Investigating roles of Clathrin-coated vesicle components in hypocotyl length in *Arabidopsis thaliana*

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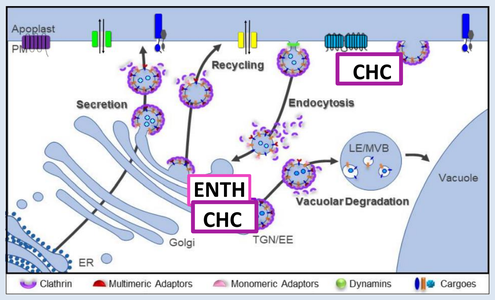
Introduction

Throughout a plant's life cycle, the cell wall provides structural support and protects intracellular components. A key molecule in the plant cell wall that aids in cell expansion is the polysaccharide cellulose. In the model plant *Arabidopsis thaliana*, cellulose synthases form large enzyme complexes that need to be localized in the plasma membrane (PM) to catalyze the synthesis of extracellular cellulose.[1]

To fine-tune cellulose production for polymer arrangement and expansibility of the cell wall, the plant cell adjusts the PM abundance of cellulose synthases using vesicular trafficking via clathrin-coated vesicles (CCVs). CCVs have emerged as the prominent vesicle type that transports cellulose synthases from one cellular organelle to another in form of small membrane-bound vesicles. Newly synthesized cellulose synthase complexes are secreted via the *trans*-Golgi Network (TGN) to the PM. With the help of CCVs, cellulose synthases are internalized from the PM by constitutive endocytosis, transported to the TGN and then recycled back to the PM to allow for a new round of synthesis of cellulose.

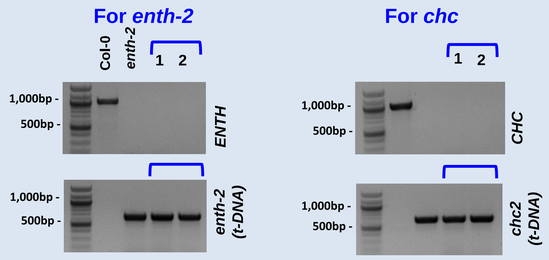
My goal in this study was to determine if there is a phenotypic difference in the hypocotyl length between loss of function CCV mutants and the wild-type when grown for 6 days in the dark. Quantifying hypocotyl length of dark-grown seedlings has previously identified trafficking components with novel roles in trafficking cellulose synthases to/from the PM.

Here, I focused on following two CCV components with yet unknown functions in cellulose synthase trafficking: 1) **ENTH** that functions as a Clathrin-Adaptor at the TGN and modulates the PM Proteome through secretion of proteins from the TGN to the PM[2]; 2) **CHC** that is recruited by ENTH to the TGN to help secrete proteins from the TGN to the PM and functions in endocytosis of proteins from the PM to the TGN.



Results

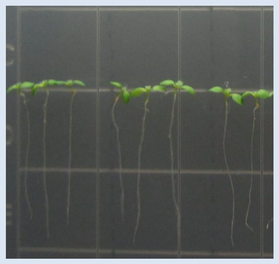
1. PCR genotyping the *enth chc* double mutant



PCR genotyping using gene specific primers for *ENTH* and *CHC* wild-type and T-DNA insertion mutant alleles *enth-2* and *chc*, respectively, confirmed the isolation of homozygous enth-2 and chc double mutants (indicated by numbers 1 and 2).

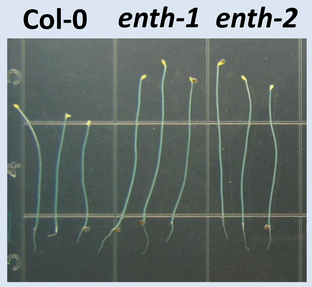
Results (cont.)

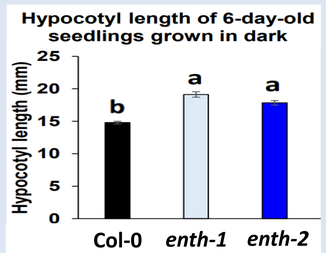
2. Two independent *enth* mutant alleles have no obvious growth defect when grown in light



