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Identification of the Biological Function of Rab-GGT β-Subunits by Reverse Techniques

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Introduction

Protein prenylation is a post-translational process where lipids are added to carboxyl end groups, which allows proteins to function properly in the eukaryotic cell. The job of prenylation is to help in targeting certain proteins to specific membrane along with promoting protein-protein interactions. We use reverse genetics techniques to understand the function of prenylation in plant development by examining the phenotypic changes caused by specific gene disruption. One of the excellent model organisms Physcomitrium patens (moss) is used due to its simple structure, limited tissue and cells, sequenced genome, and its high gene targeting efficiency.

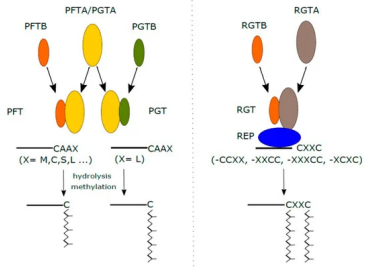

Figure 1: Protein Prenylation in Plants (Hála & Viktor, 2019)

Rab geranylgeranyl transferase-II (Rab-GGT) is one of three enzymes that can perform protein prenylation. Moss has one copy of Rab-GGT α subunit (*PpRGTA1*) and two copies of β-subunit (*PpRGTB1* and *PpRGTB2*). Rab-GGT also requires an additional protein, Rab escort protein (REP), for activity. This study focuses on the role of the Rab-GGT β subunit in the moss.

Hypothesis

Dr. Running lab previously figured out that protein prenylation plays major roles in polar cell elongation and cell fate specification in *P. patens*. It has been found that the knockout of either *PpRGTB1* or *PpRGTB2* results in no visible phenotype, which leads us to believe that these genes are functionally redundant. The knockout of both *PpRGTB1* and *PpRGTB2* genes has shown to be lethal, which means Rab-GGT is required for the plant viability. For these reasons, the actual function of Rab-GGT is largely unknown. If the Rab-GGT gene expression is reduced by knockdown instead of complete knockout, it will be possible to observe a phenotype that does not pose a threat to the survival of the plant and accordingly, the function of the gene can be examined.

Methods

To figure out the biological function of Rab-GGT, we use RNA interference (RNAi) approach to down-regulate the expression level of *PpRGTB1* in the PpRGTB2 knockout background and analyze the phenotypic consequences.

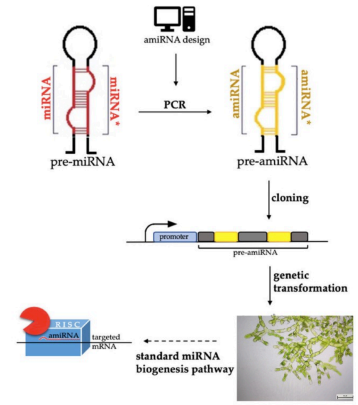

Figure 2: The process of RNA interference (Wójcik, 2020)

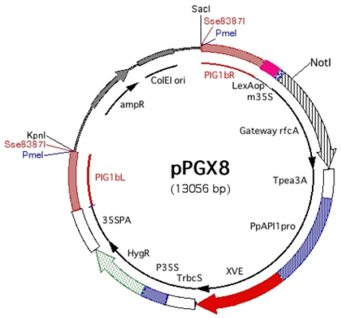

Figure 3: β-estradiol inducible vector pPGX8.

Agarose Gel Electrophoresis

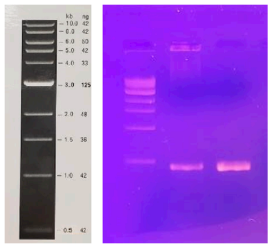

Figure 4: Confirmation of the size of amiRNA (around 0.4kb) with Agarose Gel Electrophoresis.

Results

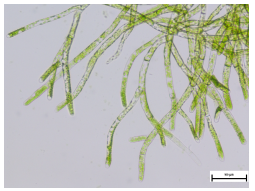

Figure 5: *PpRGTB1* knockdown in *rgtb2* knockout mutant Control (0.01% DMSO)

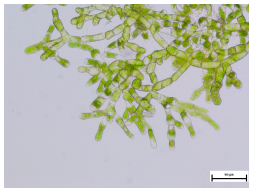

Figure 6: *PpRGTB1* knockdown in *rgtb2* knockout mutant. 1um β-estradiol induction

After 1um β-estradiol treatment, knockdown lines remain viable and show branched filamentous growth. But they result in fewer caulonemal cells and gametophores, reduced cell elongation and defects in cell polarity compared to the control.

CONCLUSION

- The moss P. patens provides us an opportunity to test the hypothesis that prenylated Rab-GTPase target proteins play major roles in polar cell elongation and cell fate determination. Its advantages are its simple body plan, sequenced genome and ability to perform targeted knockouts of any gene or multiple genes of interest at high efficiency.
- The Rab-GGT β knockdown mutants showed developmental defects, indicating that Rab-GGT plays pivotal role in tip growth and/or caulonema cell differentiation, and might work as complex. Further metabolic studies will be needed to identify and confirm that the functional roles of prenylation are conserved among disparate species of plants.

Bibliography

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