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# **The molecular basis of RPS4/RRS1-mediated defense activation in Arabidopsis**

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## TABLE OF CONTENTS

ABSTRACT.....	6
ACKNOWLEDGMENTS .....	8
LIST OF PUBLICATIONS.....	9
ABBREVIATIONS.....	10
<b>CHAPTER 1: GENERAL INTRODUCTION .....</b>	<b>12</b>
1.1 Introduction .....	12
1.2 PAMP-triggered immunity (PTI) .....	14
1.2.1 Suppression of PTI by effectors .....	15
1.3 Biochemical functions of effectors.....	16
1.4 Effector-triggered immunity .....	20
1.4.1 Plant R proteins.....	20
1.4.2 NLR R proteins.....	21
1.4.3 Mechanisms of pathogen recognition by R proteins .....	25
1.4.3.1 Direct recognition .....	25
1.4.3.2 Indirect recognition: Guard/decoy hypothesis .....	26
1.4.4 Paired R proteins .....	27
1.5 The RPS4/RRS1 NLR complex .....	28
1.5.1 The <i>avrRps4/RPS4</i> gene-for-gene model.....	28
1.5.2 The <i>popP2/RRS1</i> gene-for-gene model.....	30
1.5.3 Recognition of multiple effectors .....	32
1.5.4 RRS1- and RPS4-independent AvrRps4 recognition (RRIR) .....	36
1.6 Genes required for ETI Signaling.....	36
1.7 Transcriptional repression in immunity.....	38
1.7.1 The EAR motif.....	39
1.8 Aims of the study.....	39
<b>CHAPTER 2: MATERIALS AND METHODS .....</b>	<b>41</b>
2.1 Plant materials .....	41
2.2 Primers used .....	41
2.3 Bacterial strains.....	53
2.4 Growth conditions .....	59
2.5 Cross-fertilization of Arabidopsis.....	59
2.6 Media .....	59

2.6.1	L .....	59
2.6.2	King's B .....	59
2.6.3	Murashige-Skoog (MS) .....	60
2.7	Antibiotics .....	60
2.8	Plant pathology .....	60
2.8.1	Arabidopsis infection .....	60
2.8.1.1	Hypersensitive response (HR) assay .....	60
2.8.1.2	Ion leakage assay .....	60
2.8.1.3	<i>In planta</i> bacterial growth assay.....	61
2.9	Molecular biology .....	61
2.9.1	DNA.....	61
2.9.1.1	Polymerase chain reaction (PCR).....	61
2.9.1.2	Chelex plant genomic DNA extraction .....	61
2.9.1.3	Plasmid DNA preparation.....	62
2.9.1.3.1	Manual protocol.....	62
2.9.1.3.2	Axygen Plasmid Miniprep kit protocol .....	62
2.9.1.4	Electrophoresis of DNA.....	63
2.9.1.5	Purification of DNA from agarose gel .....	63
2.9.1.6	Ligation.....	63
2.9.1.7	Preparation of electrocompetent <i>Escherichia coli</i> (DH5alpha) and <i>Agrobacterium tumefaciens</i> (AGL1) cells .....	63
2.9.1.8	Transformation of competent <i>E. coli</i> and <i>A. tumefaciens</i> cells .....	64
2.9.1.9	Site-directed mutagenesis.....	64
2.9.1.10	Colony PCR .....	65
2.9.1.11	DNA sequencing .....	65
2.9.1.12	Golden Gate cloning .....	65
2.9.1.13	Triparental mating .....	65
2.9.2	RNA.....	66
2.9.2.1	Total RNA extraction .....	66
2.9.2.2	Reverse transcription PCR (RT-PCR).....	67
2.9.2.3	qRT-PCR.....	67
2.9.3	Protein.....	67
2.9.3.1	Protein expression <i>in planta</i> .....	67
2.9.3.1.1	<i>Agrobacterium</i> -mediated transient transformation .....	67
2.9.3.1.2	Arabidopsis stable transformation.....	68

2.9.3.2	Total protein extraction from plant tissue and Western blot .....	68
2.9.3.3	Co-immunoprecipitation (CoIP) assay .....	69
2.10	Yeast-two-hybrid (Y2H) assays.....	69