Grown on 0.5X MS
n= 20-30
Seedlings were grown 6 days under 24-hour light

3. *enth* mutants display elongated hypocotyl when grown in dark

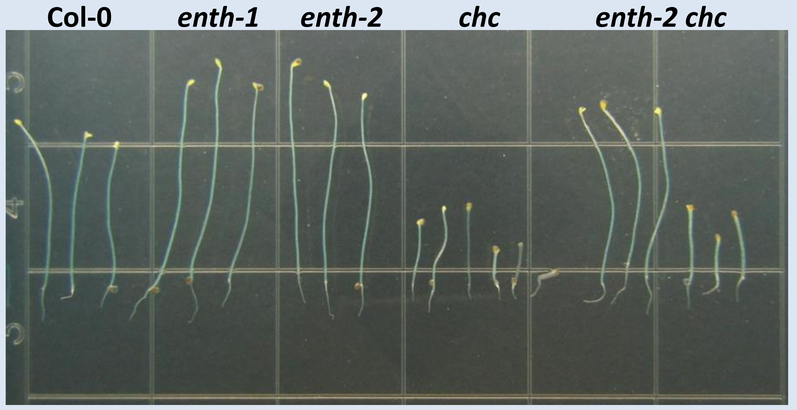




p-value ≤0.05, Tukey's Test

Grown on 0.5X MS
n=55-100
Seedlings were grown 6 days in dark conditions
3 Independent Trials
Measurements taken with Fiji ImageJ

4. *chc* mutant displays stunted hypocotyl growth that is suppressed by loss of ENTH when grown in dark



Grown on 0.5X MS
3 Independent Trials
Seedlings were grown 6 days in dark conditions
3 Independent Trials n=55-100
Measurements taken with Fiji ImageJ

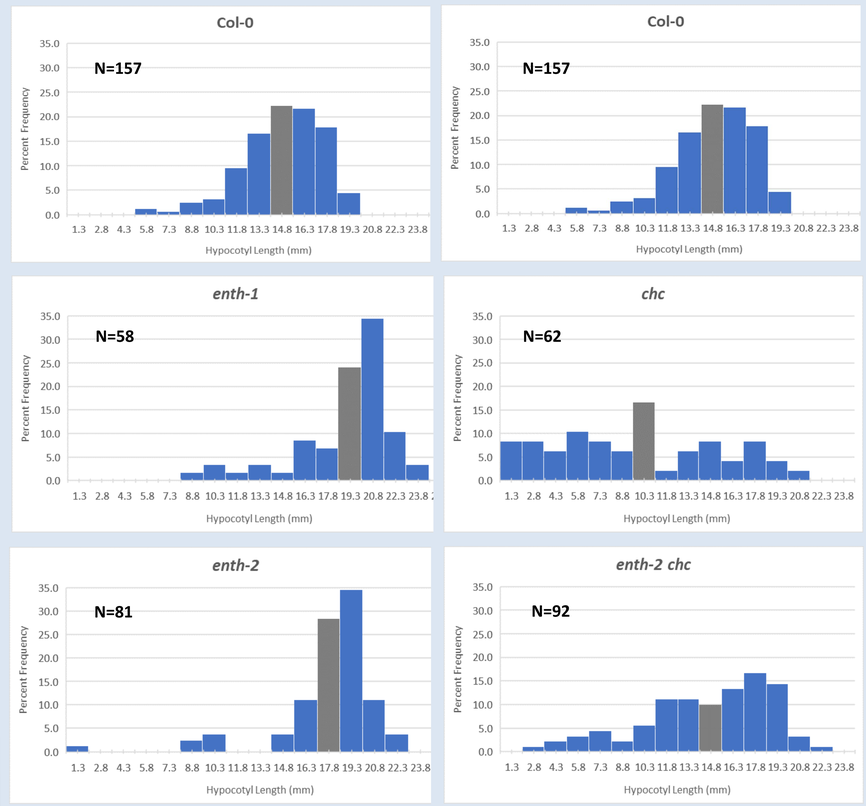
Literature Cited

[1] Xin *et al.* (2020) *Journal of Experimental Botany*, *doi: 10.1093/jxb/eraa063.*

[2] Collins *et al.* (2020) *Plant Physiology*, 182(4): 1762–1775.

[3] Mitra, Loqué (2014) *Journal of Visualized Experiments,* (87): 51381

Hypocotyl Length Frequency Graphs



"Percent Frequency" indicates the percentage of hypocotyls within each category
Highlighted bar indicates median group
Frequency graphs created using Microsoft Excel

Conclusion

We identified novel roles for the two CCV components ENTH and CHC in modulating hypocotyl length of dark grown Arabidopsis seedlings

Loss of ENTH results in elongated hypocotyl

Loss of CHC results in stunted hypocotyl

↳ **ENTH and CHC appear to have opposite roles in hypocotyl length.**

Loss of ENTH partially suppresses the CHC dependent stunted hypocotyl length

Future Directions

1. Determine whether increase in hypocotyl length can be attributed to an **increase in cell number or cell size.** Stain light and dark grown seedlings with propidium iodide stain to **visualize individual cells** underneath stereomicroscope.

2. Stain cells with Conge Red dye to **qualitatively determine cellulose content and localization**[3].

Acknowledgements

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