XVI Congress on Molecular Plant-Microbe Interactions Meeting Report

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The IC-MPMI was held in Rhodes, Greece from July 6-10, 2014. A new feature of the meeting was the inclusion of a “Student Travel Workshop”. In honor of our late colleague Ko Shimamoto, a student travel fellowship program was initiated to enrich graduate student and postdoctoral participation at this congress. The fellowship program was generously supported by IS-MPMI and two government agencies in the USA—the National Science Foundation and the United States Department of Agriculture. Sixty-five fellows spanning all continents participated in the workshop and various networking activities. The fellows contributed to the dissemination of the meeting findings by releasing daily scientific highlights via social networking. A group of fellows were selected to summarize their perspectives of the meeting highlights to share this information with our broader IS-MPMI community. A snapshot of the exciting work presented at IC-MPMI in Rhodes is summarized below. Contributions to this meeting report were made by: Cris Argüeso, Bethany Huot, Panya Kim, Derek Lundberg, Morgan Halane and Irene Serrano, in collaboration with two IS-MPMI Board members, Roger Innes and Mary Beth Mudgett.

The Plant Microbiome.

Plant microbiome research is an effort to understand plant-microbe interactions in the context of the more complicated microbial communities that are the norm in nature. On crops and wild plants, numerous microbes – many of which have never been cultured - interact not only with the plant, but also with each other. A recent expansion in this field results largely from falling costs of DNA sequencing, which have allowed detection and quantification of microbial communities at ever-higher resolution. For the first time, MPMI dedicated a concurrent session to talks related to plant microbiome research. The session was highly attended.

Paul Schulze-Lefert, at the Max Planck Institute for Plant Breeding Research (MPI-PBR) in Germany, opened the session. Paul presented new work characterizing barley root and

rhizosphere microbiomes, and contrasted them to those of *Arabidopsis thaliana*, which he presented at IC-MPMI in 2012. Shotgun sequencing of all organisms in the barley rhizosphere revealed enrichment of type III and IV secretion systems compared to surrounding soil, suggesting a wealth of unexplored molecular and effector biology in these rhizosphere organisms. Barley genotype explained about 6% of the variability in bacterial root microbiomes, lighting the path for deeper genetic work. Paul presented strong evidence that culturing is not a thing of the past. The majority of bacteria associated with roots appear to be culturable. His group is culturing and sequencing full genomes from over 1,000 root-associated bacteria from *A. thaliana*.

Gerald Tuskan, at the Oak Ridge National Laboratory in the USA, continued along similar veins with an overview of popular root microbiomes, including bacteria (using 16S rRNA amplicons) and fungal groups (using Internal Transcribed Spacer amplicons). His group is also culturing to complement their sequencing work and finding it a fruitful enterprise. Gerald demonstrated that some root bacteria enhance poplar growth in low nitrogen. Similar to barley, about 6-10% of poplar’s variability in microbial communities is explained by genotype. A major highlight was that Gerald’s team used the natural variation in wild poplar to map a gene for *Laccaria* mycorrhizal association to a D-mannose binding receptor kinase, and showed that expression of this same gene in *A. thaliana* allowed *Laccaria* to associate with *A. thaliana* roots. This type of project, using microbial abundance as a phenotype to map underlying host genes that can then be validated, provides a strong model moving forward to elucidate the molecular basis of complex plant-microbe associations.

Sarah Lebeis, at the University of Tennessee-Knoxville in the USA, described a reverse genetic approach to study the microbiome, using *A. thaliana* mutants with defects in genes controlling immune system components (e.g., phytohormones, glucosinolates, and NLR genes) to study effects on root bacterial microbiomes. The microbiomes of these *A. thaliana* mutants were mostly similar to each other, with differences seen only for specific microbes or families of microbes, although mutants in phytohormone pathways, or with severe defects (e.g., *eds1* mutants) allowed bacteria not normally found on roots to colonize.