**CHAPTER 3: A conserved EAR motif is required for avirulence and stability of the *Ralstonia solanacearum* effector PopP2 *in planta*.....70**

3.1	Introduction .....	70
3.2	Results .....	74
3.2.1	PopP2 is highly conserved among Korean <i>R. solanacearum</i> isolates and harbors a putative transcriptional repressor motif.....	74
3.2.2	Only one of the newly identified PopP2 variants loses avirulence function <i>in planta</i> .....	77
3.2.3	The conserved EAR motif is required for PopP2 avirulence activity in Arabidopsis. ....	79
3.2.4	The conserved EAR motif is required for PopP2-mediated PTI suppression. ....	85
3.2.5	PopP2 does not interact with known Arabidopsis transcriptional co-repressors in yeast. ....	86
3.2.6	The EAR motif is required for PopP2 stability <i>in N. benthamiana</i> .....	87
3.3	Discussion.....	90
3.3.1	PopP2 natural variation in virulent <i>R. solanacearum</i> strains.....	90
3.3.2	EAR motif-dependent protein stability control .....	91
3.3.3	Possible mechanisms of PopP2 EAR motif function.....	93

**CHAPTER 4: Characterization of *SUSHI* mutations in the *RRS1* disease resistance gene.....95**

4.1	Introduction .....	95
4.2	Results .....	97
4.2.1	Characterization of intragenic suppressors of <i>slh1</i> -mediated immunity	97
4.2.2	<i>RRS1 SUSHI</i> mutations differentially affect RPS4-dependent <i>RRS1</i> <sup>SLH1</sup> auto-activity and effector recognition in tobacco .....	103
4.2.3	<i>RRS1</i> <sup>C1243Y</sup> auto-activity in tobacco displays distinct features from other auto-active <i>RRS1</i> variants.....	107
4.2.4	The NB-ARC and LRR domains are required for <i>RRS1-R in trans</i> interference with auto-activity in tobacco.....	109
4.2.5	<i>RRS1</i> TIR domain <i>SUSHI</i> residues are required for TIR domain function and heterodimer formation with RPS4 .....	111
4.2.6	The NB-ARC and LRR domain <i>SUSHI</i> residues that are conserved in <i>RRS1B</i> are required for function in tobacco .....	116

4.2.7	<i>RRS1 SUSHI</i> mutations do not affect TIR domain-C-terminus intramolecular interaction.....	121
4.3	Discussion.....	125
4.3.1	Identification of <i>RRS1 sushi</i> mutants and tobacco HR characterization. 125	
4.3.2	Characterization of the auto-active <i>RRS1</i> <sup>C1243Y</sup> variant.....	126
4.3.3	The effect of <i>SUSHI</i> mutations on <i>in trans</i> interference and inter/intramolecular <i>RRS1/RPS4</i> interactions.....	128
4.3.4	<i>RRS1B</i> corresponding <i>SUSHI</i> mutations.....	128
4.3.5	Models of the effect of <i>RRS1 SUSHI</i> mutations.....	129
4.3.6	Is <i>RRS1</i> a functional transcription factor?.....	132
<b>CHAPTER 5: Investigating the RPS4 TIR domain interfaces required for defense signaling .....</b>		<b>135</b>
5.1	Introduction .....	135
5.2	Results .....	136
5.2.1	R116A and M150R mutations disable RPS4 TIR domain auto-activity 136	
5.2.2	R116A and M150R mutations do not affect RPS4 homodimerization or RPS4/ <i>RRS1</i> heterodimerization in a CoIP assay .....	139
5.2.3	R116A and M150R mutations impair RPS4 full-length signaling .....	139
5.3	Discussion.....	144
<b>CHAPTER 6: GENERAL DISCUSSION AND OUTLOOK .....</b>		<b>146</b>
6.1	Summary of findings .....	146
6.2	Comparison to other systems .....	147
6.3	Outlook.....	149
REFERENCES .....		152

## ABSTRACT

Upon pathogen invasion, each plant cell has the ability to mount an innate immune response. Plants have evolved *R* genes, which typically encode nucleotide-binding domain and leucine-rich repeat-containing immune receptors (NLRs). The model plant species, *Arabidopsis*, harbors the paired NLRs, *RPS4* and *RRS1*, the products of which function cooperatively to confer recognition of the *Pseudomonas syringae* effector, AvrRps4, and the *Ralstonia solanacearum* effector, PopP2. The exact mechanism underlying RPS4/RRS1-mediated effector recognition remains unclear; therefore, the function of RPS4 and RRS1 was further elucidated.

