unilaterally, by pre-incubation of the membranes with either an antibody against Ufe1p or the cytosolic region of the protein. The interpretation of these results was that Ufe1p in one membrane forms a complex with Ufe1p in the other membrane. It

was also shown that Ufe1p is the likely molecular target of the p97 homologue Cdc48p. For instance, overexpression of Cdc48p rescued growth in mutant strains defective in Ufe1p, and vice versa. Further, an anti-Ufe1p antibody co-immunoprecipitated both Ufe1p and Cdc48p, indicating that the two proteins normally exist complexed to each other.

Consistent with the idea that homotypic membrane fusion can occur through the self-assembly of t-SNAREs, these proteins do form higher-order structures. Patel *et al.* [12] showed that Ufe1p behaves predominantly as a homohexamer, which is stable enough to survive such extreme conditions as treatment with 4 M urea. Rabouille *et al.* [11] presented an image of syntaxin 5 complexed with p97/p47, which is shaped like a hairpin — or, perhaps more appropriately, a SNAREpin — with two syntaxin 5 'legs' dangling from the p97/p47 core. The observation that liposomes containing complexes of the proteins syntaxin and SNAP-25 do not fuse together [8] suggests that these complexes must first be disassembled to permit SNAREpin formation between membranes. Whether p97/p47 is able to disassemble t-SNARE–t-SNARE complexes, in the same way that NSF/α-SNAP disassembles v-SNARE–t-SNARE complexes, is not yet clear. In temperature-sensitive *cdc48* yeast mutants, however, the amount of monomeric Ufe1p is apparently reduced at the restrictive temperature [12], suggesting that Cdc48p might indeed function in this way. How disassembly is controlled is at present completely mysterious.

Why do some membrane fusion events require NSF, while others require p97? The requirements might depend upon which complexes predominate in the undocked membrane, as illustrated in Figure 1. If most of the t-SNARE is complexed with a v-SNARE, then NSF would be required, as in many vectorial fusion events, including synaptic vesicle exocytosis [3] and retrograde transport of vesicles to the ER in yeast, where Ufe1p interacts with the v-SNARE Sec22p [13]. If, on the other hand, the t-SNARE is predominantly self-associated, and does not interact significantly with a v-SNARE, p97 might be required. This appears to be the situation in the yeast ER membrane, where only a small proportion of Ufe1p is complexed with the v-SNARE Sec22p [12]. In the Golgi complex, there is likely to be a physical separation of the v-SNAREs and t-SNAREs, with the t-SNARE syntaxin 5 enriched in the cisternal core and the v-SNARE GOS-28 enriched in the peripheral rim [11]. It might therefore be that p97 is more important in t-SNARE–t-SNARE-dependent fusion of the cores, whereas NSF drives v-SNARE–t-SNARE-dependent fusion at the rims. This physical separation of the two types of fusion could also explain the differences in morphology between Golgi cisternae reconstituted by the NSF and the p97 pathways [9].

As mentioned above, Sec18p is required for homotypic fusion of yeast vacuoles [4], which is most efficient when both membranes contain v-SNAREs and t-SNAREs [14]. Significantly, in this system fusion still occurs when both membranes contain only a t-SNARE, at about half the efficiency seen when one membrane has a v-SNARE and the other a t-SNARE. In contrast, fusion between membranes containing only v-SNAREs is extremely inefficient. As pointed out by Patel *et al.* [12], it would be interesting to determine whether t-SNARE–t-SNARE-dependent fusion of vacuoles is enhanced when the system is supplemented with Cdc48p, as predicted by the model described here.

In both the *cis*-Golgi and the ER, the t-SNARE is able to interact with either a v-SNARE or an identical t-SNARE, and thereby mediate distinct membrane fusion events [11,12]. What determines which partner is chosen by the t-SNARE? It is possible that NSF and p97 generate different conformations of the t-SNARE that are able to bind to either a v-SNARE or another t-SNARE, although there is as yet no evidence for this. Another layer of subtlety might be added by modulation of the activity of p97/Cdc48p in a cell-cycle-dependent manner, possibly by phosphorylation [12]. This would allow the cell to tune the extents of the two types of membrane fusion to its needs at any particular time, allowing vesicle transport to predominate during interphase, and increasing organelle reconstitution after cell division. The relative importance of the two types of fusion might also vary between cells. The NSF pathway might predominate in differentiated secretory cells, controlling protein flux through the Golgi, whereas the p97 pathway might be more important in a dividing tumour cell, where a high rate of organelle biogenesis is required.

The field of membrane fusion is clearly well trodden. Nevertheless, novel proteins that are involved in fusion are still turning up. Transport between the ER and the *cis*-Golgi in yeast is known to require the protein Bet3p [15]. *BET3* interacts genetically with SNAREs involved in this transport step, but Bet3p itself does not form part of the SNARE complex. By immunoprecipitation of Myc-tagged Bet3p from a detergent extract of yeast cells, Sacher *et al.* [16] have now shown that the protein is a member of an 800 kDa complex that they call TRAPP (transport protein particle). They suggest that TRAPP is required for the targeting or fusion of ER-derived transport vesicles with the *cis*-Golgi. TRAPP consists of at least ten proteins, including Bet3p, and at least five of these have human homologues, suggesting that their role is highly conserved. But it is not yet known how, or if, the roles of TRAPP and the NSF/SNARE complexes are related.

The ability of v-SNAREs and t-SNAREs to fuse lipid bilayers together demonstrates the essential validity of the SNARE hypothesis. The challenge for the future is to

incorporate into this basic membrane fusion machinery the many control mechanisms that allow cells to maintain such a varied internal architecture and to pass it on to their progeny. We can be confident that exciting times lie ahead.

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