Subcellular Localization of *Oryza sativa* V-ATPase Subunit H

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Introduction and Background

V-ATPases are ATP-dependent proton pumps that regulate pH of endomembrane compartments in the cell.

- Subunit H (VHA-H) is thought to be the complex activator (Flannery & Stevens, 2008)
- Encoded by a single gene *VHA-H* -> evidence of two splice isoforms
- Other subunits' isoforms shown to exhibit specificity in localization and function (Dettmer et al., 2010)
- May also occur in *VHA-H* isoforms

- V-ATPases play critical role in pollen development and drought stress tolerance (Dettmer et al., 2005, Liu et al., 2018)
- Localization of *VHA-H* provides insight into V-ATPase activity regulation

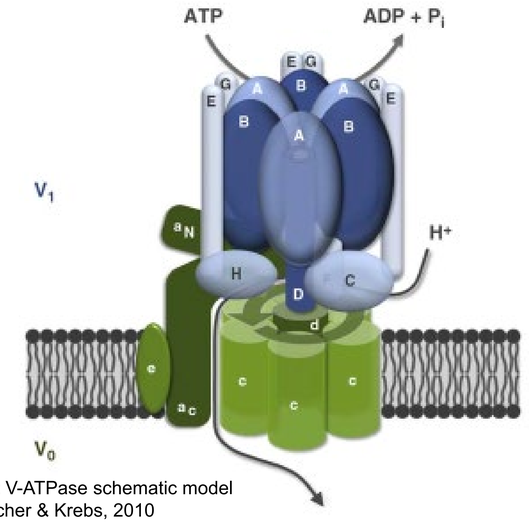


Figure 1: V-ATPase schematic model
Schumacher & Krebs, 2010

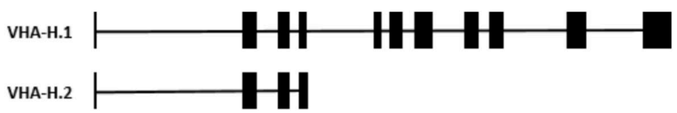


Figure 2: *VHA-H* gene schematic
Taehoon Kim

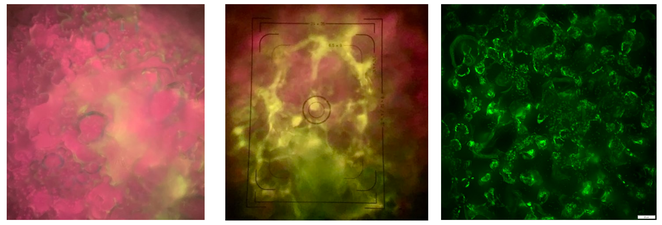
Hypothesis

It is expected that there may be differential localization between splice isoforms *VHA-H1* and *VHA-H2.*

Viewing Localization using YFP

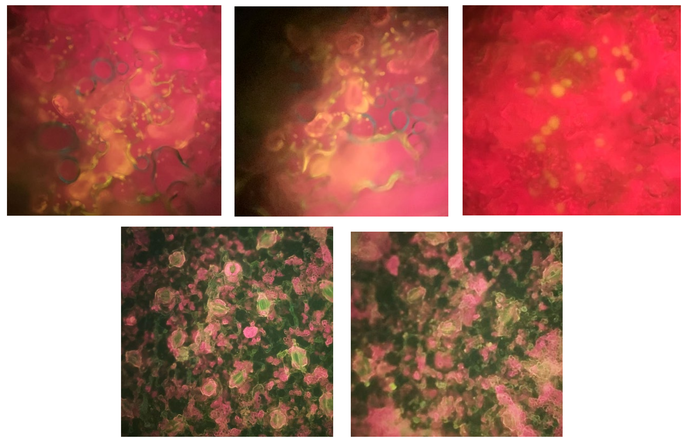
- Constructs of yellow fluorescent protein fused with H1/H2 subunit genes cloned into *Agrobacterium* Ti plasmid; transformed into tobacco leaves
- Viewing signals: fluorescent light microscope using UV light and GFP filter

YFP Control

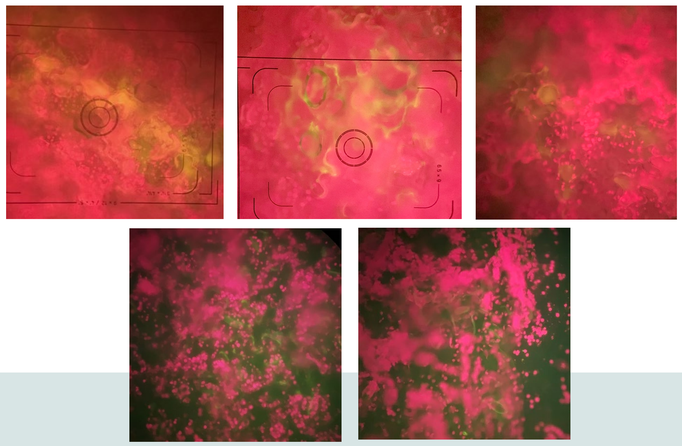


YFP-MIR control on confocal microscope

YFP-H1



YFP-H2



Transient Transformation in *N. benthamiana*

Tobacco leaves syringe-infiltrated with *Agrobacterium* constructs

YFP signals observed from 2-7 days post-inoculation using whole leaf tissue and epidermal peels

Results and Next Steps

- YFP-H1 signals exhibit localization in vesicles and cell membranes, possibly guard cell membranes and cytosol
- YFP-H2 signals exhibit localization in cell membranes and cytosol, possibly guard cell inner membranes

- Differences in localization of splice variants suggests that there may be difference in functions between *VHA-H1* and *VHA-H2*
- Resembles differential localization and function found in VHA-E isoforms

Next Steps:

- Further validation of signal localization data using laser scanning confocal microscopy
- Stable transformation of *O. sativa vha-h* CRISPR mutants via *Agrobacterium* and tissue culture organogenesis for functional analysis

References

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3. Flannery, A. R., & Stevens, T. H. (2008). Functional characterization of the N-terminal domain of subunit H (Vma13p) of the yeast vacuolar ATPase. *The Journal of Biological Chemistry, 283*(43), 29099-29108.
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5. Schumacher, K., & Krebs, M. (2010). The V-ATPase: Small cargo, large effects. *Current Opinion in Plant Biology, 13,* 724-730.

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