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Matthew Agler, a postdoctoral scholar with Eric Kemen at the MPI-PBR, described an experiment to test how *Albugo laibachii* infection of *A. thaliana* leaves affects the existing community of eukaryotic and prokaryotic microorganisms. For both wild plants and field experiments, *Albugo* infection substantially lowered microbial diversity, and *Pseudomonas* and *Burkholderia* in particular took over.

Christos Zamioudis, at the University of Utrecht in the Netherlands, presented a more reductionist angle to the session, describing an intriguing study where volatile organic compounds emitted from *Pseudomonas fluorescens* WCS417 in the rhizosphere increased a novel photosynthesis-dependent iron uptake mechanism in *A. thaliana* roots. This response first appeared to resemble a pathogenic interaction; however, Christos provided evidence that manipulation of iron homeostasis by these microbes improves the iron content of plants, thereby enhancing plant fitness in iron limiting soils.

The session closed with doctoral student James Kremer with Sheng Yang He at the Plant Research Laboratory (PRL) at Michigan State University in the USA. James described his RNA-seq studies of *A. thaliana* grown in a gnotobiotic system. He compared uninoculated plants with plants inoculated with microbes from several soil types. His observations thus far indicate that transcripts for WRKY transcription factors, LRR proteins, PTI marker genes, and superoxide dismutases are all down-regulated in sterile plants and the response to MAMPs is delayed. Ongoing work in the laboratory is to characterize the role of the microbiome in influencing plant growth and immunity by removing the entire microbiome.

Every session speaker presented some unpublished data, which is evidence of a healthy field with diverse projects. The combination of “zoomed-out” microbiome techniques to explore or explain molecular or genetic phenomena will be critical as the MPMI community moves towards translation of biology into products and interventions. It is safe to conclude that this session will continue at IC-MPMI in 2016.

Hormones and defense signaling.

A key message at this congress was the importance of plant hormones in achieving specificity, timing and balance in plant-pathogen interactions. The canonical defense hormones salicylic acid (SA) and jasmonates (JA) have been shown to be preferentially activated by biotrophic and hemi-biotrophic pathogens, or by necrotrophic pathogens and herbivorous insects, respectively, to enable invader-specific responses. The signaling pathways controlled by these two hormones have also been shown to be generally antagonistic, and Corné Pieterse, at the University of Utrecht University, presented evidence demonstrating that this is mediated at least in part by ORA59 degradation, which enables SA signaling at the expense of JA signaling when both pathways are simultaneously induced. However, Kenichi Tsuda, at MPI-PBR in Germany, showed data from a genetic study used to generate a dynamic network model indicating a positive role of JA in SA signaling, which was confirmed by measuring both SA levels and *PR1* gene expression. It was proposed that the activation of different combinations of the immune response sectors enables both specificity and robustness to the plant defense response.

In addition to regulating pathogen-specific responses, hormone crosstalk is also involved in modulating growth, defense and environmental tradeoffs to maximize plant fitness. David De Vleeschauwer, at the University of Ghent in Belgium, proposed these growth-defense tradeoffs are mediated by mutual antagonism between TOR, which promotes growth hormone signaling, and SnRK1, which promotes defense hormone signaling. Several talks demonstrated pathogen manipulation of growth hormone signaling to suppress defense, such as Avr2

promotion of brassinosteroid (BR) signaling (Eleanor Gilroy at the James Hutton Institute at the University of Dundee in the UK), pathogen-induced increases in abscisic acid (ABA) used in conjunction with HrpA to facilitate suppression of photosynthesis and defense (Murray Grant at the University of Exeter in the UK), and ABA2 dampening of MPK3 and MPK6 signaling during PTI (Kenichi Tsuda at the MPI-PBR in Germany). Abiotic stresses can also affect hormone crosstalk to negatively impact defense responses. Cold-induced elevation of ABA was shown to result in PTP-mediated antagonism of MAPK activation resulting in suppression of WRKY45 and SA-mediated defense in rice (Hiroshi Takatsui at the National Institute of Agrobiological Sciences in Japan). While ABA has also been shown to play a role in inhibition of resistance protein-mediated defense at high temperature, this effect was shown to be SA-independent. Moreover, Bethany Huot, a doctoral student with Sheng Yang He, reported the impact of high temperature on SA-mediated defense. She showed that the ability of the SA analog, benzothiadiazole (BTH), to induce SA marker genes and SA production was blocked, but BTH protection against a hemi-biotrophic pathogen was still observed. While BTH-mediated resistance at high temperature was uncoupled from induction of classical SA-signaling markers, it was shown to require NPR1, the key regulator of SA signaling, suggesting that a subset of SA signaling components are still functional and are sufficient to confer resistance under these conditions.

