

The vesicular ATPase: A missing link between acidification and exocytosis

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The vesicular adenosine triphosphatase (ATPase) acidifies intracellular compartments, including synaptic vesicles and secretory granules. A controversy about a second function of this ATPase in exocytosis has been fuelled by questions about multiple putative roles of acidification in the exocytic process. Now, Poëa-Guyon et al. (2013). *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201303104> present new evidence that the vesicular ATPase performs separate acidification and exocytosis roles and propose a mechanism for how these two functions are causally linked.

Vesicular H⁺-ATPases (V-ATPases) function as ATP-driven proton pumps in intracellular compartments, such as endosomes, Golgi-derived vesicles, secretory vesicles, synaptic vesicles, lysosomes, and vacuoles (Forgac, 2007). Acidification is important for a plethora of cell biological processes ranging from endosomal ligand–receptor dissociation to lysosomal degradation (Yan et al., 2009; Williamson et al., 2010; Zoncu et al., 2011). Consequently, interfering with V-ATPase function leads to direct and indirect defects that are difficult to tease apart. In addition, acidification-independent roles of the V-ATPase in secretion and membrane fusion have been proposed (Israël et al., 1986; Peters et al., 2001; Morel et al., 2003; Hiesinger et al., 2005; Liégeois et al., 2006; Sun-Wada et al., 2006; Peri and Nüsslein-Volhard, 2008). The difficulty to distinguish consequences of an acidification-independent mechanism from indirect effects of acidification defects is exacerbated by an unclear dependence of secretion on acidification (Cousin and Nicholls, 1997; Ungerermann et al., 1999; Hiesinger et al., 2005).

In synaptic vesicles, the V-ATPase generates a proton gradient that is used by an antiporter to fill synaptic vesicles with neurotransmitter. Hence, loss of acidification leads to “empty” synaptic vesicles and loss of neurotransmitter release. Can such vesicles still fuse and thereby “shoot blanks”? The V-ATPase comprises of two sectors that can reversibly dissociate: the cytosolic V1 sector and the membrane-bound V0 sector. Loss of the neuronal a1 subunit of the V0 sector (V0a1) leads to almost complete loss of neurotransmission in *Drosophila melanogaster*—a phenotype that may result from a defect in neurotransmitter loading or exocytosis (Hiesinger et al., 2005). Single vesicle

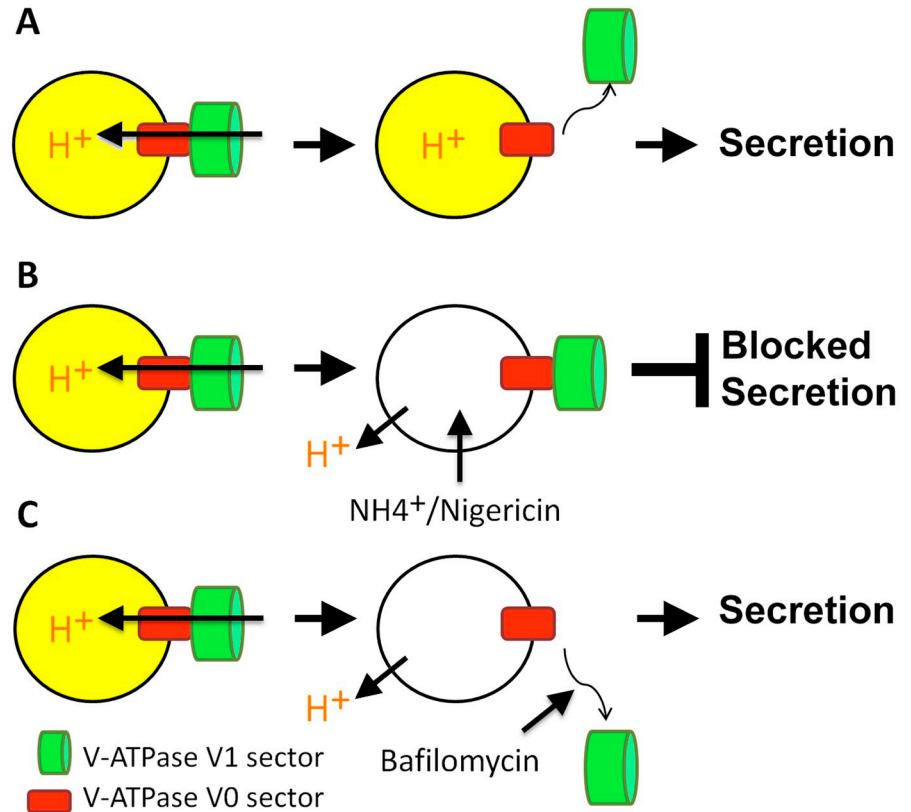
release events in the V0a1 mutant revealed a quantal postsynaptic response, suggesting that at least some vesicles are loaded. In addition, loss of V0a1 impairs synaptic vesicle cycling in an FM1-43 dye uptake assay, whereas pharmacological block of the V-ATPase with bafilomycin causes no significant defect in this assay (Hiesinger et al., 2005). These findings supported previous studies of an acidification-independent role of the yeast V0 sector and specifically the V0a1 orthologue vph1 in vacuole fusion (Peters et al., 2001; Bayer et al., 2003). They also support earlier controversial implications of the V-ATPase V0 sector in neurotransmitter release (Israël et al., 1986). More recently, numerous studies have added evidence in worm, fish, fly, and mouse for possible acidification-independent roles of various V-ATPase V0 subunits in secretion or membrane fusion (Bayer et al., 2003; Lee et al., 2006; Liégeois et al., 2006; Sun-Wada et al., 2006; Peri and Nüsslein-Volhard, 2008; Di Giovanni et al., 2010; Williamson et al., 2010; Strasser et al., 2011). However, questions about the relationship of V-ATPase-dependent acidification and the observed secretion or membrane fusion defects remained. How can one cleanly separate between two protein functions if one potentially depends on the other? The study of V0a1 has been complicated by the finding that it is not only a synaptic vesicle protein but also localizes to other organelles. Consequently, specific disruption of the acidification function of V0a1 led to endolysosomal acidification defects, even though it partially restored neurotransmission (Williamson et al., 2010). A better dissection of the two possible functions is needed.

