Title: Regulation of Vacuole Fusion in Guard Cells through (de)phosphorylation of HOPS-Specific Proteins

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As desertification and the occurrence of record-breaking drought events continue to rise, understanding how plants regulate water loss becomes an increasingly important area of study. Stomata function by allowing for the uptake of CO_2 when open and mitigating water-vapor loss when closed. The opening and closing of stomata are regulated in part by vacuole fusion and fragmentation. While vacuole fusion is well studied in yeast, it is less characterized in plants. The homotypic vacuole protein sorting (HOPS) complex tethers apposing vacuole membranes and promotes the association of SNAREs (soluble N-ethylmaleimide sensitive factor attachment protein receptors) into a *trans*-SNARE complex. Eventually, HOPS dissociates from SNAREs to allow complete vacuole fusion. The HOPS-specific subunits VPS39 and VPS41 are required for proper homotypic vacuole fusion. We characterized a viable T-DNA insertion allele of *VPS39* which demonstrates a critical role of VPS39 in stomatal vacuole dynamics. Computational modeling of plant vacuole fusion events predicted a steady-state of the HOPS:SNARE association prior to fusion, and the rapid progression of fusion after HOPS dissociation from SNARE. We hypothesized that the release of HOPS from SNARE is the result of rapid post-translational modifications including phosphorylation. In support of this hypothesis, multiple phosphorylation states of VPS39 and VPS41 have been detected in publicly-available phosphoproteomics studies. Candidate residues that may control HOPS function were selected to generate phosphorylation mutants, which are currently being characterized. Furthermore, we identified mutants of a candidate kinase with impaired vacuole fusion phenotypes in stomata. Initial Bi-Fluorescence Complementation data, support a possible interaction between the kinase and the HOPS complex. These results overall underscore a potential role of protein phosphorylation and dephosphorylation in the regulation of vacuole fusion in plant stomata.