Other talks covered advances in SA- and JA-mediated defense signaling. Thomas Griebel, a postdoctoral scholar from Jane Parker's laboratory (MPI-PRL in Germany), showed data supporting a role of TPR1, a member of the TOPLESS family of transcriptional repressors, in coordinating transcriptional reprogramming in conjunction with EDS1. Chika Tateda, a doctoral student in Jean Greenberg's laboratory (University of Chicago in the USA) explored the role of SA during PTI by studying the effect of BTH on the formation and accumulation of receptor-like kinase (RLK) complexes at the plasma membrane. BTH was shown to potentiate RLK complex formation in an ACD6-dependent manner to promote PTI. Potential SA receptors and mobile systemic acquired resistance (SAR) signals were also discussed. Xinnian Dong, at Duke University in the USA, demonstrated the roles of NPR3 and NPR4, two putative intracellular SA receptors, in regulating the stability of nuclear localized NPR1 protein in a SA-concentration-dependent manner to facilitate cell survival and SAR in uninfected cells while enabling programmed cell death in infected plant cells. Nobuaki Ishihama, a postdoctoral scholar from Ken Shirasu's laboratory (RIKEN Center for Sustainable Resource Science in Japan) presented data from a high-throughput screen of chemical agonists and antagonists of SA signaling leading to the discovery of the CUPIN1 protein, which is similar to the auxin receptor protein, ABP1. Crystal structure analysis showed that the P7 compound binds, perhaps competitively, to the CUPIN1 protein to block SA signaling, whereas the A2 compound increases CUPIN1 activity and promotes SA signaling. While the identity of the mobile SAR signal is still highly controversial, Jürgen Zeier, at Heinrich Heine-University of Düsseldorf in Germany, presented evidence showing the importance of pipelicolic acid (Pip) for the establishment of SAR, and proposed a model whereby Pip serves as the mobile signal with SA as the amplifier to mediate full SAR.

On the JA-side of defense, Corné Pieterse at the University of Utrecht in the Netherlands, presented data from an RNA-sequencing based time series study aimed to establish a JA signaling network by incorporating timing with function. As a result of this study, 27 gene clusters and 6 distinct phases in JA

signaling were identified as well as novel regulators, including new MYC and MYB transcription factors. In addition, Sheng-Yang He presented the structural analysis of a MYC3-JAZ9 co-crystal, which revealed the JAZ-binding domain of MYC3 as well as a mechanism of transcriptional repression whereby binding of MYC3 to JAZ9 blocks its interaction with the transcriptional co-regulator complex, MEDIATOR.

These and other studies shared at this congress lend insight into outstanding questions involving the role of hormones in achieving specificity, timing and balance in plant-microbe interactions

Epigenetic regulation of defense.

In eukaryotic genomes, gene expression and DNA recombination are affected by structural chromatin traits. Chromatin structure is shaped by the activity of enzymes that either introduce covalent modifications in DNA and histone proteins, or disrupt histone-DNA interactions. These genomic traits are susceptible to being altered in response to stress against pathogens. David Baulcombe, at the University of Cambridge in the UK, gave a plenary lecture on RNA silencing and epigenetics in the context of plant defense responses. David's group screened for *Arabidopsis* mutants defective in initiation of RNA-directed DNA methylation. From the results of this screen, they concluded that RNA polymerase V and 21-22 nucleotide small interfering RNAs (siRNAs) are required for such DNA methylation, while RNA polymerase IV and 24 nt siRNAs are not. Significantly, his group also determined that gametogenesis is required to establish this silenced state as the DNA modifications did not appear until the generation following the initiation event (infection by Tobacco Rattle Virus). This phenomenon appears to be mechanistically related to "paramutation" in which a silenced gene in one parent silences the homologous allele in the other parent in F2 progeny of a cross between silenced and non-silenced parents. The significant take-home from this work is that alterations in gene expression induced by a biotic stress can be heritable, thus the biotic environment of a plant's grandparents can influence the defense responses of the grandchildren.