In this issue of *JCB*, Poëa-Guyon et al. provide compelling evidence for two separable functions of V0a1 in acidification and exocytosis. Instead of a genetic dissection, they opted for an elegant temporal dissection with the idea that acute inactivation of a function of V0a1 in exocytosis should instantly block neurotransmission, whereas acute inactivation of the proton pump should leave neurotransmission functional as long as loaded vesicles are available. Indeed, Poëa-Guyon et al. (2013) found a fast disruption of secretion in both primary rat neuronal culture and chromaffin cells when they acutely inactivated V0a1 using chromophore-assisted light inactivation. In contrast, inactivation of the reversibly associated V1 sector revealed very different effects than what would be expected from a loss of the proton pump.

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Figure 1. **V-ATPase V0–V1 association blocks secretion.** (A) Poëa-Guyon et al. (2013) suggest that dissociation of V0 (red boxes) and V1 (green cylinders) sectors follows vesicle acidification (yellow) and frees the V0 sector for an acute, acidification-independent function in secretion. (B) V-ATPase-independent pharmacological disruption of vesicular acidification causes increased V0–V1 assembly of the functional proton pump, which in turn blocks secretion. (C) Pharmacological disruption of the V-ATPase disrupts both vesicle acidification and V0–V1 assembly, thereby permitting V0-dependent secretion. This mechanism can override disruption of acidification shown in B and restores secretion of nonacidified vesicles.



How about the dependence of exocytosis on acidification? Poëa-Guyon et al. (2013) developed an assay based on granule exocytosis in neurosecretory PC12 cells. Compartmental proton gradients can be abolished by a variety of means, including pharmacological inhibition of the V-ATPase with bafilomycin or concanamycin. A more acute destruction of intracompartamental proton gradients can be achieved through addition of alkalinizing ammonium chloride or the potassium ionophore nigericin, which exchanges intracompartamental protons with potassium ions. Interestingly, Poëa-Guyon et al. (2013) found that only the acute disruption of the intracompartamental proton gradients with ammonium chloride or nigericin leads to an impairment of secretion but not block of the V-ATPase. What happens to the V-ATPase and its acidification-independent function under these different conditions? The authors show that both ammonium chloride and nigericin not only abolish the proton gradient but also lead to increased association of the V0 and V1 sectors (Fig. 1). This association is required to form a functional pump. A straightforward explanation for this observation is that the cell attempts to activate the proton pump to reacidify vesicles that lost their proton gradient. In contrast, the pharmacological block of the V-ATPase itself leads to increased free V0 sectors, consistent with loss of proton pump function. In a key experiment, Poëa-Guyon et al. (2013) show that this pharmacological inhibition of the V-ATPase with bafilomycin can override the effects of ammonium chloride or nigericin and restore secretion. If the pharmacological block of the V-ATPase prevents V0–V1 association, no functional pumps assemble even in the presence of ammonium chloride or nigericin. This result

implies that the V0–V1 association itself prevents exocytosis. Pharmacological V0–V1 dissociation seems sufficient to expose V0 and exert a V1-independent function in exocytosis. This conclusion is consistent with previous findings in which the same pharmacological inhibition of the V-ATPase was found to leave exocytosis and endocytosis intact (Cousin and Nicholls, 1997; Hiesinger et al., 2005). However, the interpretation of the data changes: according to the new findings, exocytosis does not depend on vesicle acidification, per se, but on V0–V1 association that results from lack of acidification. Acidification and V-ATPase assembly thereby become a checkpoint for vesicle loading, and the assembled V-ATPase becomes a no-go signal for fusion (Fig. 1). The model is elegant and leads Poëa-Guyon et al. (2013) to suggest the V-ATPase as an acidification sensor, similar to previous observations (Hurtado-Lorenzo et al., 2006). However, whether it is really the V-ATPase itself that senses the proton gradient is not directly assessed in this study.

How general is the V-ATPase checkpoint, and what is the mechanism of V0-mediated, acidification-independent exocytosis? Both questions remain unanswered. The checkpoint idea is beautiful and does not obviously contradict current ideas on exocytic regulation. However, potential mechanisms for V0-mediated membrane fusion remain controversial (Saw et al., 2011; Ernstrom et al., 2012). In yeast, V0 proteolipid expansion in the membrane has been proposed to play a direct role in lipid mixing during vacuole fusion based on a thorough genetic dissection of fusion and acidification functions of the V0 sector (Strasser et al., 2011). No such role has hitherto been shown for neurotransmitter release, which comprises numerous different

forms of vesicle release that are differentially regulated. A better genetic or pharmacological dissection is needed to reveal when, where, and how V0 meddles with membrane fusion.

We would like to thank all members of the Hiesinger laboratory for discussion.

Work in our laboratory is supported by grants from the National Institutes of Health (RO1EY018884) and the Welch Foundation to P.R. Hiesinger (I-1657). P.R. Hiesinger is a Eugene McDermott Scholar in Biomedical Research.

Submitted: 25 September 2013

Accepted: 8 October 2013

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