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

TOBY EDWARD NEWMAN

Institute of Agriculture and Environment
Massey University

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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 analagous 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


Work in this thesis contributed to the publications below.


† These authors contributed equally
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