Lionel Navarro, at IBENS in Paris, continued on the theme of epigenetic inheritance of disease resistance. His group is studying the roles played by siRNAs in regulation of plant immunity, and the links between regulation of defense gene expression and transcription of transposable elements. The siRNA-directed DNA methylation pathway is needed to silence transcription of transposable elements, and Lionel's group has shown that the same DNA methylation pathway that keeps transposons silenced also acts to keep many defense genes expressed at a low basal state. Significantly, demethylation of defense genes acts to "prime" them for rapid activation, and demethylation is controlled in part by past exposure to biotic stress. Confirming David Baulcombe's work, such demethylated states can be heritable.

Departing from the topic of DNA methylation, Thomas Eulgem, at the University of California-Riverside in the USA, described a transposon-associated mechanism for controlling transcription of an NB-LRR immune receptor in *A. thaliana*. In that work, it was found that a retroelement insertion in the 3' end of the *RPP7* disease resistance gene influenced the choice of polyadenylation sites, with one site producing a non-functional product. Polyadenylation site choice was determined by a specific histone modification (H3K9me2) caused by the retroelement. Interestingly, this histone modification was responsive to biotic stresses, thus expression of functional *RPP7* was subject to prior biotic stress history as a direct result of a retroelement insertion.

Mark Gijzen at the Agriculture and Agri-Food Canada described effects on host immunity by epigenetic gene silencing

in pathogens. Based on his research, pathogens evolve rapidly in response to host or environmental challenges. Clonal and invasive species adapt quickly despite a lack of genetic diversity. Data from his group suggest that evasion of host immunity can result from silencing of virulence effectors; thus, epigenetic reprogramming of gene expression can aid in pathogen adaptation.

Defense priming.

Although there were separate sessions at XV IC-MPMI dedicated to epigenetics and priming, an exciting conclusion of the talks from both sessions is that epigenetics plays a central role in priming. Ana Sanchez presented her work conducted at Pablo Vera's lab at the Technical University of Valencia in Spain. Ana discussed the mechanisms regulating trans-generational-induced immunity priming, where the progeny of *A. thaliana* plants that have been previously exposed to priming agents also show reduced susceptibility to pathogens. She presented data showing that decreased susceptibility to biotrophic pathogens in plants displaying trans-generational-induced immunity priming is followed by activation of SA-regulated genes, at the expense of JA-regulated genes. Using mutants of RNA-dependent DNA methylation pathways, Ana established that priming requires DNA hypomethylation, consistent with the conclusions of David Baulcombe and Lionel Navarro. Her research has also uncovered hypo-methylated epi-alleles that maintain trans-generational priming over subsequent generations, specifically at regions of the genome that contain genes encoding transposable elements. The role of transposable elements in defense priming however is not clear. Pablo Vera's laboratory is now exploring the role of trans-generational-induced immunity priming on defense responses against necrotrophic pathogens.

