Meeting report

Plant immunity from A to Z

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A report of The Keystone Symposium on Plant Innate Immunity, Keystone, USA, 10-15 February 2008.

Plants resist potential microbial infections by deploying a wide range of innate defenses. More than 150 plant scientists assembled recently at a Keystone Conference in Colorado to discuss the latest advances in plant innate immunity. Many novel and exciting findings were presented in the spirit of the meeting. This report highlights some key presentations. In the keynote address, Brian Staskawicz (University of California, Berkeley, USA) illustrated major advances over the past half century and recent paradigm shifts (Figure 1), and encouraged his audience to integrate their studies on pathogen effector functions, the *in planta* targets and molecular bases of resistance towards the development of durable disease resistance in the field.

PAMP-triggered immunity

Pathogen-associated molecular patterns (PAMPs) are conserved microbial structures that are perceived through host receptors and induce a plethora of plant defense responses triggering immunity. In *Arabidopsis*, the best-characterized PAMPs are the bacterial proteins flagellin (flg22) and translation elongation factor Tu (elf18), which are recognized by the plant receptor kinases FLS2 and EFR, respectively, both of which use the receptor kinase BAK1 as a co-factor. Despite its importance in plant defense, little is known about the molecular basis of PAMP signaling and the components required for PAMP-triggered immunity. Cyril Zipfel (Sainsbury Laboratory, Norwich, UK) reported the isolation of more than 100 elf18-insensitive (*elfin*) *Arabidopsis* mutants that are not due to mutations in the *EFR* gene. The mutant *elfin27-6* appeared to be unique in being affected in both elf18 and flg22 signaling and the mutated gene responsible turned out to be *BAK1*. Zipfel also identified *elfin1* as *SDF2* (stromal-derived factor 2). *Sdf2* mutants are specifically impaired in responses to elf18 and are highly susceptible to bacterial infection. He provided evidence that SDF2 localizes to the endoplasmic reticulum (ER) and noted that three other *ELFIN* genes encode proteins with known roles in protein quality control in the ER.

Paul Schulze-Lefert (Max-Planck-Institute for Plant Breeding Research, Cologne, Germany) described the isolation of 'priority for sweet life' (*psl*) mutants that exhibit derepression of sucrose-induced flavonoid accumulation (a stress response) in the presence of elf18. Cloning of the responsible genes revealed components of the ER protein quality control system: *PSL1* encodes a member of the calreticulin family involved in the ER chaperone system and *PSL2* codes for an UDP-glucose glycoprotein glucosyl-transferase that functions as a folding sensor in the ER. Both presentations pointed to the significance of the ER protein folding system during EFR-mediated PAMP-triggered immunity. It remains to be shown whether EFR itself is the client of the ER quality control system.

Pathogen effector molecules

Over the past few years the virulence-promoting function of pathogen effectors and their host targets has become a center of attention. Effectors appear to be modular proteins, an adaptation to the everlasting evolutionary arms race between plant and pathogen. Gregory Martin (Boyce Thompson Institute for Plant Research, Ithaca, USA) has defined the roles of different modules in the bacterial effector AvrPtoB from *Pseudomonas syringae* pv tomato DC3000 (Figure 1). The amino terminus (amino acids 1-307) of AvrPtoB interacts with the tomato Pto kinase, which triggers resistance via recognition of the resistance (R) protein Prf. This region of AvrPtoB is also important for the virulence function of the protein, and Martin reported that it associates with the LysM-receptor kinase Bti9, a potential virulence target. A longer amino-terminal fragment (1-387)

