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A new life for an old pump: V-ATPase and neurotransmitter release

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Neurons fire by releasing neurotransmitters via fusion of synaptic vesicles with the plasma membrane. Fusion can be evoked by an incoming signal from a preceding neuron or can occur spontaneously. Synaptic vesicle fusion requires the formation of trans complexes between SNAREs as well as Ca^{2+} ions. Wang et al. (2014. *J. Cell Biol.* http://dx.doi.org/jcb.201312109) now find that the Ca^{2+}-binding protein Calmodulin promotes spontaneous release and SNARE complex formation via its interaction with the V_0 sector of the V-ATPase.

Evoked release of synaptic vesicles occurs after an action potential (or series of action potentials), whereas spontaneous release occurs in the absence of a presynaptic action potential (Kochubey et al., 2011). Both forms of release require the formation of trans complexes between SNARE ("soluble NSF attachment receptor") proteins, one in the vesicle and the other anchored in the plasma membrane. SNARE proteins exist in two cognate forms: R-SNAREs (e.g., Synaptobrevin, which is located in the synaptic vesicles) and Q-SNAREs, which can contain up to three subunits (e.g., Syntaxin1A and SNAP-25, located in the plasma membrane). Both forms of neurotransmitter release also require Ca^{2+} ions. Synaptotagmins act as a Ca^{2+} sensor for evoked release. Little is known about how the Ca^{2+} dependence of spontaneous release is generated.

The V-ATPase is a proton pump consisting of two subcomplexes that can reversibly dissociate from each other: The membrane-integral V_0 sector and the peripheral V_1 sector, which carries the ATPase activity. V_0 contains a cylinder of proteolipids (consisting of highly homologous isoforms of subunit c); the large, membrane-integral subunit a; and the peripheral subunit d and subunit e (Fig. 1 A). V-ATPase provides the electrochemical potential that contributes to loading of the secretory vesicles with neurotransmitters. V-ATPase–dependent lumenal acidification can influence protein trafficking, e.g., between endolysosomal compartments (Wada et al., 2008; Huotari and Helenius, 2011). In addition, in vivo evidence points to a physical role of V_0 in exocytosis and membrane fusion, which is independent of proton pumping. Observations that have uncovered a function for V_0 in exocytosis and membrane fusion stem from regulated secretion in both *Drosophila melanogaster* (Hiesinger et al., 2005) and mammalian cells (Sun-Wada et al., 2006; Di Giovanni et al., 2010), secretion of multivesicular bodies in *Caenorhabditis elegans* (Liégeois et al., 2006), phagosome–lysosome fusion in the zebrafish *Danio rerio* (Peri and Nüsslein-Volhard, 2008), and vacuole fusion in yeast (Bayer et al., 2003; Strasser et al., 2011). A recent study challenged a physical role for V_0 in vacuole fusion in vivo (Coonrod et al., 2013); however, the main assay in that study suffered from a conceptual flaw, i.e., it could not measure vacuole fusion but only biosynthetic transport of the indicator proteins to the vacuoles. At the same time, another study provided a compelling in vivo demonstration that a lack of vesicle acidification impairs exocytosis, but that it does so by reducing the pool of free V_0 sectors and thus impeding their physical function in the fusion process (Poëa-Guyon et al., 2013).

V_0 subunits interact with Calmodulin (Peters et al., 2001; Zhang et al., 2008) and with Q- and R-SNAREs (Galli et al., 1996; Peters et al., 2001; Takeda et al., 2008; Di Giovanni et al., 2010). In this issue, Wang et al. provide compelling evidence that Calmodulin regulates SNARE complex assembly via V_0. They used the V_0 subunit a from *Drosophila* (v100) with point mutations in its Calmodulin binding site to selectively disrupt the interaction of Calmodulin and v100. This allele ($v100^{WFI}$) rescues most defects resulting from the loss of v100 (Hiesinger et al., 2005; Williamson et al., 2010), notably endolysosomal acidification and endolysosomal protein sorting. Thus, $v100^{WFI}$ retains its functionality as part of the V-ATPase proton pump. $v100^{WFI}$ rescues evoked neurotransmitter release but it diminishes spontaneous transmitter release by >90%. An extensive biochemical characterization revealed that v100 disrupts the assembly of Q-SNARE complexes by competitively binding to Syntaxin1A and SNAP-25. Ca^{2+}–Calmodulin can disrupt the competitive interactions of v100 with the SNAREs, permit the Q-SNAREs to form a complex, and also incorporate the R-SNARE VAMP and catalyze fusion. These results indicate that v100, when associated with Calmodulin, can serve as a regulator of SNARE complex formation (Fig. 1 B).

