DISSECTING TRANSCRIPTIONAL REGULATION OF *RPP8* IN *ARABIDOPSIS THALIANA*

BY

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ABSTRACT

Plants have evolved physical barriers and inducible defense responses to combat microbial pathogens. Inducible responses are mediated by R proteins, which recognize invading pathogens. R proteins must be precisely regulated to provide effective resistance, without inhibiting normal plant growth. However, little is known about *R* gene regulation under defense-inducing conditions. The interaction between the oomycete *Hyaloperonospora parasitica* and the model plant *Arabidopsis thaliana* provides an excellent model system to explore *R* gene regulation. My research focuses on *RPP8*, a CC-NBS-LRR gene, which provides resistance to the *H. parasitica* isolate Emco5. Previous work in the McDowell lab suggested that *RPP8* is upregulated during defense responses. My research shows that *RPP8* alleles from the Columbia and Landsberg *erecta* ecotypes are upregulated by *H. parasitica* and the defense signaling molecule salicylic acid, suggesting a potential feedback loop. *RPP8-Ler* is also systemically upregulated after infection of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. Additionally, *RPP8-Ler* expression is increased during wounding and heat stress. I also examined the role of regulatory *cis* elements in the *RPP8* promoter. Three W-boxes are essential for basal and inducible *RPP8* expression, and are required for resistance to Emco5. The X-box, a unique *cis* element in the *RPP8* promoter, is essential for strong basal expression and wound-induced upregulation, and affects spatial expression of *RPP8-Ler*. However, the X-box is not required for *RPP8-*

Ler upregulation during pathogen or SA treatment. *R* genes may be induced as part of global defense responses, which could prime the host for more effective pathogen recognition.

Dedication

To Ben, for everything.

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Glossary

- *ACT2* – Arabidopsis actin gene, used as a normalization control in real-time and semiquantitiative PCR experiments.
- Avirulent pathogen – A pathogen that is recognized by plant gene-for-gene resistance, and is therefore unable to cause disease.
- *Avr* – (Avirulence determinant) A pathogen gene product recognized by a host resistance protein; such an interaction activates defenses.
- Basal resistance – Defenses activated in susceptible plants to limit the spread of infection. Basal responses are weak and slow, compared to gene-for-gene resistance.
- Benzothiadiazole – (BTH) A synthetic analog of salicylic acid.
- *Blumeria graminis* f. sp. *hordei* (*Bgh*) – Barley powdery mildew.
- CC-NBS-LRR – A major structural class of R genes, composed of 3 domains: 1) CC = coiled coil domain, 2) NBS = nucleotide binding site, and 3) LRR = leucine-rich repeats.
- *Brassica oleracea* – Cabbage species.
- ChIP – Chromatin immunoprecipitation.
- Col-0 – (Columbia) Ecotype of Arabidopsis.
- CMV-Y – Cucumber mosaic virus strain Y.
- CW84 – Arabidopsis hybrid, derived from a cross between Col and Ws. This line was selected for non-functional alleles of several *RPP* genes.
- DC3000 – Strain of *Pseudomonas syringae*, virulent on the Columbia ecotype.

- Di-17 (Dijon 17) – Line of Arabidopsis derived from the Dijon ecotype. This line was selected for consistent HR, *PR* gene expression, and SA accumulation after inoculation of turnip crinkle virus.
- *EDS1* – (Enhanced disease susceptibility 1) A signaling protein required for the function of many TIR-NBS-LRR R proteins.
- *EDS5* – (Enhanced disease susceptibility 5) A signaling protein required for basal and gene-for-gene resistance to multiple pathogens.
- Emco5 – Isolate of *Hyaloperonospora parasitica,* specifically recognized by *RPP8* in the Ler ecotype.
- EMSA – Electrophoretic mobility-shift assay.
- Ethylene – A plant hormone; involved in some disease resistance pathways.
- flg22 – Purified flagellin protein, used as an elicitor of basal defense responses.
- *FLS2* – Arabidopsis receptor that activates basal defenses upon detection of bacterial flagellin.
- Hiks1 – Isolate of *Hyaloperonospora parasitica* specifically recognized by *RPP7*.
- HR – (Hypersensitive response) A plant response to pathogens that involves host cell death at the infection site. Can be initiated by R proteins.
- *HRT* – (Hypersensitive response to turnip crinkle virus) Allele of *RPP8* in the Di-17 ecotype of Arabidopsis
- *Hpa (Hyaloperonospora parasitica)* – Oomycete pathogen of Arabidopsis thaliana and other *Brassica* species; causes downy mildew disease. Formerly called *Peronospora parasitica*.