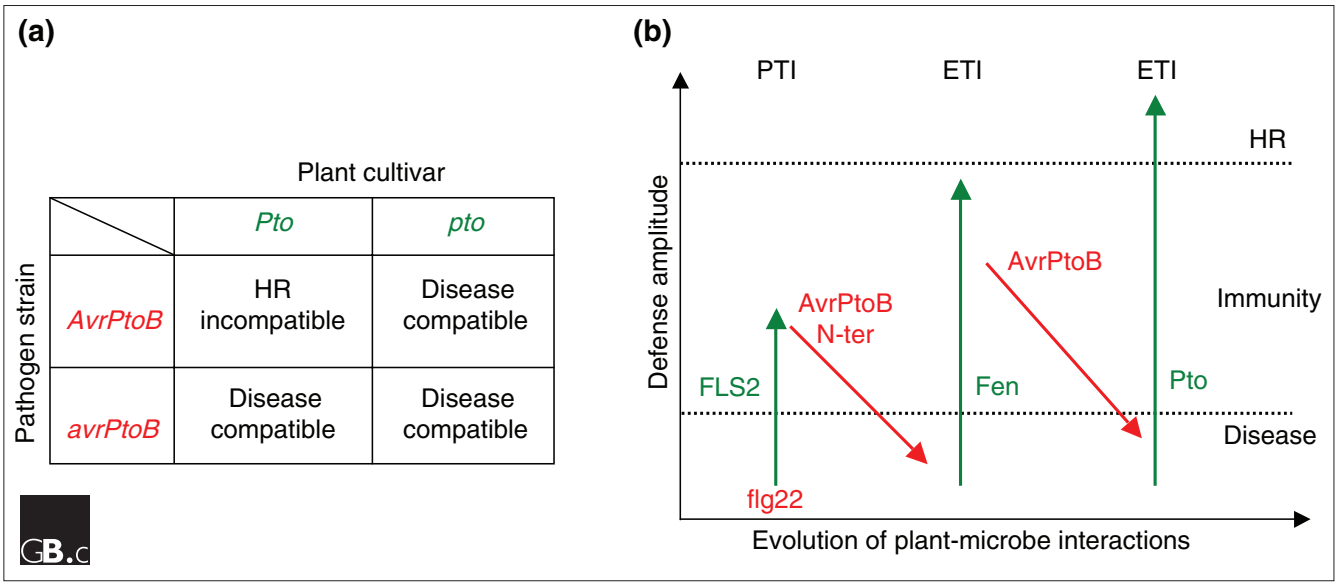

Figure 1
Classic and current views of plant-microbe interactions. **(a)** The 'gene-for-gene' model proposed by HH Flor in 1946 is illustrated by the interaction of *P. syringae AvrPtoB* and tomato *Pto*, which is guarded by the resistance (*R*) gene *Prf*. A plant cultivar expressing a given *R* gene is resistant to a pathogen strain delivering a cognate avirulence (*Avr*) gene. This host-pathogen incompatibility is typically accompanied by the hypersensitive response (HR). If either component of the *Avr*/*R* gene pair is missing, the plant-microbe interaction becomes compatible and disease occurs. **(b)** The 'zigzag' model proposed by Jonathan Jones and Jeffery Dangl in 2006. The perception of PAMPs (for example, flg22) by a cognate pattern recognition receptor (for example, FLS2) mediates PAMP-triggered immunity (PTI). Pathogens secrete effectors (for example, AvrPtoB N-ter) that suppress PAMP signaling and PAMP-triggered immunity, and thus confer disease in the absence of R protein activation. In resistant tomato plants, Prf recognizes AvrPtoB N-ter via Fen and induces a reinforcement of defense termed effector-triggered immunity (ETI). To counteract this, *P. syringae* has acquired a new function for AvrPtoB by the addition of a carboxy-terminal E3 ligase domain that targets Fen for degradation. In turn, tomato plants have evolved Pto that resists AvrPtoB-mediated degradation and triggers strong ETI involving the HR. Compared with the gene-for-gene relationship, this model integrates the multi-layered/stacked plant immune responses of different amplitudes and highlights the constant evolutionary adaptation in plant-microbe interactions. Components of plant immunity are shown in green, pathogen-derived molecules are depicted in red.

interacts with the tomato Fen kinase, which mediates Prf resistance in the absence of Pto. The carboxyl terminus of AvrPtoB shares structural homology with eukaryotic E3 ubiquitin ligases and ubiquitinates Fen, but not Pto. Martin discussed the means by which Pto resists the AvrPtoB E3 ligase activity and hypothesized that Pto actively inhibits ubiquitination (by phosphorylation of the ligase) or passively resists it (due to a lack of key lysine residues). He identified wild tomato accessions in which AvrPtoB-triggered resistance occurs independently of Pto, and thus Fen also appears resistant to AvrPtoB-mediated degradation in these plants, possibly due to the substitution of key lysine residues.

Martin also noted that both Pto and Fen phosphorylate AvrPtoB, but at different residues. In line with this, John Rathjen (Sainsbury Laboratory, Norwich, UK) reported that in *Nicotiana benthamiana* Pto phosphorylates AvrPtoB in the E3 ligase domain and that the Pto kinase activity is required for preventing AvrPtoB-mediated ubiquitination of Pto. In regard to the virulence function of AvrPtoB, Rathjen reported that AvrPtoB associates with the *Arabidopsis* kinase chitin elicitor receptor kinase 1 (CERK1), another receptor that activates plant defenses. Thorsten Nürnberger (University of Tübingen, Germany) described work done in collaboration with Jen Sheen (Harvard Medical School, Boston, USA) showing that AvrPto and AvrPtoB both interact with FLS2 and BAK1 and inhibit flg22-triggered FLS2/BAK1 complex formation. AvrPtoB thus has multiple functions and, as it can suppress both PAMP-triggered immunity and effector-triggered immunity, it provides clues to trace how plant-microbe coevolution has shaped the virulence strategy of a pathogen.

