Characterization of Pollen-predominant RALFs

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Background

FERONIA (FER) receptor kinase is a transmembrane protein found in flowering plants like those of *Arabidopsis thaliana.* FER plays a crucial role in the plant's life and is a key player in mediating stress responses. FER acts as an upstream regulator of RAC/ROPs (RHO GTPases of plants) by receiving and transducing outside signals to regulate multiple cellular pathways to regulate plant responses to environmental stimuli. (Duan et al., 2010)

The Cheung and Wu lab have shown in previous work that the Glycosylphosphatidylinositol (GPI)-anchored proteins, Lorelei-Like-GPI-anchored proteins (LLGs) act as a coreceptor for FER. Specifically, we have seen that LLG1 binds to the extracellular juxtaposition region of FER and is pivotal for FER functionality. (Li et al., 2015)

Rapid Alkalinization Factors (RALFs) are peptide hormones that are involved in various aspects of plant development and growth. RALF signaling is dependent on FER/LLG complex. In FER/LLG knockout mutants RALF mediated signaling was found to be insensitive (Li et al., 2015) The RALF peptide family contains more than 30 members. (Figure 1) Despite having shorter amino acid sequences, peptides in the RALF family have conserved motifs found important for its biological properties. Among many of these biological properties, certain RALFs are predominately expressed. The highlighted (red rectangular boxed) RALFs in Figure 2, are predominately expressed in pollen. (Zhong et al., 2022)

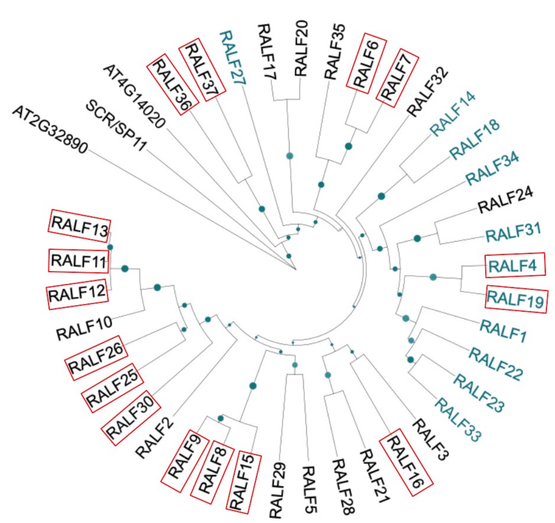
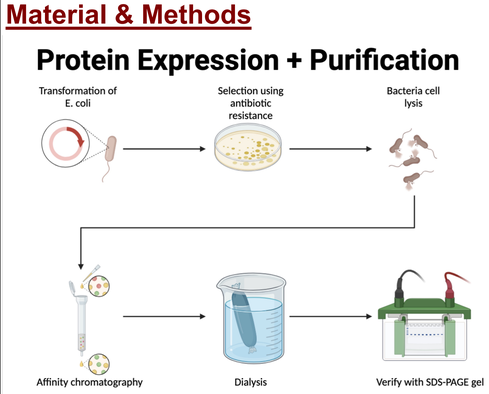


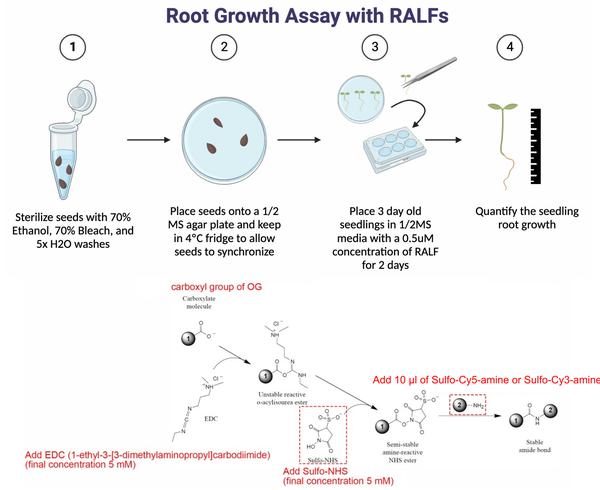
Figure 1. Phylogenetic tree of the RALF peptide family (Abarca et al., 2021)

Figure 2. Sequence alignment of mature pollen predominantly expressed RALF sequences

RALF1 has been studied for its ability to inhibit root growth and its ability to form particles through liquid-liquid phase separation with Oligogalacturonides (OG). Over the course of this summer, I studied the cell-wall derived OG on how they mediate intracellular signaling along within pollen predominantly expressed RALFs.

