Delineating day length-dependent autoimmune responses in clathrin-coated vesicle mutants

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INTRODUCTION:

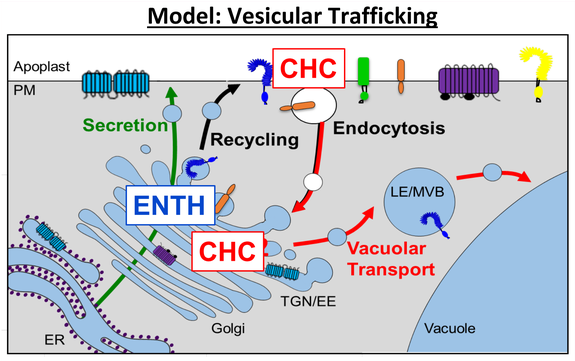
Agriculture is one of the most important industries that exists to clothe and feed the world, as they are used to make a variety of commodities such as food, fiber, biofuels, and medicine. By understanding how model plants respond to different pathogens, we can translate that knowledge to engineer more resistant plants that may help to reduce crop loss for farmers.

Proteins located in the plasma membrane (PM) play important roles in a plants ability to perceive and transduce the presence of bacterial pathogens. Our lab focuses on vesicular trafficking proteins and their roles in regulating the PM protein composition, so that proteins with defense functions are present in the PM at the right time and in the right abundance. In plants, clathrin-coated vesicles (CCV) is the main vesicle type that transports PM proteins with immune functions from the *trans*-Golgi Network (TGN) to the PM [1,2]

Our lab has published that in the model plant *Arabidopsis thaliana*, the vesicular trafficking protein ENTH is required to defend plants against infection by bacterial pathogens [3]. ENTH is a TGN-localized CCV adaptor protein that helps recruit CLATHRIN HEAVY CHAIN (CHC) for transport of PM proteins from the TGN to the PM [1]. We also discovered that *chc enth* double null mutant but not the *chc* or *enth* single mutant seedlings displayed elevated defense responses in the absence of bacterial pathogen (no stimulus) when grown under 24 hour day-length conditions. The (auto)immune response in Constitutive defense response (CPR) mutants can be triggered by environmental conditions such as temperature, humidity, light intensity, and day length [4].

My hypothesis is that for the *enth chc* double mutant, constitutive activation of immune responses is dependent on 24-hour/long day-length conditions. To test this hypothesis, I compared two different autoimmune responses (callose deposition and *PR1* mRNA accumulation) in *chc enth* double to the appropriate single mutant and wild-type seedlings that were grown for seven days under three different day-length conditions.

Model: Vesicular Trafficking



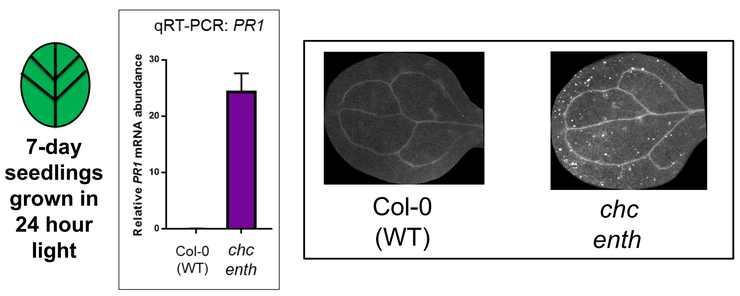
[2]

Model: Clathrin Coated-Vesicle Formation

[1]

BACKGROUND:

1. *chc enth* displays autoimmune defects

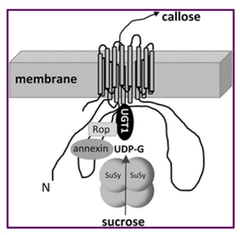


- The *enth chc* double mutant seedlings have elevated levels of callose and *Pathogenesis-Related 1* (*PR1*) mRNA in the absence of any pathogen.
- The triple mutant *enth chc sid2*, which cannot produce the plant defense hormone salicylic acid (SA) has high levels of callose, while it has no *PR1* mRNA.
- This indicates that callose deposition is independent of SA while *PR1* mRNA is dependent on SA.
- For all of these assays, seedlings were grown under continuous 24 hour day-length, a growth condition that can cause autoimmune responses for some mutants.

