# Recognition of *Pseudomonas* Effector Proteins by Tomato and Profiling of Plant Gene Expression Changes that Occur During Plant-Pathogen Interactions

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## Bacterial Speck Disease Resistance in Tomato

The molecular basis of "gene-for-gene" resistance in *Pseudomonas syringae* pv *tomato* infection of tomato lies in the interaction of Pto (a host R protein) and AvrPto (a bacterial Avr protein)(Scofield et al. 1996). Disease occurs when either the *Pto* or *avrPto* genes are lacking from the corresponding organism. The *Pto* gene encodes a serine/threonine protein kinase that is likely to be localized in the plant cell cytoplasm (Martin et al. 1993). The *avrPto* gene encodes a small hydrophilic protein that likely enters the plant cell through a type III secretion system (Ronald et al. 1992; van Dijk et al. 1999). The recognition of AvrPto by Pto is determined by a single threonine residue in Pto and leads to changes in gene expression (Frederick et al., 1998; Zhou et al. 1998). Additional defense responses such as the oxidative burst, nitric oxide production, and the hypersensitive response (HR) ultimately leads to disease resistance.

The recent characterization of several other genes involved in Pto-mediated resistance further enhances the potential of this system for understanding host recognition of a bacterial effector proteins. We have isolated the *Pti1* (Pto-interactor 1) gene, which encodes a protein kinase that is phosphorylated by Pto, and three genes, Pti4, Pti5, and Pti6, that encode defense related transcription factors (Zhou et al. 1995; 1997). By using a yeast three-hybrid system five AvrPto-Pto dependent interactor (Adi) proteins have been identified that interact specifically with the AvrPto-Pto

complex and may play a role in resistance to pathogens (Bogdanove and Martin 2000).

## Recognition and Signaling Events Involving the Pto Kinase and *Pseudomonas* Effector Proteins

### LOCALIZATION OF PTO AND AVRPTO PROTEINS IN THE PLANT CELL

AvrPto contains a myristylation motif (MGNICV) at its N terminus which is required for localization of the protein to the plasma membrane of the plant cell (Shan et al. 2000a). Substitution of the potentially myristylated glycine residue with alanine (G2A) abolishes the avirulence activity of AvrPto in the plant cell as well as its localization to the plasma membrane. However, this G2A mutation in AvrPto does not affect its interaction with Pto (Shan et al. 2000a). These data suggest a model in which the localization of AvrPto to the plant cell membrane recruits Pto to the cell membrane as an initial event in the recognition process. In order to study this process *in vivo* we have generated expression constructs to produce AvrPto:GFP and Pto:GFP chimeric proteins. Analysis of the transiently expressed GFP chimeric proteins in *Nicotiana benthamiana* by confocal microscopy for GFP expression indicates that the AvrPto:GFP protein is localized to the plasma membrane while the AvrPto:GFP (G2A) myristylation mutant is not. While Pto contains a putative myristylation motif, Pto:GFP does not appear to be localized to the plasma membrane. Experiments to analyze Pto:GFP cell localization when co-transiently expressed with AvrPto:GFP are underway.

### DISCOVERY AND CHARACTERIZATION OF A SECOND *PSEUDOMONAS* EFFECTOR PROTEIN THAT INTERACTS WITH PTO

We have recently identified another *Pseudomonas* effector gene, *avrPto2*, from *P. syringae* pv. *tomato* strain DC3000 that encodes a protein which interacts with the Pto kinase in a yeast two-hybrid system. AvrPto2 protein is about 40% similar to VirPphA from *P. s. phaseolicola* (Jackson et al. 1999). Southern hybridization analysis revealed that almost all *P. syringae* pathovars contain *avrPto2* homologs and alignment of the predicted amino acid sequences of these *avrPto2* genes indicates conservation at the N- and C- terminus with high variability in the center portion of the proteins. Like AvrPto, AvrPto2 is predicted to be secreted into the plant cell by the type III secretion system and it elicits resistance in tomato plants containing *Pto*. However, AvrPto2 does not contain a myristylation motif. Transient expression of an AvrPto2::GFP construct in *N. benthamiana* and analysis of GFP expression by confocal microscopy indicates that AvrPto2 is not localized to the plant cell periphery.

Alignment of AvrPto and AvrPto2 indicates that overall the proteins do not share a high degree of similarity. However, several small regions appear to be conserved and may indicate the region by which they interact with Pto.

## TRANSCRIPTION FACTORS MEDIATING DEFENSE GENE EXPRESSION IN THE PTO – AVRPTO INTERACTION

We are also investigating signal transduction steps that lie downstream of the Pto kinase. By using a yeast two-hybrid screen for Pto interacting proteins three transcription factors, Pti4, Pti5, and Pti6 were identified and later found to bind the GCC-box cis element present in the promoters of many pathogenesis-related (*PR*) genes (Zhou et al. 1997). Pti4 and Pti5 are similar to ethylene-responsive element binding proteins (Thara et al. 1999). Thus, they potentially define a key link between pathogen recognition and activation of defense response genes. We have found that the Pti4 protein is specifically phosphorylated by the Pto kinase, and that this phosphorylation enhances binding of Pti4 to the GCC-box. Pti4 is also induced by salicylic acid (SA) and ethylene which is consistent with SA and ethylene being implicated as mediators of defense responses (Gu et al. 2000). In addition, we have found that overexpression of Pti4 in Arabidopsis leads to overexpression of a variety of defense-related genes and to a decrease in symptoms after bacterial or fungal infection of leaves.