This finding adds a novel aspect to the role of V_0 in exocytosis. However, other known effects and interactions of V_0 subunits illustrate that regulating SNARE complex formation cannot be the only way in which V_0 influences membrane fusion and

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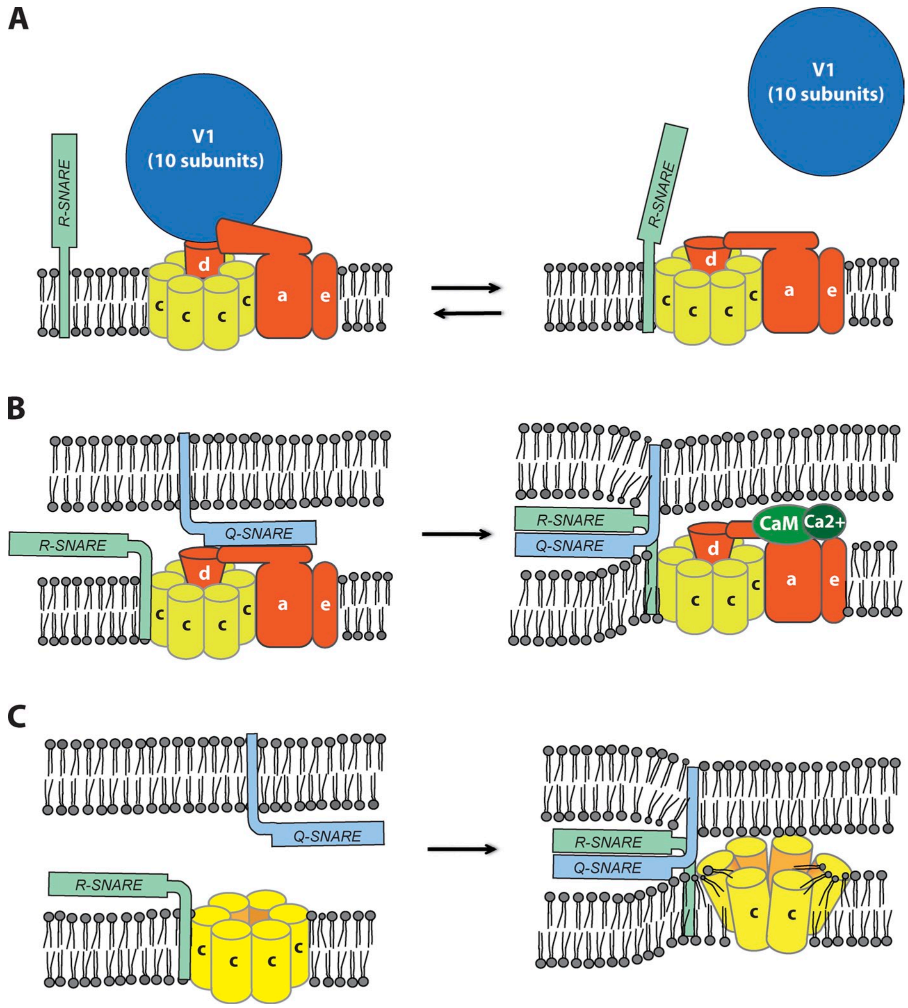