- ISR – (Induced systemic resistance) A systemic resistance activated by nonpathogenic rhizobacteria, mediated by jasmonic acid and ethylene.
- JA – Jasmonic acid, a plant compound; involved in some disease resistance pathways.
- Ler-0 – (Landsberg *erecta*) Ecotype of Arabidopsis.
- LUC – (Luciferase reporter enzyme) Reacts with its substrate, luciferin, to produce one photon of light.
- *NahG* – A bacterial salicylate hydroxylase transgene; degrades salicylic acid.
- *NDR1* – (Non-race specific disease resistance 1) A signaling protein required for the function of many CC-NBS-LRR R proteins.
- Nonhost resistance – prevents infection of all known isolates of a pathogen between species.
- *NPR1* – (Non-expresser of PR genes 1) Also known as *NIM1*. A transcriptional regulator of salicylic acid signal transduction; also required for induced systemic resistance.
- *PAD4* – (Phytoalexin deficient 4) A signaling protein that interacts with *EDS1* and regulates SA signaling.
- PAMP – Pathogen-associated molecular pattern.
- *PR* – (Pathogenesis-related) Defense genes activated by salicylic acid and pathogen attack.
- *Pseudomonas syringae* – A bacterial pathogen of numerous crops and Arabidopsis.

- *RAR1* – A defense signaling protein that interacts with *SGT1*, possibly to mediate stability of R proteins.
- *RCY1* – (Resistance to cucumber mosaic virus-Y 1) Allele of *RPP8* in the C24 ecotype.
- ROI – Reactive oxygen intermediate.
- *RPP* genes – Resistance to *Hyaloperonospora parasitica* in Arabidopsis.
- *RPP7* – Mediates resistance to the Hiks1 isolate of *Hyaloperonospora parasitica;* found in the Columbia ecotype of Arabidopsis.
- *RPH8A* – Closely related homolog of *RPP8* in the Landsberg *erecta* ecotype of Arabidopsis; unknown function.
- *RPH8A-LUC* – *RPH8A* translationally fused to luciferase.
- *RPP8-Col* – Allele of *RPP8* in the Columbia ecotype of Arabidopsis; unknown function.
- *RPP8-Ler* – Found in the Landsberg *erecta* ecotype of Arabidopsis; mediates resistance to the *Hyaloperonospora parasitica* isolate Emco5.
- *RPP8-LUC* – *RPP8-Ler* translationally fused to luciferase.
- SA – (Salicylic acid) A phenolic compound; functions as a signaling molecule in systemic acquired resistance and local R protein-mediated defense responses.
- SAR – (Systemic acquired resistance) A long-lasting form of resistance in which the entire plant becomes resistant to a broad range of unrelated pathogens after a localized infection.
- *SGT1* – A defense signaling protein that interacts with *RAR1,* and is associated with ubiquitin-mediated proteolysis.

- SHL – (Spontaneous hypersensitive-response-like lesions) Cell death in the absence of a pathogen.
- TIR-NBS-LRR – A major structural class of *R* genes, composed of 3 domains: 1) TIR = resembles the cytoplasmic domain of Drosophila Toll transmembrane LRR receptor and mammalian interleukin-1 receptor, 2) NBS = nucleotide binding site, and 3) LRR = leucine-rich repeats.
- TCV – Turnip crinkle virus.
- TMV – Tobacco mosaic virus.
- UTR – (Untranslated region) Transcribed regions outside the coding region of a gene.
- Virulent pathogen – A pathogen that is not recognized by plant gene-for-gene resistance pathways, and is therefore able to cause disease.
- W-box – A consensus DNA sequence ([T/C]TGAC[T/C]) recognized by WRKY transcription factors.
- WRKY transcription factors – Family of transcription factors, characterized by the conserved WRKY domain, that interact with W-box *cis* elements; implicated in defensive gene regulation.
- X-box – A promoter sequence (TAGAGAATCCTCGG**C**CAAGAGAT) highly conserved in alleles of *RPP8*; the C at -189 (bolded and oversize) is essential for *RPP8* transcription and function.
- *xbox-LUC– RPP8-Ler* translationally fused to luciferase, with a C to T transition at -189 in the promoter X-box element that greatly reduces transcription.