Membrane fusion as a team effort

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Since the discovery that tetanus and botulinum toxins inhibit synaptic vesicle fusion by cleaving three proteins (synaptobrevin/VAMP, SNAP-25, and syntaxin/HPC) at the synapse, it has been known that these proteins, and, by extension, their many homologs [subsequently referred to as SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors)] are essential for fusion (1–5). The discovery that these three proteins bind to each other (6), and do so in a parallel fashion that forces the membranes in which they reside into close proximity (7, 8), prompted a facile model of fusion: namely, that SNAREs generally form trans complexes that link the two membranes destined to fuse with each other and that the full assembly of the SNARE complexes then forces the membranes into such a close proximity that their phospholipid surfaces disintegrate and reanneal to form a bilayer in which the SNAREs are now in cis (reviewed in ref. 9). Initial experiments using liposome containing reconstituted SNARE proteins supported this model and led to the hypothesis that SNAREs by themselves are sufficient to account for the membrane fusion process, i.e., constitute a minimal fusion machinery (10). However, two lines of evidence raised doubts about this hypothesis. First, members of two other protein families, the SM proteins (Sec1/Munc18-like proteins) and the Rab proteins, were found in many *in vivo* systems to be required for fusion (reviewed in ref. 11). At the synapse, for example, deleting the SM protein Munc18–1 causes a more severe block of fusion than deleting the SNARE proteins synaptobrevin/VAMP or SNAP-25 (12–14). Second, experiments with liposomes containing SNAREs revealed that unphysiologically high concentrations of SNAREs were required for fusion and that the fusion process observed was leaky, i.e., involved breakage of membranes, whereas physiologically, fusion is not leaky (15, 16). These findings demonstrated that SNAREs are not sufficient to account for biological membrane fusion and raised the issue of whether the SNAREs can, in fact, actually initiate fusion, or whether they just bring membranes together in preparation for the subsequent fusogenic activity of SM and/or Rab proteins. The feature article in this issue of PNAS by Starai *et al.* (17) addresses this issue by using yeast vacuole fusion as a model system. The

Fig. 1. Models of membrane fusion catalyzed by an excess of SNAREs in a membrane (Left) or by a team effort between SNAREs, Rab, and SM proteins (Right). SNAREs mediate fusion by forming a complex between R-SNAREs and Q-SNAREs (red and magenta, respectively; reviewed in ref. 11). In the case of the yeast vacuole, the Rab protein is Ypt7p (green), and the SM protein Vps33 is part of HOPS (blue). Both types of membrane fusion involve formation of trans-SNARE complexes between the two membranes, which, in turn, requires activation of SNAREs by Sec17p and Sec18p in yeast and soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment proteins (SNAPs) and NSF in animals (data not shown). The two types of fusion differ, however, in the organization and effectiveness of the SNARE complexes, which are disordered in SNARE-only fusion, leading to lysis of membranes in addition to fusion, but are orchestrated into a single fusion machine in the case of the physiological SNARE/Rab/SM protein fusion.

article elegantly demonstrates that SNARE proteins acts as the motor of fusion as first shown by Weber *et al.* (10) but that control of this motor by a Rab and SM protein is required for fusion. This requirement is not simply regulatory and cannot be overcome by brute force (i.e., by increasing the concentration of SNARE proteins), but involves Rab and SM proteins as essential organizers of the fusion reaction. Thus, these experiments compare in a biologically relevant *in vitro* system “SNARE-only fusion” with fusion mediated by SNARE, Rab, and SM proteins (Fig. 1).

Extensive previous studies from the Wickner laboratory had demonstrated that fusion of yeast vacuoles requires not only SNARE proteins but also the Rab protein Ypt7p and the SM protein Vps33p that is present in a high-molecular-weight complex called HOPS (homotypic

fusion and vacuole protein sorting complex; reviewed in ref. 18). The yeast vacuole is a suitable system for studying membrane fusion because it constitutes an *in vitro* system that allows direct manipulation of its components but at the same time makes it possible to correlate a particular *in vitro* result with an *in vivo* function in a whole cell. Starai *et al.* (17) ask whether vacuole fusion, which normally depends on the HOPS and Ypt7p in addition to SNARE proteins, can be rendered Ypt7p- and/or HOPS-independent by increasing the levels of SNARE proteins, and how vacuole fusion under various

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conditions relates to vacuole lysis. Starai *et al.* start by modifying the *in vitro* vacuole fusion assay in a way that allows measuring not only the mixing of the contents of the two vacuoles but also their lysis, and they demonstrate that SNARE/Rab/SM protein-driven fusion does not involve lysis. They then show that the simple addition of an excess of a single SNARE protein causes vacuoles to lyse during the fusion reaction, or more impressively, the same effect can be achieved by overexpression of all four SNAREs involved in vacuole fusion. Importantly, fusion of vacuoles containing overexpressed SNARE proteins was independent of the Rab protein Ypt7p but needed to be activated either with Sec18p [the yeast homolog of *N*-ethylmaleimide-sensitive factor that is involved in activating SNARE proteins by dissociating non-productive SNARE complexes (19)] or with HOPS.

The importance of the study by Starai *et al.* (17) is several-fold. First, it bridges the gap between purely *in vitro* liposome fusion studies using reconstituted

SNAREs and genetic studies on membrane fusion. Second, it directly compares fusion and lysis in a biologically

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meaningful system, demonstrating that fusion is physiologically nonleaky. Overall, these results provide strong support for the notion that SNARE proteins are indeed the motor of membrane fusion, driving the energetics of the reaction (11), but that the SNAREs by themselves are powerful but hapless, unable to channel the energy released by their complex formation, and need to be

organized by Rab and SM proteins. Finally, the results from Starai *et al.* continue an important string of results obtained in previous studies from the Wickner laboratory emphasizing the importance of the supramolecular organization of the fusion machine, the positioning of this machine into vertices at the edge of the contacts between the membranes of the two fusing membranes, and the partitioning of specific lipids around the fusion site, for the overall fusion reaction.

Although this study provides a significant advance in our understanding of biological fusion, it also poses new questions that can be productively addressed in particular with the vacuole system, because this system allows such a beautiful coupling of genetics with *in vitro* assays. Among these questions, the most important probably is: what is the exact role of the Rab and SM proteins in organizing fusion and how conserved is this role among different intracellular fusion reactions, given the uniform requirement of these proteins for most fusion reactions? Stay tuned.

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