Uwe Conrath, at the RWTH Aachen University in Germany, presented exciting new work on the epigenetics of SA-dependent defense priming in *A. thaliana*. In prior work using northern blot analysis, the group showed that while some genes are directly up-regulated by SA treatment, others are up-regulated only upon exposure to pathogens, defining candidate "primed" genes. One of these genes, *WRKY29* was analyzed for epigenetic modifications after priming induction. Acetylation of Histone H3 at lysine residues 9 and 12 was found to be important for *WRKY29* priming. Interestingly, Histone H3 acetylation at the *WRKY29* locus is dependent on NPR1, pointing to a role for this critical defense regulator in the epigenetic control of priming activation. The epigenetic modifications at *WRKY29* in the *sncl* and *cpr1* mutants, which display constitutive defense responses, were found to be most often associated with Histone H3 tri-methylation at lysine residue 4. These data indicate that defense activation status is linked to different epigenetic signatures. Using FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements), a chromatin occupancy technique adapted from yeast, Uwe's laboratory has now begun probing genome-wide chromatin status upon priming induction. The ability to detect epigenetic changes and associated regulatory elements at the whole genome level is expected to substantially increase our knowledge of the epigenetic mechanisms controlling priming activation.

Jürgen Zeier, at the University Düsseldorf in Germany, discussed the role of pipecolic acid (Pip) in the establishment of priming and SAR in *A. thaliana*. Pip is a lysine catabolite that was previously shown to accumulate in primary and systemic leaves after pathogen inoculation, suggesting a possible role for this compound in priming and SAR. Pip appears to act as a real priming agent, as Pip inoculation is not sufficient for direct activation of defense genes. Pathogen attack is still required to initiate defense activation. Using GC/MS, Jürgen's

group performed metabolite profiling of leaf exudates of *A. thaliana* leaves infected with *Pst*. Plants that were treated with Pip by soil drenching showed less susceptibility to *Pst* and activation of priming as early as 10 hours post-inoculation. RNAseq experiments confirmed these results and indicate that most Pip-regulated genes are SA-independent. The current model predicts that upon pathogen attack lysine is catabolized by ALD1, a lysine aminotransferase, leading to the formation of Pip. Pip is then translocated systemically throughout the plant, where upon pathogen exposure it leads to the activation of the SA-independent defense gene expression. Scientists have long proposed that priming of defense responses is somehow associated with changes in energy partitioning for increased defense activation. The work by Jürgen's laboratory provides a link between primary metabolism (i.e., amino acid metabolism) and activation of priming, paving the way for more exciting discoveries in this field.

Finally, Jurriaan Ton, at the University of Sheffield in the UK, reported on the identification of the receptor for one of the most well studied priming agents, the non-protein amino acid, β -aminobutyric acid (BABA). In a mutant screen for BABA insensitivity, Jurriaan's group identified the *ibi1* mutant, which displays insensitivity to BABA-induced priming. *IBI1* encodes an aspartyl t-RNA synthetase. Interestingly, *ibi1* mutants, while impaired in BABA-induced priming, are hypersensitive to BABA-induced growth inhibition. These data suggest that IBI1 regulates defense activation and plant growth through separate pathways. Jurriaan's laboratory is now focusing on further elucidating IBI1 function by identifying IBI1 interactors and downstream targets using TAP-tagging and transcriptomics approaches, respectively. Jurriaan presented preliminary data showing IBI1 interaction with MIOX4, a myo-inositol oxygenase, suggesting a possible function for MIOX4 in priming activation and resistance to pathogens. Notably, priming agents have been successfully used in agriculture as a way to promote resistance to pathogens without the severe fitness costs usually associated with chemicals that promote direct defense activation. The identification of the BABA receptor and further elucidation of its molecular function may lead to new strategies of disease control without penalties on yield.

Ubiquitination in defense and symbiosis.

Protein modification via covalent attachment of ubiquitin has emerged as a crucial regulatory process during plant immune signaling. The noticeable number of posters and talks in which the role of ubiquitination in defense signaling was stated reflected this. New research showed the involvement of E3 ubiquitin ligases at different stages of the plant defense response against pathogens and in the establishment of symbiotic relationships.

