COMMENTARY

What are the roles of V-ATPases in membrane fusion?

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At the dawn of the 20th century, Metchnikoff (1) fed particles of litmus to various eukaryotic cells. The endocytosed particles changed color from blue to red, indicating their residence in acidifying endosomes. This acidification is driven by a highly conserved multisubunit H^+ pump, the vacuolar ATPase (V-ATPase). Acidification activates luminal hydrolases, and V-ATPase–generated pH and voltage gradients (ΔpH and ΔΨ) are harnessed to transport solutes (including neurotransmitters) into the lumen (2). However, the V-ATPase is also proposed to regulate or even execute membrane fusion events by generating electrochemical gradients or as a structural component of the fusion machinery. The V-ATPase is also proposed to signal to TORC1, a core metabolic regulator, and it has roles in stress and cellular aging (3–5). This complexity has made it hard to resolve how V-ATPases might function in fusion. Now, Sreelatha et al. (6) report studies of yeast lysosomal vacuoles and VopQ, a type III effector secreted by *Vibrio parahaemolyticus*. VopQ binds the V-ATPase, collapses ΔpH and ΔΨ, and inhibits fusion. Clostridial neurotoxins were key tools in the quest to understand the roles of SNARE proteins in fusion. Similarly, VopQ could aid ongoing efforts to delineate V-ATPase functions in membrane traffic and fusion.

Most fusion in eukaryotes is driven by SNAREs and their cofactors (7). Studies in vivo, and with minimal in vitro systems, revealed SNAREs and SNARE cofactors as necessary and sufficient to drive fusion (8, 9). In minimal assays, neither V-ATPase nor electrochemical gradients are needed. In contrast, genetic studies in several organisms suggest V-ATPase functions in traffic or fusion. Like the mitochondrial F-ATPase, the V-ATPase is shaped like a mushroom (2). There are two subcomplexes: V_1 and V_0. V_1, the "cap," comprises the sites of ATP hydrolysis; V_1 sits atop the V_0 "stalk," which conducts H^+ ions. V_0 consists of six small hydrophobic subunits (proteolipids) and a large subunit, V100.

Proton Pumping and Membrane Fusion?

Impaired V-ATPase activity causes defects in membrane traffic, and early studies suggested that ΔpH or ΔΨ promote fusion of yeast lysosomal vacuoles (10). Sreelatha et al. (6) provide the most compelling evidence to date that vacuoles fuse without ΔpH or ΔΨ. VopQ causes accumulation of undigested autolysosomes in mammalian cells and fragmentation of the yeast vacuole (11–13), implying a fusion block. VopQ forms a 40 Å membrane pore that collapses ΔpH and ΔΨ (6, 14), and Sreelatha et al. (6) identify a VopQ mutant that collapses ΔpH and ΔΨ without substantially inhibiting fusion. Treatments with ionophores and ammonium also show efficient fusion when ΔpH and ΔΨ are collapsed (6). Thus, ΔpH and ΔΨ are dispensible for fusion per se, and VopQ inhibits fusion through a mechanism entailing activities other than pore formation. VopQ binds V_0 directly, and VopQ does not arrest SNARE-mediated fusion of liposomes lacking V_0. Hence, VopQ inhibits fusion only when V_0 is present.

Sreelatha et al. report studies of yeast lysosomal vacuoles and VopQ, a type III effector secreted by *Vibrio parahaemolyticus*.

In contrast, Coonrod et al. (15) argue that ΔpH or ΔΨ promote homotypic vacuole fusion and challenge the hypothesis that the V-ATPase has a structural role in fusion. Their approaches include a yeast mating assay in which homotypic fusion is monitored by maturation of a vacuolar hydrolase (proPho8). Here, V-ATPase (and V_0 specifically) could be replaced by a pyrophosphate-driven plant H^+ transporter (AVP1) with no evolutionary similarity to V_0. However, AVP1, even at high levels, only partially rescues V_0 deletion mutants. In some experiments, V_0 subunits were depleted through repressible promoters, raising the possibility that tiny numbers of V_0 subunits still drive fusion. Finally, the assay design should result in dilution of the ALP reporter, potentially harming the signal to background ratio. Nevertheless, in a critical control, *nyv1* mutant vacuole pairs (which cannot fuse homotypically) had the expected defect. If ΔpH or ΔΨ influence fusion in vivo, they apparently do so through mechanisms not recapitulated with isolated native vacuoles. ΔpH or ΔΨ seem to be involved in vacuolar fission (vs. fusion), and could control a wide array of other regulatory events. It remains unclear how ΔpH and ΔΨ shape organelle morphology.