Regine Kahmann (Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany) presented a genomic approach to identifying effectors of the smut fungus *Ustilago maydis*. Genome sequencing of *U. maydis* has revealed about 550 proteins that are predicted to be secreted. Comparison of the genomes of *U. maydis* and *Sporisorium reilianum*, a close relative that, unlike *U. maydis*, does not trigger tumor formation during infections, uncovered a high degree of sequence conservation and synteny, whereas genes in previously defined pathogenicity clusters were only poorly conserved. The deletion of one poorly conserved pathogenicity cluster resulted in loss of pathogenesis in *U. maydis*, but *S. reilianum* retained full virulence. The deletion mutants of *U. maydis* proliferated inside the plant but failed to elicit

tumors, and so Kahmann suggested that this cluster might be responsible for *U. maydis* tumor formation. This study emphasizes the power of comparative genomics combined with mutant analysis for the identification of effectors responsible for different stages of the infection process.

The oomycetes include some serious plant pathogens, such as *Phytophthora infestans*, the cause of potato blight. In these pathogens, as in bacteria, genome sequencing is a powerful tool for identifying the effector inventory. Jim Beynon (Warwick University, UK) reported the genome sequencing of the oomycete *Hyaloperonospora parasitica*, and predicted the presence of about 200 so-called RxLR effectors (named after a shared RxLR amino acid motif). Sophien Kamoun (Sainsbury Laboratory, Norwich, UK) presented a genome-wide catalogue of oomycete effectors. He focused on the *Phytophthora* CRINKLER family of effectors, which trigger cell death in plants and comprise about 200 members carrying an LxLFLAK motif. Jean Greenberg (University of Chicago, USA) formulated an interesting question: why do pathogens encode so many effectors, for example, the 30-40 effectors in *P. syringae*? She proposed that pathogens require different sets of effectors in different infection phases, such as epiphytic growth and growth inside the plant.

Networks in defense and hormone signaling, growth control and disease

Corné Pieterse (Utrecht University, The Netherlands) described the antagonism between salicylic acid (SA) and jasmonic acid (JA) signaling pathways in diverse plant-microbe interactions. He has observed the suppression of JA-induced expression of the defensin gene *PDF1.2* in *A. thaliana* upon application of SA during infection with the fungus *Alternaria* or with a combined *H. parasitica* infection and infestation by caterpillars of the cabbage white butterfly (*Pieris rapae*). He found that this effect is long lasting, and is conserved in different *A. thaliana* accessions. Interestingly, the SA-mediated repression of JA signaling is abolished by the loss of non-expressor of PR-1 (NPR1) or glutathione biosynthesis; the NPR1-dependence is, however, rescued by ethylene. Gene-expression profiling revealed that 30% of JA-responsive genes and 20% of SA-responsive genes are under the antagonistic control by the other hormone. Pieterse proposed that regulation of the SA/JA cross-talk occurs at the transcriptional level.

Jane Glazebrook (University of Minnesota, Minneapolis-St Paul, USA) described work with Fumi Katagiri on the use of DNA miniarrays to study the gene-expression profiles of pathogen-responsive genes in a large number of known defense and signaling mutants of *Arabidopsis* upon challenge with *P. syringae* DC3000 AvrRpt2. Systems-biology principles were applied to the data to determine positive and negative interactions among components of plant immunity. The analysis indicated that SA signaling is part of PAMP signaling, and also revealed a calmodulin-binding protein as a node in the network of SA and PAMP signaling. Mutations in the gene encoding this protein enhanced disease susceptibility and reduced PAMP-induced accumulation of SA.