Yoshitake Desaki, in Naoto Shibuya's group at the Meiji University in Japan, described the involvement of ubiquitin ligases in basal defenses responses after the PAMP chitin recognition. His poster gives new insights into the interaction between a U-box ubiquitin ligase and the chitin receptor CERK1 (Miya et al. 2007). This interaction takes place only when CERK1 is phosphorylated, which enables the ubiquitination of CERK1, contributing thus to chitin signaling. Chil-Woo Lee, a research associate in Marco Trujillo's group at the Leibniz Institute of Plant Biochemistry in Germany, showed a connection between vesicular trafficking and PAMP-triggered signaling via the U-box ubiquitin ligase PUB22. PUB22 interacts with Exo70B2, a subunit of the exocyst complex, required for both immediate and later responses triggered by PAMPs and pathogens. The interaction of Exo70B2 with the E3 ligase PUB22 leads to its degradation, attenuating PAMP-induced

signaling. Jacqueline Monaghan, a postdoctoral scholar in Cyril Zipfel's group at the Sainsbury Laboratory in the UK, provided another example of the control of PAMP signaling by ubiquitination. Although the E3 ligase remains unidentified, her work showed that BIK1 phosphorylation by CPK28 leads to BIK1 degradation via the proteasome. Such fine tune regulation of BIK1 levels appears to prevent an over-activation of defense responses.

It has been previously shown that the C-terminus of the *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) effector AvrPtoB is able to ubiquitinate the tomato kinase Fen, undermining its activity to activate ETI. AvrPtoB targets an additional tomato kinase, Pto, which gained the ability to escape AvrPtoB-mediated degradation. It has been reported that Pto has a higher kinase activity than Fen and that this allows Pto to inactivate AvrPtoB via phosphorylation. Johannes Mathieu, a postdoctoral scholar in Greg Martin's group at the Boyce Thompson Institute for Plant Research at Cornell University in the USA, showed that Pto interacts with a second site distal to the E3 ligase domain in AvrPtoB, and that this interaction, rather than a phosphorylation-dependent inactivation of AvrPtoB, confers Pto its ability to evade AvrPtoB ubiquitination.

Beside the role of ubiquitination in plant defense responses, new research has started to elucidate the role of ubiquitin-ligases in the establishment of symbiotic relationship. Boglárka Oláh, at the Biological Research Centre of the Hungarian Academy of Sciences in Hungary, investigated the role of two U-box containing E3 ubiquitin ligase proteins, MtUSA and MtLIN, in the process of nitrogen-fixing symbiosis in *Medicago truncatula*. These proteins seem to have an opposite role in the formation of the symbiotic nodules; *usa* mutants showed a classic symbiotic phenotype for a negative regulator, while *lin* mutants were unable to form symbiotic nodules, indicating the positive role of MtLIN in this process. Another U-box containing E3 ubiquitin ligase of *Medicago truncatula*, MtPUB1, was found to have a negative role in the common symbiotic signaling pathway. New research by Tatiana Vernié, a postdoctoral scholar in Julie Cullimore's group at the Laboratory of Plant Microbe Interactions (LPMI) at INRA in France, showed that MtPUB1 ubiquitin activity is required for its negative role in root endosymbiosis. In addition, the group elucidated new components of the MtPUB1-dependent signaling pathway. MtPUB1 interacts with and is phosphorylated by DMI2, suggesting that this modification could lead to MtPUB1 activation/inhibition, relocalization and/or interaction with its targets.

The role of ubiquitination as post-transcriptional modification in plant-microbe interactions has been traditionally underestimated, but emerging research in this field is showing high potential for new discoveries in the establishment of both pathogenic and symbiotic relationships.

Effector-mediated acetylation.

Pathogen effector biology continued to be a very popular topic at the conference. While a variety of effector virulence and avirulence mechanisms were discussed, effector-triggered acetylation of plant proteins emerged at this conference as a key virulence strategy to alter immune signaling.

Previously, it was reported that YopJ-like effectors in *P. syringae* (HopZ1a) and *Ralstonia solanacearum* (PopP2) possess acetyltransferase activity, analogous to that of YopJ from *Yersinia*, an animal bacterial pathogen. The nature of the plant substrates has been a subject of debate. Throughout the meeting, several groups reported on the acetylation of distinct plant proteins, highlighting the diversity of host substrates regulated by pathogen acetyltransferases.

