Binding of RALF1 to the FERONIA receptor kinase downregulates the plasma membrane H^+- ATPase and reduces cell elongation in roots

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Cell elongation is an essential process for plant growth and development. A 5-kDa peptide, Rapid Alkalinization Factor 1 (RALF1) regulates root cell elongation via binding to a receptor kinase, FERONIA. Using an *in vitro* fluorescent polarization binding assay we have now demonstrated that RALF binding to the FERONIA ectodomain is sequence specific, reversible, dose responsive, and pH dependent. The effect of a kinase negative mutation of FERONIA on root growth was compared to the effects of this mutation on fertilization. While the kinase negative mutation does not affect fertilization, reduced kinase activity results in reduced sensitivity to RALF1-induced root growth inhibition, indicating that there may be differences in the signaling pathway utilized in reproductive versus vegetative tissue. The molecular mechanism for changes in RALF1 sensitivity of the kinase-negative mutant is also being interrogated by measuring changes in RALF1-induced cytoplasmic calcium concentration. RALF1-induced phosphorylation changes in a plasma membrane proton pump, AHA2, correlates with the peptide's ability to induce extracellular alkalinization. To genetically test AHA2 involvement in RALF-FERONIA physiology, we examined the growth of plants containing mutations in AHA2. A phosphomimetic mutation of AHA2 at Ser899, an amino acid whose phosphorylation was increased by RALF, caused reduced ability to rescue the *aha2* mutant. Moreover, a double mutation, *fer/aha2*, suppresses the longer root phenotype seen with *feronia* single mutants. AHA2 acting downstream of RALF-FERONIA pathway is also supported by an observation that AHA2 protein abundance decreases in WT when treated with RALF1. This AHA2 abundance change was not seen in *feronia* mutant. Overall, our results support a model in which FERONIA and RALF1 kinase signaling restricts cell expansion by down-regulating AHA2 function.