## AVRPTO PROMOTES DISEASE PROGRESSION WHEN PTO IS ABSENT FROM THE PLANT HOST

It has always seemed counterintuitive that a pathogen would deliver a protein to a host cell that allows it to be recognized. Although the original work with *avrPto* gene focused on its Avr activity it has recently been shown that expression of *avrPto* in the pathogen when *Pto* is absent from the plant host leads to increased pathogen growth (Chang et al. 2000; Shan et al. 2000b). Interestingly, three mutations in AvrPto that abolished interaction with Pto and eliminated AvrPto avirulence activity had no effect on virulence activity of this protein (Shan et al. 2000b). Other possible manifestations of virulence activity are observable when *avrPto* is expressed directly in leaves of tomato and tobacco using a transient expression system. In tobacco, expression of *avrPto* in leaf cells causes chlorosis within 2-3 days while expression in tomato produces cell death within the same time frame. Possible host targets of susceptibility have been identified by using AvrPto in a yeast two-hybrid system with a tomato cDNA library as the prey (Bogdanove and Martin 2000). Four such AvrPto-interacting (Api) proteins were identified: 1) Api1, similar to a stress-related protein from field bean (*Phaseolus vulgaris*); 2) Api2, a Rab8-like GTP binding protein; 3) Api3, another Rab-like protein; and 4) Api4, a

myristyl-CoA protein N-myristyltransferase. The possible involvement of the Api proteins in promoting AvrPto virulence is currently being investigated.

## A Model for the Compatible and Incompatible Interactions of *Pseudomonas syringae* pv *tomato* and Tomato

Based on the findings discussed above, a model has been proposed for AvrPto avirulence activity (incompatible interaction) in which AvrPto is secreted into the plant cell and localized to the cell membrane. This recruits Pto to the cell membrane and the physical interaction of Pto and AvrPto (possibly involving the Adi proteins and/or the Prf protein) activates the Pto kinase. Activated Pto then phosphorylates and activates diverse downstream target proteins, each with a unique role in the resistance response (Fig. 1, right; Cohn and Martin 2001). The Pti1 kinase is one such target and is involved in the HR leading to localized cell death. The Pti4/5/6 transcription factors are involved in a separate pathway leading to activation of certain PR genes. In the absence of Pto, AvrPto exhibits virulence activity (compatible interaction) and Api1/2/3/4 are host proteins that may be targets of AvrPto. Inhibition of these proteins may inhibit plant defense responses and lead to disease development (Fig. 1, left).

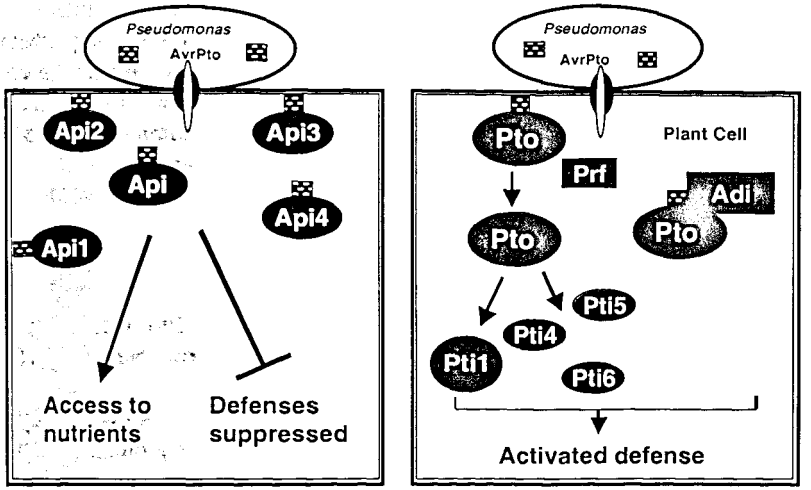


Fig. 1. A model for AvrPto virulence activity (left) and avirulence activity (right) during infection of tomato leaves by *Pseudomonas syringae* pv. *tomato*.

## Profiling Plant Gene Expression Changes that Occur in Response to Pto Recognition of AvrPto

We have utilized three methods to identify genes that are differentially expressed in the incompatible Pto/AvrPto interaction in order to array these genes on microscope slides for a detailed analysis of gene expression during the Pto/AvrPto interaction. The first two methods are cDNA and computer based subtraction methods to identify differentially expressed genes between *Pseudomonas syringae* pv *tomato* (*avrPto*) infected Pto overexpressing plants and plants lacking Pto. The third method is a gel based, mRNA-profiling technique termed GeneCalling (done in association with CuraGen Corporation) that was utilized to identify differentially expressed genes from both a compatible and incompatible *Pseudomonas*-tomato interaction. All together over 700 EST clones and controls were arrayed, in triplicate, on microarray slides and probed with fluorescently labeled RNA from resistant and susceptible tomato plants challenged with an avirulent strain of *P. syringae* pv *tomato*. Analysis of the microarrays indicated that over 100 genes were differentially expressed in response to the pathogen. Genes whose expression was induced in response to the pathogen include genes from the ubiquitination pathway, PR genes, the phenylpropanoid pathway, transcription factors, genes related to the oxidative burst and programmed cell death, and the jasmonic acid pathway. Genes whose expression was suppressed include those associated with cell wall degradation and photosynthesis. This method is allowing us to analyze alterations in total gene expression and to build an overall picture of the cell processes affected in response to pathogen attack on tomato.

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