Discovery and characterization of a second *Pseudomonas* protein recognized by Pto

Young Jin Kim

Korea University

Plants have evolved complex mechanisms to recognize, and defend themselves against many potential pathogens. These mechanisms include a rapid, localized cell death at the site of infection (Hypersensitive response, HR), increased expression of defense-related genes, and the oxidative burst. In many plant-pathogen interactions, recognition of pathogen is mediated by a plant disease resistance (R) gene that responds to the presence of corresponding avirulence (avr) gene in the pathogen. In interactions where the specific R gene in the plant or the corresponding avr gene in the pathogen is lacking, there is no concerted defense response and disease ensues. 'Gene-for-gene interactions' can be envisaged to involve four steps including: (i) delivery of a pathogen-produced elicitor molecule to the plant cell; (ii) recognition of this signal molecule by the plant cell; (iii) signal transduction, which may involve several pathways; and (iv) the activation of a variety of defense responses. Elucidation of the molecular mechanisms by which plant defense systems are activated after specific recognition of a pathogen offers great potential for increasing the effectiveness of natural plant resistance by genetic engineering.

Over the past ten years, many R genes have been isolated that confer resistance to various pathogens including virues, bacteria, fungi or nematodes (Martin et al. 2003). With a few exceptions (e.g. *Hm1, mlo, Hspro-1*), these R genes condition disease resistance in a gene-for-gene manner (Dangl 2001 Martin et al. 2003). Based on predicted protein sequences, these R gene products can be divided into five classes: (i) intracellular protein kinases (e. g. Pto); (ii) proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic protein kinase region (e.g. Xa21); (iii) intracellular proteins containing a region of a LRRs and a nucleotide binding site (NBS; e.g. RPS2, RPM1); (iv) intracellular proteins containing a region of homology to the Toll/IL-1R proteins in addition to LRRs and a nucleotide binding site (e.g. N, L6, RPP5); and (v) proteins with LRRs that appear to encode membrane-bound extracellular proteins (e.g. Cf-4, Cf-9). Proteins with these motifs are known to have important roles in signal recognition and transduction in mammals. For example, LRRs have been implicated in protein-protein interactions and the binding of peptide hormones by

transmembrane receptors, the NBS may play a role in activation of kinases or G-proteins by binding to the nucleotide triphosphate ATP or GTP, and protein kinase participate in phosphorylation cascades that are central to many signal transduction pathways.

The Pto resistance gene in gene-for-gene interaction with the avrPto avirulence gene governs resistance to bacterial speck of tomato. A member of a small gene family in tomato, Pto encodes a serine/threonine kinase that interacte in the yeast two-hybrid system with the production of avrPto, an 18-kDa hydrophilic protein. AvrPto likely enters the plant cell via the *Pseudomonas* type III secretion system where it physically interacts with Pto kinase and activates signaling pathways leading to variety of defense responses. We have recently identified another *Pseudomonas* effector protein, Over the past decade, studies of these genes, their products, and the defense response signaling pathway they govern have led to significant advances in our understanding of the biochemical pathway of Pto, the bacterial delivery and Pto recognition specificity for AvrPto, and candidate components in the pathway and their potential functions.

AvrPtoB, that also interacts specifically with Pto. AvrPtoB and AvrPto are similar in several discrete regions which might define their contact points with Pto. Both proteins also appear to confer virulence of *Pseudomonas* when the Pto kinase is not present in plant. We developed an avrPtoB deletion mutant of Pst DC3000 and found that it also retains Pto-specific avirulence on tomato. These observations suggested that avrPto and avrPtoB both contribute to avirulence (Kim et al., 2002). The AvrPtoB type III effector protein is conserved among diverse plant pathogens suggesting it plays an important but hitherto unknown role in pathogenesis. Here we report that *Pseudomonas* AvrPtoB suppresses plant immunity and does so by inhibiting defensive programmed cell death (PCD). AvrPtoB acts inside the plant cell and inhibits PCD initiated by disease resistance proteins and the pro-apoptotic mouse protein Bax (Abramovitch et al., 2003). Using deletion mutants, we identified distinct AvrPtoB domains that are necessary for host recognition and PCD inhibition. We also discovered a suppressed host resistance activity that triggers AvrPtoB-dependent immunity only in the absence of AvrPtoB PCD inhibitory activity. These findings suggest a new model, with mechanistic and structural details, of how a type III effector protein promotes disease. Recently, several *P. syringae* effectors have been identified as suppressors of HR and other associated with defense. The HR elicited by several different Avr proteins were suppressed by the *P. syringae* effectors AvrPtoB, AvrPphE, AvrRpt2, HopPtoD, HopPtoE, HopPtoF, and HopPtoN. While the mechanism of suppression is unknown, suppression of PCD suggests that these effectors may act on conserved pathways in plant.

References

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