

The acid test of v-ATPase function

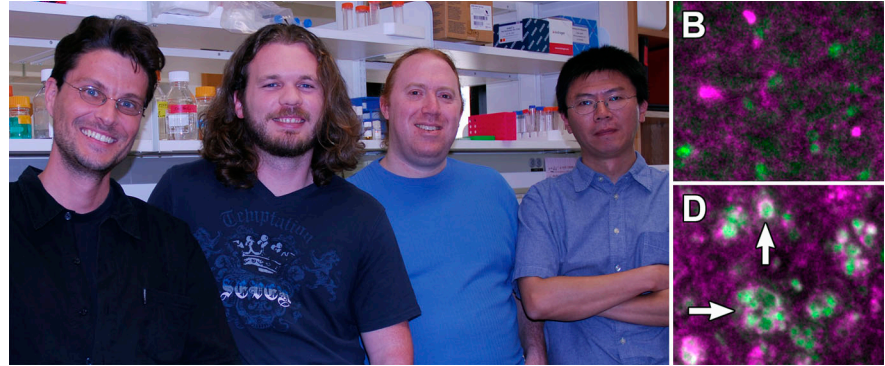
The vesicular protein complex has a dual role in sorting and degrading neuronal proteins.

Many neurodegenerative diseases are linked to defects in the membrane transport pathways that deliver proteins to lysosomes for degradation. Problems with autophagy or endosomal trafficking lead to the accumulation of toxic proteins and neuronal death. Williamson et al. (1) uncover a neuron-specific degradation pathway that relies on two distinct functions of the vesicular (v)-ATPase—a multi-subunit complex whose role in membrane trafficking has long been controversial.

The v-ATPase is best known for pumping protons across membranes to acidify intracellular compartments (2). But the complex has also been proposed to promote membrane fusion between various organelles, including yeast vacuoles (3) and zebrafish phagosomes and lysosomes (4). In 2005, Robin Hiesinger—then a postdoc with Hugo Bellen, now with his own lab at UT Southwestern Medical Center in Dallas—discovered that a neuron-specific subunit of the v-ATPase called *v100* is required for synaptic vesicle secretion by *Drosophila* neurons (5). Synaptic vesicles in *v100* mutants are loaded with neurotransmitter, a process that requires vesicle acidification, suggesting that this function of the v-ATPase doesn't involve proton translocation.

To dissect the acidification-dependent and -independent functions of the v-ATPase in neurons, Hiesinger and colleagues rescued *v100* mutant flies with a version of the protein unable to pump protons (1). Pump-deficient *v100* successfully restored synaptic vesicle exocytosis and neurotransmission, but Williamson et al. were surprised to see a completely new effect that they hadn't observed in flies lacking *v100* completely: the “rescued” neurons were unhealthy and prone to cell death. Yet the same acidification mutant didn't cause neurodegeneration in wild-type flies.

“It's inexplicable from a geneticist's point of view,” Hiesinger says. “A partial



(Left to right) Robin Hiesinger, Ryan Williamson, Adam Haberman, and Dong Wang describe a dual function for the vesicular ATPase in a neuron-specific degradation process. The complex sorts proteins into the pathway by promoting vesicle fusion with early endosomes, and then degrades them by acidifying the endosomes as they mature into degradative lysosomes. When this second step is blocked by selectively removing the v-ATPase's proton translocation function (bottom), neuronal proteins like choptin (green) accumulate in immature endosomes (purple), damaging fly neurons and triggering neurodegeneration.

loss-of-function mutant shouldn't cause a novel phenotype. The only way to understand it was to look at the cell biology.”

A closer look at *v100*'s behavior in neurons revealed that the protein promotes vesicle delivery to early endosomes as well as synaptic vesicle exocytosis. Just like synaptic vesicles, early endosomes retained a normal pH in the absence of *v100*, but they failed to become more acidic as they matured into late endosomes and lysosomes, restricting the organelles' degradative capacity. “So we think the v-ATPase has a dual function in the endo-lysosomal system of neurons,” Hiesinger explains. “First it sorts proteins into the early endosomes and then it becomes a pump to help the endosomes mature into degradative compartments.”

The pump-deficient version of *v100* blocks this second step, causing proteins sorted into the endosomes by the v-ATPase to accumulate, damaging the mutant neurons. This fatal buildup is slowed when *v100* is completely absent because proteins aren't sorted into this degradative pathway in the first place. Instead, they are probably consumed

by the regular endolysosomal pathway, which is common to all cell types and still exists in neurons lacking *v100*.

“*v100* is only required in neurons and may represent an additional, specialized part of the neuronal endolysosomal machinery,” Hiesinger says. “It's intriguing when you consider that many neurodegenerative diseases are linked to degradation defects.”

The dual function of the v-ATPase is an ideal way to coordinate endosomal sorting and maturation. But how does the complex deliver cargo into the pathway? Williamson et al. found that *v100* binds syntaxin7, a SNARE protein that controls the fusion of vesicles with early endosomes. *v100* also binds the plasma membrane SNARE syntaxin1, which fits with the v-ATPase's involvement in synaptic vesicle secretion. “We think it increases the fusion probability of vesicles with different target membranes marked by specific syntaxins,” Hiesinger says. “We now need to find out exactly how it does that.”

1. Williamson, W.R. et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003062.
2. Nishi, T., and M. Forgac. 2002. *Nat. Rev. Mol. Cell Biol.* 3:94–103.
3. Peters, C., et al. 2001. *Nature.* 409:581–588.
4. Peri, F., and C. Nusslein-Volhard. 2008. *Cell.* 133:916–927.
5. Hiesinger, P.R., et al. 2005. *Cell.* 121:607–620.

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