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Protein Prenylation in the moss Physcomitrium patens

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Protein Prenylation in the moss *Physcomitrium patens*

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Introduction

- Protein prenylation is the addition of a 15- or 20-carbon lipid to a cysteine near carboxyl terminus of target proteins.
- Prenylation increases hydrophobicity, which facilitates membrane associations and protein-protein interaction
- Protein prenylation is generally conserved among eukaryotes, and mutations in genes that carry out prenylation are lethal in animals and yeast.
- In plants prenylation mutations are not always lethal, but they do affect development, disease resistance, biofuel production, and drought response, among other processes of agricultural interest.

Purpose

Our goals for the study included:

- To understand the evolutionary and developmental implications of plant protein prenylation
- To search for proteins that meet minimal criteria for prenylation in *Physcomitrium patens*

Methods

- Searched for the presence of a sequence that includes cysteine and one of six specific amino acids at the C terminus of proteins in the *P. patens* annotated genome.
- Analyzed these proteins with an online prenylation prediction program to assess their likelihood of being prenylated based on additional sequence motifs.
- Determined potential biological function of putative target proteins by using BLAST sequence similarity searches to identify related genes with known function

Results

Prenylation Enzymes

The three-known protein prenylation enzymes are farnesyltransferase (PFT), geranylgeranyltransferase-I (PGGT), and Rab geranylgeranyltransferase (Rab-GGT). The gene names of the subunits of each enzyme in the model dicot *Arabidopsis thaliana* and the model moss *Physcomitrium patens* are indicated in Table 1. Some subunits are present in multiple copies. An additional putative prenyltransferase alpha subunit, *PPAL*, is also present in both genomes.

Enzyme	α subunit gene name (s):		β subunit gene name (s):	
PFT	At PLP	Pp PLP	At ERA1	Pp ERA1
PGGT	At PLP	Pp PLP	At GGB	Pp GGB
Rab-GGT	At RGTA 1, RGTA 2	Pp RGTA1	At RGTB 1, RGTB 2	Pp RGTB 1, RGTB 2
Unknown	At PPAL	Pp PPAL 1, PPAL 2		

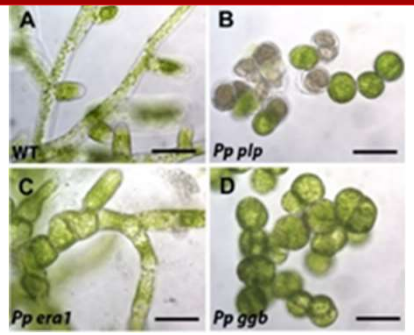
Table 1. Protein prenylation components in *Arabidopsis thaliana* (*At*) and *Physcomitrium patens* (*Pp*)

Prenylation Enzymes

Knockouts of prenylation components in moss result in dramatic developmental and physiological phenotypes (Figure 1), including a complete reversion to unicellular algae-like plants seen in *plp* and *ggb* knockouts (Fig 1B and 1D). They also hyperaccumulate oils, showing a potential use in biofuels.

PFT, PGGT, and Rab-GGT all target specific proteins for prenylation, but they differ slightly in their target sequence. At a minimum, a cysteine to which the prenyl group is attached must be present at or near the C-terminus, with a strong preference for cysteines that are fourth from the terminus. In addition, PFT prefers alanine, cysteine, glutamine, methionine, or serine as the terminal amino acid, while PGGT prefers leucine in that position (Running, 2014).

Figure 1. Knockout phenotypes of prenylation subunits in *P. patens* (Thole et al., 2014)



Target Sequences

We used the *P. patens* annotated genome sequence v3.3 to search for putative prenylation target proteins, finding approximately 2,000 that meet minimal target criteria. We then used an online tool, the GPS Lipid prediction site (http://lipid.biocuckoo.org), to assess the likelihood of prenylation (and other lipid modifications) of each protein based on additional sequence motifs. We assessed potential protein function by using BLAST to find proteins of similar sequences with known functions. An example protein target is shown in Figure 2.

Figure 2. Example sequence, putative function, and prenylation prediction analysis of a *P. patens* protein.

```
>Pp3c10_190V3.2.p pacid=32900597 transcript=Pp3c10_190V3.2 locus=Pp3c10_190 annot-
version=v3.3
MNPEYDYLFKLLLIGDSGVGKSCLLLRFADDSYLESYISTIGVDFKIRTVELDGKTIKLQIWDTAGQERFRTITSSYYRG
AHGIIIVYDVTDQESFNNVKQWLNEIDRYASENVNKLLVGNKSDLASKKVVDYATAKAFADEIGIPFLETSAKNATNVEQ
AFMTMAAEIKNRMASQPALSSTSRPNNVQLRGQTILFKGGCCS*
```

Ras-related protein aka GTP-binding protein

ID	Position	Peptide	Score	Cutoff	Type
Unnamed	201	TILFKGGCCS*****	3.533	1.072	S-Palmitoylation: Cluster C
Unnamed	201	TILFKGGCCS*****	9.207	3.71	S-Farnesylation: Non-consensus
Unnamed	201	TILFKGGCCS*****	9.14	0.474	S-Geranylgeranylation: CC/CXC
Unnamed	202	ILFKGGCCS*****	2.052	1.072	S-Palmitoylation: Cluster C
Unnamed	202	ILFKGGCCS*****	24.407	3.71	S-Farnesylation: Non-consensus
Unnamed	202	ILFKGGCCS*****	11.023	0.474	S-Geranylgeranylation: CC/CXC

Conclusions

- We identified proteins with putative prenylation motifs in the sequenced genome of the moss *P. patens*.
- We assessed the likelihood of each protein to be prenylated prenylated or otherwise lipid modified based on additional sequence motifs using online tools.
- We assigned putative function to each protein using BLAST to identify proteins with sequence similarity that have an experimentally verified function.
- Several proteins we identified as likely prenylation targets are homologous to those found in other organisms to be prenylated, many of which are involved in developmental signaling processes and cytoskeletal function, such as small GTPases.
- A high number of *P. patens* putative prenylation targets do not show similarity to proteins of known function, indicating fruitful avenues of future study to understand the role of prenylation in plants.

Future Study

We plan to use these data to select prenylated proteins with functions of interest for in vivo studies using genetic and molecular tools to investigate their roles in plant development and environmental response.

Acknowledgements

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