Firstly, by investigating the avirulence activity of natural variants of PopP2 isolated from *R. solanacearum* strains from across the Republic of Korea, *popP2* was demonstrated to be well-conserved and RPS4/RRS1-mediated recognition of PopP2 could tolerate multiple natural polymorphisms in the *popP2* sequence. Moreover, a conserved PopP2 EAR motif was identified and characterized; the EAR motif was shown to be required for *in planta* PopP2 stability and recognition.

Secondly, utilizing *suppressor of slh1 immunity (sushi)* mutants generated in a forward genetic screen on *slh1* mutant seeds, insight was gained into the differential requirements for RRS1 auto-activity and effector perception. A leucine-rich repeat (LRR) mutation, L816F, was identified, which affected auto-activity but not effector recognition. Furthermore, a WRKY domain mutation, C1243Y, was identified, which conferred auto-activity with distinct features compared to other known auto-active RRS1 variants. Notably, a TIR mutant harboring a C15Y mutation was identified that impaired RPS4/RRS1 TIR/TIR heterodimer formation and full-length RRS1 function.

Finally, an analogous self-association interface (DE) identified in the crystal structure of the TNL, SNC1, was investigated for its role in RPS4 function. It was demonstrated that the DE interface mutations, R116A and M150R,

disabled RPS4 TIR domain effector-independent cell death induction and impaired full-length RPS4 signaling.

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All research conducted by myself unless otherwise stated in figure legends.

## LIST OF PUBLICATIONS

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Work in this thesis contributed to the publications below.

Zhang, X., Bernoux, M., Bentham, A.R., **Newman, T.E.**, Ve, T., Casey, L.W., Raaymakers, T.M., Hu, J., Croll, T.I., Schreiber, K.J., Staskawicz, B.J., Anderson, P.A., Sohn, K.H., Williams, S.J., Dodds, P.N., Kobe, B. (2017). Multiple functional self-association interfaces in plant TIR domains. *Proceedings of the National Academy of Sciences*, p.201621248.

Segonzac, C.<sup>†</sup>, **Newman, T.E.**<sup>†</sup>, Choi, S., Jayaraman, J., Choi, D.S., Jung, G., Cho, H., Lee, Y.K., Sohn, K.H. (In Revision). A conserved EAR motif is required for avirulence and stability of the *Ralstonia solanacearum* effector PopP2 *in planta*. *Frontiers in Plant Science*.

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## ABBREVIATIONS

aa: amino acids

Avr: avirulence

bp: base pair

CC: coiled-coil

cDNA: complementary deoxyribonucleic acid

cfu: colony forming unit

DNA: deoxyribonucleic acid

dpi: day post-inoculation

EDS1: enhanced disease susceptibility 1

ETI: effector-triggered immunity

HR: hypersensitive response

kb: kilobase

kDa: kilodaltons

LRR: leucine-rich repeat

ml: milliliter

mg: milligram

mM: millimolar

NLR: nucleotide-binding domain and leucine-rich repeat-containing protein

NLR-ID: NLR-integrated domain

OD: optical density

PAMP: pathogen-associated molecular pattern

*Pf. Pseudomonas fluorescens*

PCR: polymerase chain reaction

PR: pathogenesis-related

PRR: pattern recognition receptor

PTI: PAMP-triggered immunity

*Pto. Pseudomonas syringae pv. tomato*

R: resistance

RNA: ribonucleic acid

ROS: reactive oxygen species

RPS4: resistance to *Pseudomonas syringae* 4

RRS1: resistance to *Ralstonia solanacearum* 1

RT-PCR: reverse transcription polymerase chain reaction

SA: salicylic acid

*slh1* mutant: *sensitive to low humidity 1* mutant (single leucine insertion in *RRS1* WRKY domain)

*sushi* mutant: *suppressor of slh1 immunity* mutant

TAE: tris acetate EDTA

Tris: tris(hydroxymethyl)aminomethane

TTSS: type-three secretion system

μl: microliter

μM: micromolar

WT: wild type