2. *PR1* mRNA

- *PR1* mRNA is an immune marker gene
- *PR1* mRNA production levels can depend on the amount of Salicylic acid (SA) present in the plant.
- CPR mutant plants can have a stunted phenotype.

3.Callose Synthases in Arabidopsis



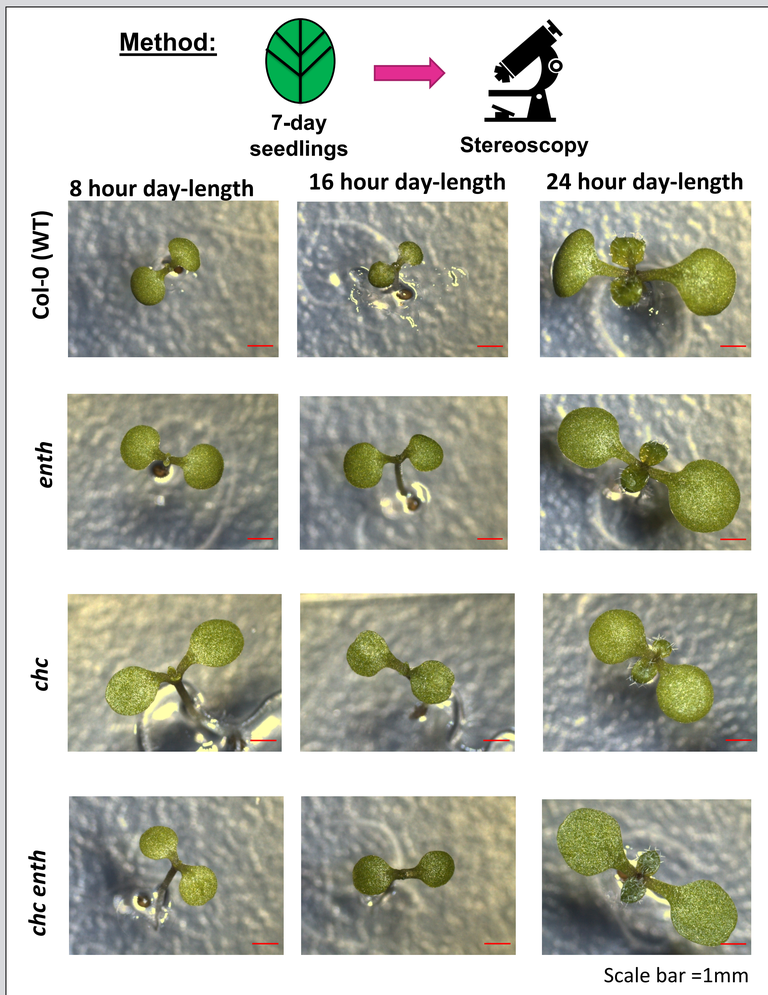
- Callose Synthases produce callose in plants.
- The model plant *Arabidopsis thaliana* expresses 12 callose synthases.

Callose

- Callose is a β-1,3-glucan polysaccharide is deposited in the plant cell wall (in the extracellular space) [5].
- It is produced in plant growth and during defense responses and is regulated by the plant defense hormone Salicylic Acid (SA)

RESULTS:

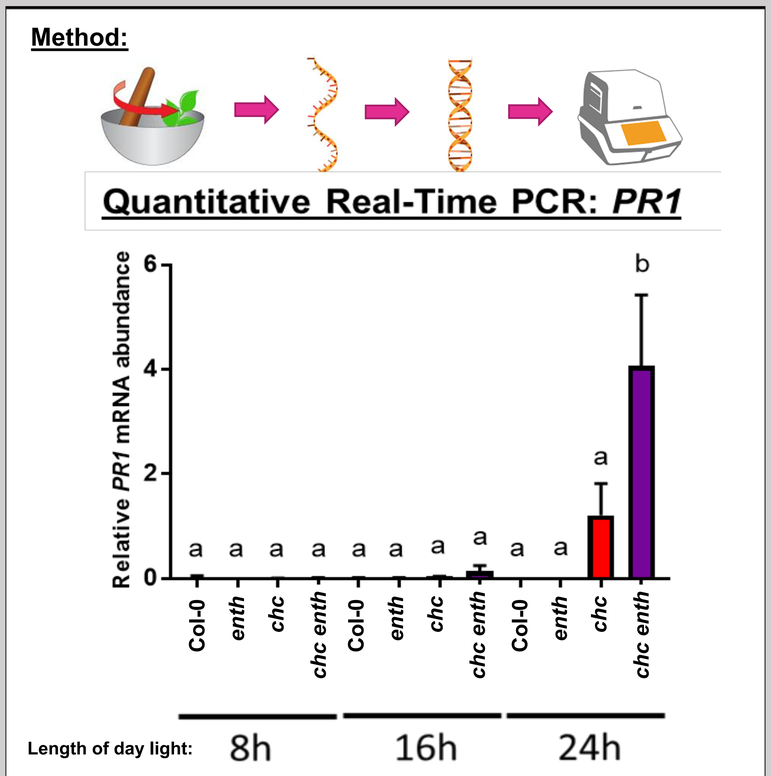
1. Representative images of mutant seedlings grown under three different day-length conditions