One highlight was the identification of PopP2 host targets. After the discovery that PopP2 directly interacts with the A.

thaliana disease resistance protein RRS1-R, the question of whether RRS1-R is a direct substrate of PopP2 arose. PopP2 auto-acetylation occurs at lysine residue 383 and mutation of this residue abolishes RRS1-R-mediated immunity, implying that acetylation of RRS1-R may be required for defense activation. Laurent Deslandes, at LPIM-INRA, confirmed that PopP2 acetylates RRS1-R and showed that modification occurs at lysine residue 1221 in its WRKY DNA-binding. Importantly, Laurent showed that the RRS1-R K1221R mutant does not bind DNA *in vivo*, indicating that acetylation interferes with RRS1-R DNA-binding activity. It is known that a heterodimer of RRS1-R and the R protein RPS4 is required for effector-triggered immunity against cross-kingdom pathogens including *Ralstonia solanacearum*, *Pseudomonas syringae*, *Colletotrichum higginsianum*, and *Colletotrichum orbiculare*. Laurent proposed the model that RRS1-R in complex with RPS4 serves as bait for PopP2. Acetylation displaces RRS1-R from DNA, leading to activation of defense transcription. Moreover, Laurent proposed that the virulence role of PopP2 is to target WRKYs transcription factors to dampen defense transcription. He provided evidence that several WRKYs are acetylated and showed that *A. thaliana wrky53* mutants are more susceptible to *R. solanacearum* infection.

Jonathan Jones, at the Sainsbury Laboratory in the UK, reported similar findings and proposed that the RRS1-R/RPS4 complex is functioning as an “effector detector” by recognizing acetylated-WRKYs. Jonathan added additional pieces to the puzzle by showing that AvrRps4 from *P. syringae* interacts with the WRKY domain of RRS1-S, the susceptible form of RRS1, and acetylation of RRS1-S by PopP2 blocks AvrRps4 binding to RRS1-S. He also described the putative function of another protein pair – RRS1B and RPS4B. The WRKY domain of RRS1B is required for AvrRps4-triggered immunity through RRS1B/RPS4. Jonathan suggested that the mode of AvrRps4 recognition has similarities to the PopP2 model previously discussed. In the latter case, AvrRps4 has other protein targets in the plant cell but is being detected by the WRKY domain of RRS1B. RPS4, therefore, is serving as a guard for RRS1B. Given that two unrelated effectors were shown to

interfere with multiple WRKY domains, this should remain a very active area of future research.

The complexity of effector acetylation was not restricted to nuclear substrates. Jean Greenberg, at the University of Chicago in the USA, reported the *P. syringae* effector HopZ3 is a potent acetyltransferase and modifies serine, threonine, and lysine residues. Interestingly, Jean showed that HopZ3 acetylates RIPK at lysine 122 resulting in the inhibition of RIPK kinase activity. Given that RIPK exists in a complex with RPM1 and RIN4, Jean’s group explored the possibility that HopZ3 inhibits RPM1-mediated defense. She showed that HopZ3 acetylates RIN4 and HopZ3 action prevents RIN4 phosphorylation *in planta*. Moreover, Jean showed that HopZ3 acetylates AvrB, an effector that activates the RIPK/RIN4/RPM1 complex. These data suggest the model that HopZ3 interferes with RPM1 signaling by acetylating multiple components of this complex. Lastly, Jean provided an example of “effector epistasis” whereby competitive post-translational modifications, including acetylation, mediated by effectors leads to the inhibition of immune signaling.

Finally, Mary Beth Mudgett, at Stanford University in the USA, provided the first evidence that a YopJ-like effector (AvrBsT) from *Xanthomonas euvesicatoria* possesses acetyltransferase activity. AvrBsT interacts with ACIP1, an unknown protein that associates with microtubules and is required for PTI and ETI. Mary Beth showed that AvrBsT acetyltransferase activity is required for dramatic changes in ACIP1 localization within plant cells, changes linked to that activation of strong defense responses. Given that HopZ1a was shown to acetylate tubulin, this work suggests that YopJ-like effectors may have evolved to target different components of the plant cytoskeleton.

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