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The Study of PPAL and its Role in the Development of Physcomitrella patens

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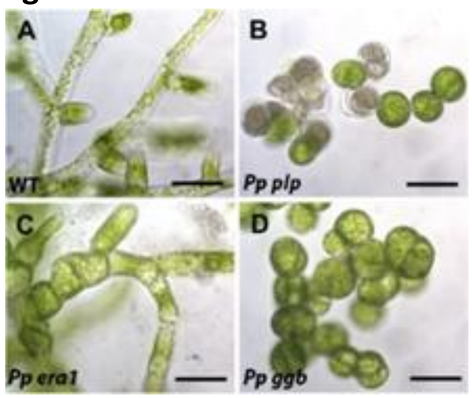
Introduction

Protein Prenylation is the addition of lipids to proteins and plays a vital role in the cell. It enhances protein-protein interactions and promotes protein-membrane targeting and association.

Protein Prenylation plays a key role in various plants' developmental processes. The key enzymes that are involved in prenylation are protein farnesyltransferase (PFT), protein geranylgeranyl-transferase-I (PGGT), and Rab geranylgeranyltransferase (Rab-GGT).

There has been prior research that examined the role of prenylation in plants. A study was done on Arabidopsis (At) and Physcomitrella *patens* (Pp) that analyzed the role of protein prenylation enzymes. A knockout of PGGT and PFT a and b subunit genes in P. patens resulted in phenotypic changes.

Figure 1



Wild Type (A) demonstrates elongation, filament formation, and adhesion which Ppplp and Ppggb (B and D) lack. Ppera1 mutants show elongation but it is not as well as WT.

The knockout of PpPPAL1 and PpPPAL2 has resulted in the death of the cell. This suggests that these genes are necessary for moss growth and survival; however, their specific role is unknown. Previous studies have shown that prenylation plays a role in ABA response, polar cell elongation, and cell fate determination in Arabidopsis.

Further studies of the role of PpPPAL1, PpPPAL2, and prenylation in P. patens needed to be done. More specifically, whether these enzymes play a role in growth, hormone response, cell adhesion and determination, sugar response, and polar cell elongation.

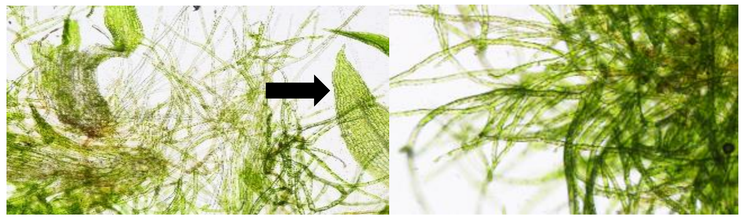
Method

• To study the role of PPAL, a knockdown approach was used to reduce gene expression of PpPPAL1 and PpPPAL2 and characterize the resulting phenotype.
•Then, in-depth expression studies, metabolic analysis, and developmental studies of *P. patens* mutants was done.

Results

1. Reduction of gene expression of PpPPAL1 and PpPPAL2 was performed through gene knockdown:
   - Artificial microRNA was created and used to target PpPPAL1 and PpPPAL2 genes
   - PpPPAL1 and PpPAL2 amiRNA transformants were grown in DMSO and β-Estradiol medium.

Figure 2. *PpPPAL1* Knockdown:



PpPPAl1 knockdown mutants showed less growth and propagation and less gametophore (arrow) compared to PpPPAL1 in control

PpPPAL1 DMSO Control **PpPPAL1 knockdown mutants**

2. The moss efficient moss transformants was used for RNA extraction and in-depth expression studies:
   - DNA extraction from moss transformants and PCR to perform genotyping
   - RNA extraction from moss transformants and perform RT-PCR to examine gene expression
   - The most efficient moss transformants was selected via gel electrophoresis visualization.
3. Studying the developmental and metabolic process in *P. patens:*
   - Cytoskeleton markers of knockdown plants was immunostained to examine the organization of cytoskeleton and cell division.
   - RabA4b markers was transformed into knockdown plants to study vesicle transport and cell polarity.
   - Certain metabolic process was analyzed to see if they are altered in the mutants.

Conclusion

- Study of gene expression of PpPPAL1 and PpPPAL2 knockdown lines will provide insights into the functional and conserved roles of PpPPAL1 and PpPPAL2 in *P. patens.*
- Preliminary results have shown that PpPPAL1 and PpPAL2 knockdowns inhibit the growth and propagation of P. *patens.* It also resulted in less gametophore.
- More in-depth studies in P.patents will be done to test whether PpPPAL1 and PpPPAL2 and prenylation play a role in growth, hormone response, cell adhesion and determination, sugar responses, and polar cell elongation.

Significance

- Provide an understanding of how early plants adapt to land and the evolution of multicellularity.
- Can be used in agriculture to create plants that can tolerate drought.
- Provide uses in the field of algae biofuels by creating algae that can adhere to each other to lower the cost of harvesting.
- Provide an understanding of prenylation in humans, as defects in prenylation could lead to health problems.

Acknowledgement

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