IN THIS ISSUE

Mechanism of Pto-Mediated Disease Resistance: Structural Analysis Provides a New Model

Race-specific disease resistance in plants typically requires the action of complementary genes in the pathogen and the host: a functional avirulence (Avr) gene in the pathogen and a corresponding resistance (R) gene in the host. There is solid evidence that Avr and R gene products influence disease resistance pathways in the host and that in the absence of a corresponding R gene, Avr proteins contribute to virulence of the pathogen (Dangl and Jones, 2001). Thus, plants and pathogens are thought to be engaged in an evolutionary arms race wherein pathogens evolve Avr proteins that help them to overcome basal defense responses in specific host plants, and host plants in turn evolve R proteins that interact (directly or indirectly) with the corresponding Avr proteins to activate defense pathways, resulting in the hypersensitive response (HR) that limits the growth of the pathogen and the spread of disease. Avr-activated R proteins often also act to induce systemic acquired resistance (Yang et al., 1997). The largest class of plant R genes that have been identified to date encodes nucleotide binding site leucine-rich repeat (NBS-LRR) proteins, and a number of plant species contain hundreds of different R gene–encoded NBS-LRR proteins, supporting the notion that this gene class has undergone adaptive evolution in response to corresponding evolution of pathogen Avr genes (Michelmore and Meyers, 1998; Dangl and Jones, 2001).

Tomato Pto, which mediates resistance to bacterial speck disease caused by Pseudomonas syringae pathovar tomato strains carrying the cognate Avr genes AvrPto or AvrPtoB, was the first plant gene cloned that participates in a gene-for-gene interaction with a pathogen (Martin et al., 1993), and it is one of the best-characterized and most intensively studied R genes (Pedley and Martin, 2003). In contrast with the canonical NBS-LRR R genes, Pto encodes a Ser/Thr kinase. AvrPto and AvrPtoB encode proteins that are delivered into host cells via the bacterial type III secretion system and are known as type III effectors. AvrPto was found to have virulence activity and cause increased bacterial growth in host plants lacking a functional Pto pathway (Chang et al., 2000). Both AvrPto and AvrPtoB interact with Pto in the yeast two-hybrid assay (Scofield et al., 1996; Kim et al., 2002), and AvrPto was found to be localized to the plasma membrane of host cells (Shan et al., 2000). It has been shown that Pto-mediated resistance to bacterial speck disease also requires the NBS-LRR gene Prf (Salmeron et al., 1996). Rathjen et al. (1999) showed that Prf does not act upstream of Pto and thus may act either downstream of or at the same level as Pto. It is possible that Pto participates in a receptor complex with Prf and AvrPto proteins, but the precise in vivo interactions among Pto, Prf, and the Avr proteins remain unknown.

Structural Modeling of Tomato Pto Kinase Reveals a Surface-Exposed Negative Regulatory Patch That Overlaps with the Avr Interaction Domain and Presumed Avr Docking Site. Colors indicate residues required solely for Avr protein interactions (orange), residues required solely for negative regulation of Pto signaling (i.e., that confer constitutive gain-of-function Pto signaling when mutated) (red), and the overlapping regions of these two domains (brown). The P+1 loop lies within the brown area. Additional residues are shown that are uniquely required for AvrPtoB binding (magenta), and the kinase catalytic residue D164 is shown in yellow.
After Pto was identified as an active kinase, researchers began to look for phosphorylation substrates, and a number of proteins were found that interact with Pto in yeast two-hybrid assays and are phosphorylated by Pto kinase activity in vitro. These include another kinase called Pt1 and a small family of transcription factor–like proteins called Pt4/5/6 (Zhou et al., 1995, 1997). Thus, a prevailing model of Pto mechanism of action states that Avr-activated Pto phosphorylates downstream targets, including the various Pt proteins, which in turn activate other downstream components of plant defense pathways (Pedley and Martin, 2003).

In this issue of *The Plant Cell*, Wu et al. (pages 2809–2821) use structural modeling to investigate the mechanism by which the interaction of tomato Pto kinase with AvrPto effector proteins activates disease resistance. The authors define a patch of surface residues on Pto that confers negative regulation on Pto signaling and overlaps the AvrPto interaction domain (see figure). Importantly, the work shows that although Pto kinase activity is required for AvrPto-mediated activation of Pto, it is not necessary for signaling by constitutive gain-of-function mutant forms of Pto. These results point to a model for Pto mechanism of action wherein Pto signaling is effected by a phosphorylation-dependent conformational change in protein structure, rather than by Pto phosphorylation of downstream substrates.

Previous studies demonstrated that a region within the kinase domain called the P+1 loop is important both for AvrPto binding and for Pto signal transduction (Rathjen et al., 1999 and references therein). Wu et al. demonstrated that the P+1 loop forms part of a negative regulatory domain of Pto signaling leading to HR because mutations both within and adjacent to the loop give rise to gain-of-function alleles (many of which no longer bind AvrPto) that trigger HR in a Prf-dependent manner. The authors next constructed a three-dimensional homology model of Pto kinase structure, based on crystal structures of closely related kinases, that predicted a patch of surface-exposed residues surrounding the P+1 loop that is critical for negative regulation and that overlaps the region of AvrPto binding (see figure). These results led the authors to predict the existence of another, as yet unidentified, peptide that normally occupies the negative regulatory patch and represses Pto signaling. Surprisingly, and in contrast with the prevailing model, they next showed that Pto kinase activity is dispensable for the downstream signaling function of Pto by construction of a Pto double mutant that carries constitutive gain-of-function activity for triggering the HR response but completely lacks kinase activity. Kinase activity was found to be required for the AvrPto activation of wild-type Pto.

The new model supported by these results is that AvrPto proteins bind to Pto, displacing the as yet unidentified negative regulator and activating the Pto kinase. This activation causes a conformational change in the protein that is the signal perceived by downstream effectors of HR (possibly including Prf). The double mutant protein described above presumably maintains a constitutive conformation of the P+1 loop that is perceived by downstream targets in the same manner as is the kinase-dependent conformational change mediated by Avr activation of the wild-type protein.

This model of Pto activation of the HR response does not preclude the possibility that phosphorylation of certain targets by Pto kinase plays a role in other pathways. For example, the Pt 4/5/6 proteins share sequence similarity with ethylene responsive factors and similarly bind to GCC-box cis elements present in the promoters of many *PR* genes (Zhou et al., 1997). *Pti4* gene expression also has been shown to be induced by salicylic acid and ethylene (Gu et al., 2000). Pto has not been demonstrated to phosphorylate any of the Pti proteins in vivo; yet an in vivo role for Pto function via Pti phosphorylation has not been ruled out. For example, it is possible that Pto regulates the Pti proteins during ethylene responses or during basal defense responses to virulent pathogens (Van der Biezen and Jones, 1998). Positive identification of the putative negative regulator predicted by Wu et al. could help to shed light on this question because the negative regulatory patch identified overlaps the kinase catalytic site.

The work of Wu et al. demonstrates the importance of protein structure modeling in functional analyses. A solution structure of AvrPto reported by Wulf et al. (2004) provides insights into the type III secretion mechanism and to the interactions with Pto and several other plant proteins previously identified as AvrPto-interacting proteins in yeast two-hybrid screens. These two reports provide important examples of how structural analyses are helping to elucidate the mechanisms underlying race-specific disease resistance in plants.

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### REFERENCES


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