Structural Roles of V_0 in Membrane Fusion?

SNARE binding to V_0 was first reported in 1996 (16). In parallel, Mayer's group reported a role for Ca^{2+}-bound calmodulin (Cmd) in yeast vacuole fusion. In screens for targets, they found that Ca^{2+}-Cmd bound V_1 and V_0 subunits (17). During docking, V_0 subunits on opposite membranes appeared to interact *in trans* with one another, as well as with SNAREs, in a reaction contingent on *trans*-SNARE complex formation. Peters et al. (17) proposed that V_0 complexes form a channel between docked membranes. The channel would initiate a pore that then dilates through lipid infiltration between the proteolipid subunits (17, 18).

This model has some appealing features. First, it might explain "flickering" fusion pores and kiss-and-run fusion events (7). Second, if V_0 does indeed function in vivo as a gated pore, its function may be conserved: the analogous F_0 subcomplex of the mitochondrial F-ATPase was recently proposed to be the Ca^{2+}-triggered apoptotic inner membrane permeability transition pore (19, 20). However, several features

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of this model require revision. First, the Cmd1-3 mutant used in initial studies (21) was inappropriate to test a Ca^{2+} requirement, as it exhibits strong pleiotropic defects. Exhaustive studies show that only the Cmd1-6 mutant is appropriate for testing a Cmd Ca^{2+} requirement (22, 23). Second, the fusion phenotype of the Cmd1-3 mutant is contested, with a newer study, indicating no defect in fusion (21, 24). Third, neither a ΔCa^{2+} gradient nor Ca^{2+} at the cytosolic face of the vacuole membrane is needed for fusion (6, 24, 25).

The role of V_0 in fusion is unresolved. VopQ blocks fusion at an early stage, either before or during docking (6). If VopQ indeed inhibits fusion through its effects on V_0, the results of Sreelatha et al. (6) are difficult to reconcile with models where V_0 acts late (17). Screens in *Drosophila* revealed neurotransmission and trafficking defects when the V_0 subunit V100 was mutated (26). In these mutants, H^+ pumping was partially intact, and V100 interactions were observed with syntaxin SNAREs (26, 27). More recently, biochemical experiments indicated that the *Drosophila* V100 binds syntaxin, inhibiting its function (28). In a working model, Ca^{2+}-Cmd relieves V100-syntaxin inhibition, allowing SNAREs to complex. Similarly, the mammalian SNARE synaptobrevin is reported to interact with V_0 proteolipid and Cmd (29). V100 is linked to insulin secretion in mice, and to phagolysosomal disposal of dead cells in fish (30).

The fusion-related phenotypes of V_0 or V_1 mutants often diverge, as in genetic analyses of apical epithelial secretion of multivesicular bodies in *Caenorhabditis elegans* (31). Do fusion defects in V_0 mutants reflect a structural role in fusion, or do they reflect subtle defects in electrogenic activities? In several of the above studies, and in experiments with yeast proteolipid mutants (18), fusion was impaired in cases where H^+ pumping still occurred. However, most of these pH assays are semiquantitative or qualitative, and $\Delta\Psi$ is seldom assessed. A final question is whether V_0 phenotypes reflect direct losses of function or regulatory responses. Metabolic signals control reversible V_1 dissociation (2). $PI(3,5)P_2$, the product of the lipid kinase Fab1, causes vacuolar fragmentation, by opposing fusion or stimulating fission. $PI(3,5)P_2$ triggers assembly of active V-ATPase (32), which raises the possibility that $PI(3,5)P_2$ inhibits fusion by reducing the amount of unpaired V_0. A century after Metchnikoff, V-ATPases and the concentration gradients they generate continue to pose critical and unresolved questions for cell biologists. Vigorous scrutiny of experimental results and controls, close attention to $\Delta\Psi$ and ΔpH, and new tools like VopQ will propel the field into its second century.

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