Investigating the Role of the Circadian Clock Genes *PRR5*, *PRR7*, and *PRR9* in Regulating Plant Immunity

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Abstract

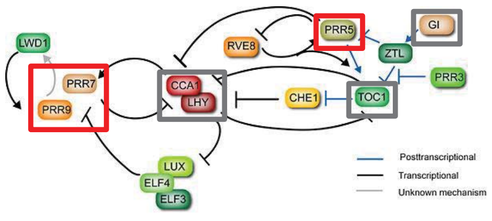
Successful defense against pathogens is critical for plant survival. Recent studies have shown that the circadian clock, the internal time measuring machinery, is involved in disease resistance in addition to its roles in plant development. One such protein, LUX, binds to the promoters of the clock genes *PRR5, 7,* and *9;* and we have preliminary results to show that these genes are involved in defense. To confirm if these genes affect SA-mediated defense, we introduced individual single mutants of the genes *PRR5, 7,* and *9* into *acd6-1*, a small mutant plant with constitutive defense whose size change predicts the defense levels. We have isolated the double mutants (*acd6-1prr5, acd6-1prr7, acd6-1prr9*), two triple mutants (*acd6-1prr5prr9* and *acd6-1prr7prr9*), and the quadruple mutant (*acd6-1prr5prr7prr9*). We are currently assessing the plant phenotypes by measuring their sizes, cell death levels, SA levels, and the expression of defense genes. Analysis of *acd6-1* phenotype suppression, if any exists, will show whether the *PRR5, 7,* and *9* genes act in a synergistic manner in the SA pathway. Significant phenotypic recovery would be evidence for roles of these genes in defense control.

Introduction

- The circadian clock is known to regulate the immune response in *Arabidopsis thaliana.*
- Salicylic acid (SA) is a key signalling hormone in plant defense.
- Preliminary results from the Lu lab show that the circadian clock genes *PRR5, PRR7,* and *PRR9* have a role in regulating the immune response in *A. thaliana.*

Project questions:

- ❖ Do the homologous genes *PRR5, PRR7,* and *PRR9* regulate the immune response through SA?
- ❖ If so, is the regulation through one pathway, or different pathways?



Grey boxes: genes known to regulate immune response
Red boxes: genes of interest

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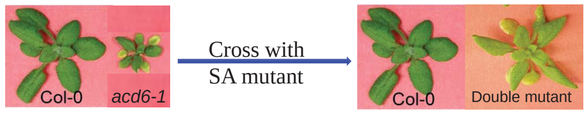
acd6-1 is a useful defense mutant whose size correlates to defense level. Knocking out a gene involved in SA-mediated defense within this background will lead to a reduction of phenotype.



- Constitutive defense
- Severe cell death
- Extreme dwarfism
- High levels of SA

- Lower defense
- Less cell death
- Increased size
- Lower SA level

Approaches and Results

We obtained knockout mutants for each *PRR* gene, and crossed the mutations into the *acd6-1* background. We genotyped seedlings by PCR to find individuals homozygous for each combination of mutations. In addition to the double mutants (*acd6-1* and one *PRR* gene knockout), we also identified two triple mutants and the quadruple mutant.

Table 1: *acd6-1 PRR* Gene Mutants Identified

acd6-1prr5-1	*acd6-1prr5-1prr9-1*
acd6-1prr7-3	*acd6-1prr7-3prr9-1*
acd6-1prr9-1	*acd6-1prr5-1prr7-3prr9-1*

We visually screened changes in the mutants. Most of the phenotypic differences between the strains were suppressed by the growth conditions, but there were small differences in leaf color and shape between the genotypes.

Table 2: Initial Phenotype Results (obtained 02/07)

Genotype Name	Phenotype	Genotype Name	Phenotype
Colombia-0	Dark green leaves, rounded shape	*acd6-1*	Light green, elongated leaves
acd6-1prr5-1	Light green, elongated leaves	*acd6-1prr5-1prr9-1*	Leaves slightly less light green than acd6-1
acd6-1prr7-3	Light green, elongated leaves	*acd6-1prr7-3prr9-1*	Did not grow
acd6-1prr9-1	Darker, more rounded leaves	*acd6-1prr5-1prr7-3prr9-1*	Lighter, more rounded leaves

We have planted another set of mutants and will collect more quantitative phenotype data beginning 04/17. We plan to measure:

- Size
- Cell death in leaves
- Defense levels as seen by callose deposition in leaves
- SA levels
- Expression of the defense gene *PR1* (by measuring the amount of its mRNA)

We also plan to measure defense levels by measuring each strain's resistance to infection by bacterial (*Pseudomonas syringae*) and oomycete (*Hyaloperonospora arabidopsidis*) pathogens.

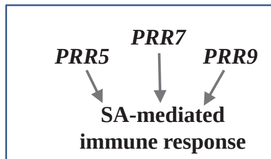
Acknowledgments

We would like to thank William Angel and the rest of the Lu lab for assistance in running experiments. We would also like to thank Dr. McClung at Dartmouth College for providing us with *PRR* gene knockouts.

This project was supported by a grant from the National Science Foundation (NSF 1456140) to H. L. and an Undergraduate Research Award from UMBC to A. H.

Discussion

If the loss of a gene suppresses the *acd6-1* phenotype and exhibits a return toward wild type (Columbia-0) phenotype, then that gene positively regulates the SA-mediated defense.



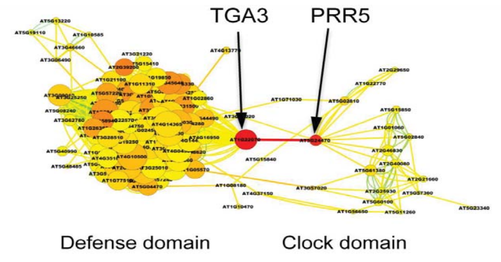
Predicted model for the role of *PRR5, PRR7,* and *PRR9* in regulating SA-mediated defense.

Col-0 | *acd6-1prr5-1* *prr7-3* *prr9-1* | *acd6-1prr5-1* *prr9-1,* *acd6-1prr7-3* *prr9-1* | *acd6-1prr9-1,* *acd6-1prr5-1,* *acd6-1prr7-3* | *acd6-1*

Chart showing the expected phenotypic recovery. Spacing of mutants along the axis shows recovery relative to other mutants.

Future Directions

- Ongoing: quantitatively measure mutant phenotypes
  - Are the *PRR* genes involved in regulating the SA-mediated immune response?
  - What pathways do they use?
- What are the downstream targets of these genes?
  - Bioinformatics analysis suggests the gene *TGA3* may be connected to *PRR5*.
  - *TGA3* is a transcription factor controlling the expression of many SA-mediated immune response genes.
  - It may be regulated by a circadian component.



References

Farre Lab website, http://farrelab.openwetware.org/Research.html

Ng, G., S. Seabolt, C. Zhang, S. Salimian, T. A. Watkins, and H. Lu. "Genetic Dissection of Salicylic Acid-Mediated Defense Signaling Networks in Arabidopsis." *Genetics* 189.3 (2011): 851-59.