Material & Methods



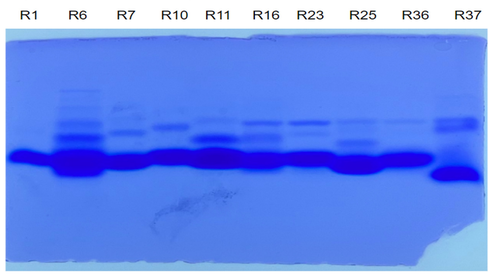


In-vitro-Particle Formation Protocol

- In a PCR tube, prepare a total of 50 µl of the reaction mixture to view under the Nikon Ti Microscope, This solution contains 0.1 mg/ml of chemically labeled $OG_{10\text{-}15\ dp}$, 5 µM of RALF peptide and the rest contains RALF buffer (50 mM Sodium acetate, pH 5.7, 100 mM NaCl)
- After all three components are added, mix thoroughly and wait for 5 minutes
- Pipette 4~5 µL from the solution and add onto a slide, place a coverslip. On 60x objective oil lens, view the samples at the corresponding wavelengths of excitation and emission of the fluorescent tags

Results

Figure 3. 20% Acrylamide Gel of RALFs



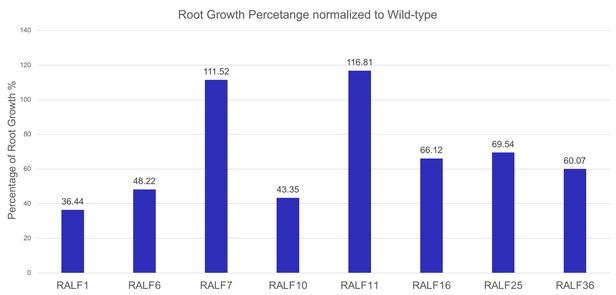


Figure 4. Root Growth Percentage normalized to Wildtype

Figure 5. In-vitro particles Cy3-SOG (dp10-15) +/- RALF1

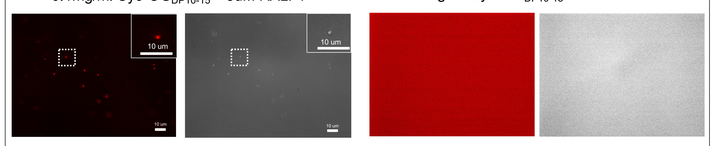
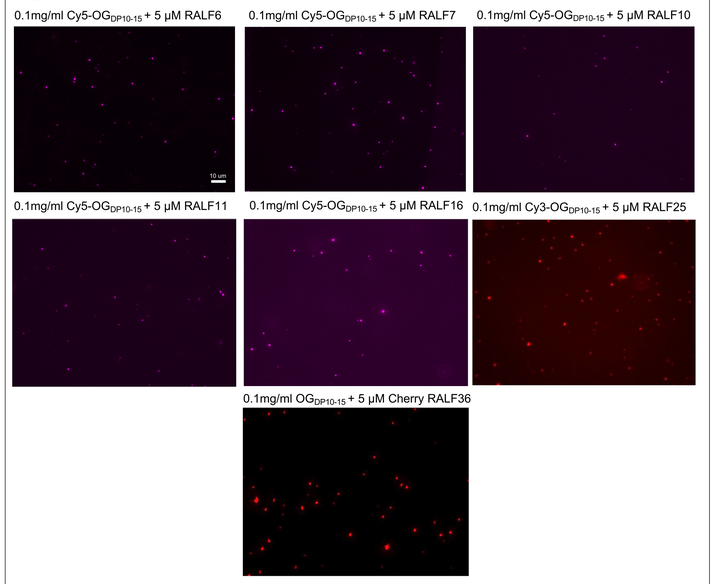


Figure 6. In-vitro particles of other RALFs



Future Directions

Over the summer, I was able to take my purified protein into experiments that allowed me to study the role of RALFs in the plant model Arabidopsis. As I enter my last year of undergraduate studies, I would like to continue the functional characterization of these RALFs, especially those expressed predominately in pollen.

Acknowledgements

First and foremost, I would like to extend a heartfelt thanks to my research advisors Alice Cheung and Hen-Ming Wu. I would like to also thank Ming-Che James Liu and Fang-Ling Jessica Yeh for their patience and tireless willingness to teach and offer their insights.

5. References

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