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 semiquantitative 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 (TAGAGAATTCCTCGGC\textC\textAAGAGAT) 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.