- **The *chc enth* double mutant does not appear to have a stunted phenotype.**

1.25X Magnification in bright field using Leica EC3 stereoscope

2. *PR1* mRNA is dependent on long day-length stress

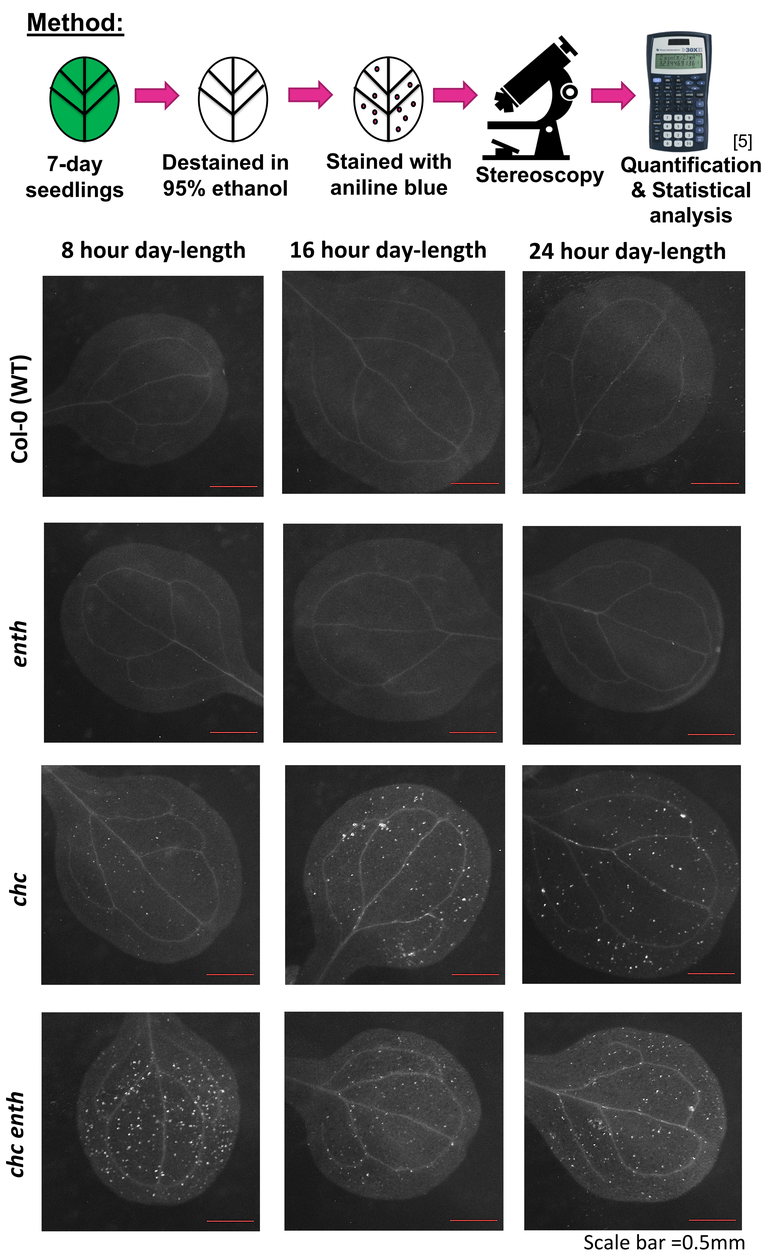


- ***PR1* mRNA in the *chc enth* double mutant is elevated under 24 hour day-length conditions.**
- **In contrast, *PR1* mRNA in the *chc enth* double mutant is not elevated in 8 hour or 16 hour day-length conditions.**
- ***PR1* mRNA is not elevated in Col-0 or the *chc* and *enth* single mutants across all three day-length conditions.**

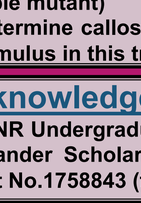
Experiment was repeated four times with similar results

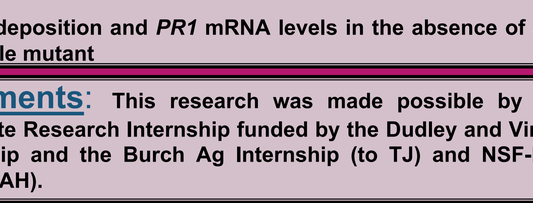
p value ≤0.05
One-Way ANOVA
n=3-5

3. Callose deposition is independent of long day-length stress



Method:





- **Callose deposition in the *chc enth* double mutant is elevated in 8-hour, 16-hour, and 24-hour daylight conditions.**

Callose deposits were visualized at 3.5x magnification by UV epifluorescence using a Leica MZFL III stereoscope

p value ≤0.05
One-Way ANOVA
n=24
Experiment was repeated three times with similar results

CONCLUSIONS:

1. **Elevated levels of *PR1* mRNA in *chc enth* double mutant is dependent on long day-length stress.**
2. **Constitutive callose deposition in *chc enth* double mutant is independent of long day-length stress.**

Some, but not all autoimmune defects in the *chc enth* are dependent on 24 hour light/long daylight stress

FUTURE DIRECTIONS:

1. **Determine whether constitutive callose deposition in *chc enth* is genetically dependent on Callose Synthase 12 (CalS12) known to be responsible for defense-related callose**
2. **Isolate plant line lacking *CalS12* in a c*hc enth* background (*chc enth cals12* triple mutant)**
3. **Determine callose deposition and *PR1* mRNA levels in the absence of any stimulus in this triple mutant**

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Literature Cited:

1. LaMontagne and Heese (2017). Current Opinion in Plant Biology. 40:114-121
2. Ekanayake, LaMontagne, and Heese (2019). Annual Review in Phytopathology. 57:387-409.
3. Collins et. al (2020). Plant Physiology. 182(4):1762-1775.
4. Vlot, Dempsey, and Klessig (2009). Annual Review in Phytopathology. 47:177-206.
5. Chen XY, Kim JY (2009). Plant Signaling and Behavior.4(6):489-92.
6. Mason et. al (2020). Methods in Cell Biology. 160:181-199