We were reminded by Jonathan Jones (Sainsbury Laboratory, Norwich, UK) that many plant pathogens produce plant hormones, such as auxin or gibberellic acid. He reported that auxin promotes pathogen propagation and compromises PAMP-induced expression of *PR-1*, a well known marker gene for the SA pathway of defense responses. Jones tested whether the auxin effect occurs through JA signaling antagonizing responses to SA, and found that virulence of the disarmed *P. syringae* DC3000 COR$^-$ strain, which is deficient in the synthesis of the JA mimic coronatin, was partially restored in mutant plants with elevated auxin levels. In addition, he reported that DELLA proteins, which are negative regulators of gibberellic acid signaling, are stabilized in response to PAMP treatment, and repress defense against *P. syringae*. Accumulation of DELLA proteins leads to repression of SA signaling but activation of JA signaling. Jones concluded that plant hormone pathways influence each other strongly, and thus the modulation of a single hormone pathway can greatly affect plant defenses.

Resistance protein function

Plant resistance (R) proteins monitor the actions of isolate-specific pathogen effectors, and can trigger programmed cell death, a defense reaction known as the hypersensitive response (HR). One prominent class of R proteins comprises the so-called nucleotide-binding site leucine-rich repeat (NB-LRR) proteins, which carry either a coiled coil (CC) domain or a Toll-interleukin receptor (TIR)-like domain at their amino termini. Frank Takken (University of Amsterdam, The Netherlands) updated the model of the mechanism of NB-LRR protein activation. He described the coexpression in *N. benthamiana* of the CC-NB-ARC domains and TAP-tagged LRR domains derived from the tomato NB-LRR protein Mi-1, which conferred full activity as detected by HR, supporting the model of NB-LRR protein activation by intramolecular interactions. Takken pointed out the role of the MHD motif in the NB-ARC domain for activation. Substitution of the conserved aspartate (D) residue in this motif caused autoactivation of four NB-LRR proteins tested, whereas replacement of the histidine (H) conferred either autoactivation or loss of function. The generalization of this updated model will require further studies, however. Takken also reported that the LRR domain interacts with the chaperones Hsp90 and PP5.

Kirsten Bomblies (Max-Planck-Institute for Developmental Biology, Tübingen, Germany) described temperature-sensitive hybrid necrosis that occurs in crosses of normally

healthy *A. thaliana* accessions. She first focused on the Uk-1/Uk-3 hybrid, in which pathogen-responsive genes are expressed constitutively, and which show cell death reminiscent of an HR. The Uk-1/Uk-3 incompatibility was mapped to two semi-dominant loci: *DM1* in Uk-3 and *DM2* in UK-1. Bomblies observed a complex variation at the *DM1* locus, which carries two *TIR-NB-LRR* genes in the reference sequence, but only a single *TIR-NB-LRR* gene in Uk-3. This *NB-LRR* was shown to cause constitutive defense activation. Although the responsible gene at the *DM2* locus is still unknown, it should be noted that *DM2* was mapped to another complex *NB-LRR* cluster carrying two *RPP1* homologs. A further survey for a potential involvement of *R* genes in other hybrid necroses revealed that *RPW8* seems to play a role in the Mrk-0/KZ10 hybrid. *R* genes have been proposed to be costly for plant growth, and the risk of necrosis could cause constraints on their evolution. Bomblies also referred to the Est-1 accession, in which an allele of *ACD6* (a positive regulator of defense) causes HR-like lesions. Thus, the risk of necrosis caused by auto-activation of cell-death inducers in plant immunity might in some situations present a barrier to gene flow.

Although the plant HR has been seen as the ultimate restriction of pathogen propagation ever since HH Flor first described gene-for-gene resistance in the 1940s, little is known about how HR cell death is initiated and regulated. Jeffery Dangl (University of North Carolina, Chapel Hill, USA) reviewed the LSD1-mediated control of cell death adjacent to infection sites. LSD1 (for lesion simulating disease resistance 1) is a plant-specific zinc finger protein that regulates the oxidative stress response that accompanies the HR. Dangl described how a gradient of SA determines a cell for death. He also found that the plant NADPH oxidase AtRbohD restricts systemic cell death in *lsd1* mutants, which indicates that reactive oxygen species have a role in limiting the HR. He reported that LSD1 interacts with the zinc-finger-containing metacaspases AtMC1 and AtMC2. Mutation of *AtMC1* suppresses runaway cell death in *lsd1* and enhances the basal level of defense. Consistent with this, AtMC1 appears to be stabilized in *lsd1* mutants, and constitutive overexpression of *AtMC1* induces cell death. Whereas the metacaspase AtMC1 promotes cell death, AtMC2 negatively regulates cell death via repression of AtMC1.

In his concluding remarks, Dangl noted that one reason for the successful growth of the field of plant-microbe interactions had been the sharing of resources and unpublished information between researchers, and he hoped that this would continue. In this respect, this meeting was a prime example of the further nourishment of our plant immunity studies.

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