Figure 1. **The V-ATPase V_0 sector affects trans-SNARE pairing and lipid mixing via its subunits a and c.** (A) The V-ATPase is composed of a peripheral sector V_1 (blue, 10 subunits) and a membrane integral sector V_0 (red and yellow). V_0 contains a cylinder of proteolipids (subunits c, yellow), the proton-conducting subunit a, and subunits d and e (red). V_1 and V_0 can dissociate from each other in a regulated and reversible fashion. This equilibrium can be influenced by SNAREs. B and C show two roles of V_0 in membrane fusion, which are not mutually exclusive. The emphasis is on subunit a or subunits c, respectively. (B) Regulation of SNARE complex formation by subunit a. Binding of subunit a to Ca^{2+}–Calmodulin (CaM, green) alleviates the block of SNARE complex assembly caused by the interaction of subunit a with the Q-SNARE. (C) Model of how proteolipids (yellow) might enhance the capacity of SNAREs to stimulate lipid mixing. Stimulated by their interaction with trans-SNARE complexes, the proteolipids undergo a conformational change, which might expose hydrophobic surfaces between the proteolipid subunits and facilitate lipid reorientation and membrane fusion (Strasser et al., 2011). V_0 subunits e and d are not shown because there are no data implicating them in fusion. V_0 subunit a is not shown for the sake of clarity.

exocytosis. If V_0 subunit a were only a negative regulator of SNARE complex formation, as suggested by the results of Wang et al. (2014), its deletion should leave fusion intact or even stimulate it. The opposite is the case, which suggests that V_0 also serves to promote fusion. This is also evident from the effects of mutations in other V_0 subunits, such as the central ring of proteolipids (subunit c), which interfere with fusion. Proteolipid rings can adopt at least two conformations (Clare et al., 2006) and they can form Ca^{2+}-inducible pores in the membrane that are permeable to hydrophilic molecules (Morel, 2003). Single amino acid substitutions in proteolipid transmembrane domains and proteolipid fusion proteins impede lipid mixing, but, unlike $v100^{WFI}$, they permit the formation of normal levels of trans-SNARE complexes (Strasser et al., 2011). These effects could only be explained by a conformational change in V_0. Therefore, the current working model postulates that V_0 proteolipids can exist in at least two conformations: one conductive to fusion and one supporting V-ATPase assembly and proton pumping (Strasser et al., 2011; Poëa-Guyon et al., 2013).

According to this model, the molecular interactions of V_0 with SNAREs (Peters et al., 2001; Di Giovanni et al., 2010) might influence V_0 conformation and/or compete with the attachment of V_1 to V_0 (Fig. 1 A). The recruitment of V_0 into the V_1–V_0 V-ATPase holoenzyme can inhibit exocytosis by depleting the pool of free V_0 sectors that are necessary to support fusion (Poëa-Guyon et al., 2013). Experimental observations from yeast support a role for SNAREs in regulating this pool of free V_0: Deletion of the vacuolar R-SNARE NYV1 (a Synaptobrevin homologue in yeast) increases V_0–V_1 association, and NYV1 overexpression reduces it (Strasser et al., 2011). Furthermore, structural data suggest that there is substantial space between the subunits of the proteolipid cylinder that might be invaded by lipids (Clare et al., 2006). These data gave rise to a hypothesis explaining how V_0 might promote membrane fusion. In this hypothesis, it is assumed that SNAREs destabilize the V_0–V_1 interaction and favor a V_0 conformation that supports fusion by allowing lipids to invade the space between subunits of the proteolipid cylinder, facilitate their reorientation, and thereby promote the merger of the membrane leaflets (Fig. 1 C). The role of v100 in SNARE complex assembly that Wang et al. (2014) describe is not mutually exclusive with a role of V_0 in lipid reorientation (Fig. 1, B and C). It adds an interesting new aspect of V_0 function and suggests that V_0 is intimately linked with the membrane fusion apparatus. This illustrates that much remains to be discovered before we will fully understand the mechanisms by which V_0 subunits regulate and promote the different stages of the fusion process.

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