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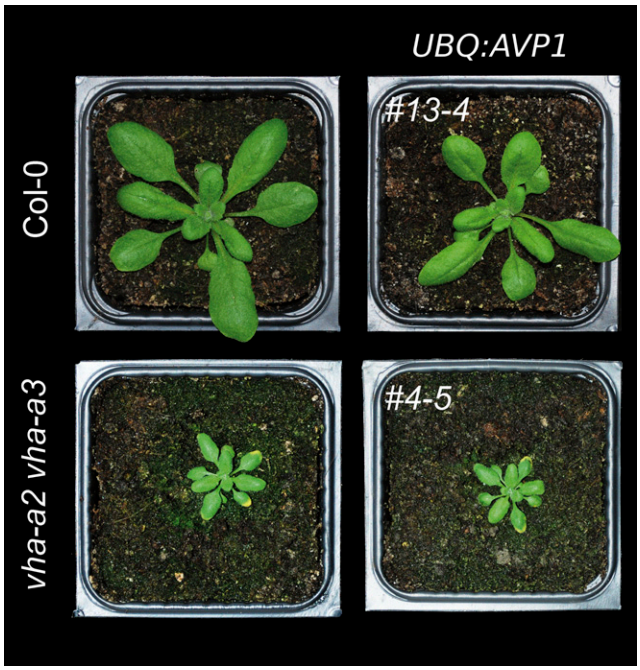
IN BRIEF

A TGN/EE-Localized V-ATPase Contributes to Vacuolar Acidification

Mature plant cells are characterized by a large tonoplast-bound central vacuole that detoxifies harmful substances and stores ions and metabolites. Vacuolar function depends on massive fluxes of molecules across the tonoplast. These fluxes are energized by two types of proton pumps, vacuolar H^+-pyrophosphatases (V-PPases) and H^+-ATPases (V-ATPases). Whereas V-PPases are homodimers of a single polypeptide, V-ATPases are multisubunit structures consisting of a V_1 subcomplex, which catalyzes ATP hydrolysis, and a V_0 subcomplex, which translocates protons across the membrane. *Arabidopsis thaliana* contains three isoforms of the V_0 subunit VHA-a; pumps harboring the VHA-a1 subunit are targeted to the *trans*-Golgi network/early endosome (TGN/EE), whereas those containing VHA-a2 or VHA-a3 are localized to the tonoplast (Dettmer et al., 2006).

Five years ago, Krebs et al. (2010) showed that an Arabidopsis mutant lacking tonoplast V-ATPase activity (*vha-a2 vha-a3*) had a severely reduced rosette size and a slight reduction in vacuolar pH, but maintained a proton concentration in the vacuole that was 10-fold higher than that in the cytosol. The authors wondered whether the remaining proton gradient in the mutant was entirely due to V-PPase activity or whether other pumps contributed to vacuolar acidification. Now, **Kriegel et al. (2015)** have dissected the contribution of V-ATPases and V-PPases to vacuolar acidification using a panel of Arabidopsis mutants. In addition to *vha-a2 vha-a3*, they obtained mutants lacking the tonoplast-localized K^+-stimulated V-PPase (*fugu5-1*) and generated plants in which the K^+-stimulated V-PPase was overexpressed in the *vha-a2 vha-a3* background (*UBQ:AVP1 vha-a2 vha-a3*) and a triple mutant lacking both V-ATPases and the V-PPase (*fugu5-1 vha-a2 vha-a3*).

Overexpression of AVP1 did not restore wild-type growth in *vha-a2 vha-a3* under standard growth conditions (see figure).



V-PPase overexpression does not restore wild-type growth in a mutant lacking tonoplast V-ATPase activity. Constitutive overexpression of *AVP1* (*UBQ:AVP1*) did not affect plant size in the wild-type (Col) background (line *13-4*) or the *vha-a2 vha-a3* background (line *4-5*) after 3 weeks of growth under normal conditions. (*Adapted from Kriegel et al. [2015], Figure 2B.*)

Furthermore, even though *UBQ:AVP1* overexpression increased V-PPase activity in both the wild-type and *vha-a2 vha-a3* background, it did not affect cell sap or vacuolar pH. Therefore, elevated AVP1 activity does not compensate for a lack of tonoplast V-ATPase activity. Prompted by the finding that V-ATPase activity is stimulated during cold acclimation (Schulze et al., 2012), the authors next examined whether this increased activity depended on the V-PPase. Interestingly, the increase in V-ATPase activity normally following cold acclimation was limited in the *fugu5-1* mutant, but amplified in *UBQ:AVP1*. Thus, the enhanced V-ATPase activity triggered by cold acclimation relies on V-PPase activity.

Finally, the authors showed that the *fugu5-1 vha-a2 vha*-a3 triple mutant was viable, but dwarfed, and maintained a 10-fold proton gradient across the tonoplast of root cells. To identify the source of vacuolar protons in the triple mutant, the authors examined the effect of ortho-vanadate (a P-type H^+-ATPase inhibitor) and concanamycin A (ConcA; a V-ATPase inhibitor). ConcA, but not ortho-vanadate, eliminated vacuolar acidification in the triple mutant. Since TGN/EE-localized V-ATPase complexes are the only remaining targets of ConcA in the triple mutant, this finding shows that the TGN/EE-localized V-ATPase contributes to vacuolar acidification.

Together, this work highlights the complexity of interactions between the tonoplast-localized proton pumps and reveals that the TGN/EE-localized V-ATPase also contributes to vacuolar acidification. The next challenge is to determine whether the TGN/EE-localized V-ATPase affects vacuolar

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acidity directly (by delivering proton-packed vesicles to the vacuole) or indirectly (by stimulating the transport of an unidentified proton pump to the tonoplast).

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