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# Molecular mechanisms involved in bacterial speck disease resistance of tomato

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An important recent advance in the field of plant–microbe interactions has been the cloning of genes that confer resistance to specific viruses, bacteria, fungi or nematodes. Disease resistance (*R*) genes encode proteins with predicted structural motifs consistent with them having roles in signal recognition and transduction. The future challenge is to understand how *R* gene products specifically perceive defence-eliciting signals from the pathogen and transduce those signals to pathways that lead to the activation of plant defence responses. In tomatoes, the Pto kinase (product of the *Pto R* gene) confers resistance to strains of the bacterial speck pathogen, *Pseudomonas syringae* pv. *tomato*, that carry the corresponding avirulence gene *avrPto*. Resistance to bacterial speck disease is initiated by a mechanism involving the physical interaction of the Pto kinase and the AvrPto protein. This recognition event initiates signalling events that lead to defence responses including an oxidative burst, the hypersensitive response and expression of pathogenesis-related genes. Pto-interacting (Pti) proteins have been identified that appear to act downstream of the Pto kinase and our current studies are directed at elucidating the roles of these components.

**Keywords:** Pto kinase; AvrPto; disease resistance; signal transduction; recognition specificity; *PR* genes

## 1. INTRODUCTION

Plants have evolved complex mechanisms to recognize, and defend themselves against, many potential pathogens (Lamb *et al*. 1989; Lamb 1994). These mechanisms include a rapid, localized cell death at the site of infection (the hypersensitive response, HR), increased expression of defence-related genes, and the oxidative burst (Cutt & Klessig 1992; Goodman & Novacky 1994; Levine *et al*. 1994; Mehdy 1994). In many plant–pathogen interactions, recognition of the pathogen is mediated by a plant disease resistance (*R*) gene that responds to the presence of a corresponding avirulence (*avr*) gene in the pathogen (figure 1). In interactions where the specific *R* gene in the plant or the corresponding *avr* gene in the pathogen is lacking, there is no concerted defence 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 defence responses (see figure 1). Elucidation of the molecular mechanisms by which plant defence systems are activated after specific recognition of a pathogen offers great potential for increasing the effectiveness of natural plant resistance by genetic engineering (Keen *et al*. 1993; Staskawicz *et al*. 1995).

Over the past five years, many *R* genes have been isolated that confer resistance to various pathogens including viruses, bacteria, fungi or nematodes (Bent 1996; Baker *et al*. 1997). With a few exceptions (e.g. *Hm1*, *mlo*, *Hspro-1*), these *R* genes condition disease resistance in a gene-for-gene manner (Dangl 1995; Martin 1995; Staskawicz *et al*. 1995). 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 with a region of 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 (Kobe & Deisenhofer 1995), the NBS may play a role in activation of kinases or G-proteins by binding to the nucleotide triphosphate ATP or GTP (Pelech & Sanghera 1992), and protein kinases participate in phosphorylation cascades that are central to many signal transduction pathways (Hunter 1995).

## 2. BACTERIAL SPECK DISEASE RESISTANCE AND FENTHION SENSITIVITY

*Pseudomonas syringae* pv. *tomato* is a common bacterial pathogen that causes leaf speck disease when infecting susceptible tomato plants. An *R* gene to the pathogen, *Pto*, was originally discovered in a wild-type species of

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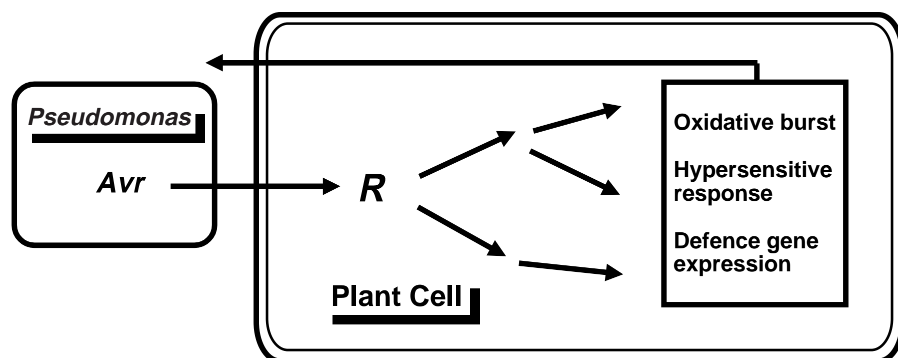


Figure 1. A general model of plant–pathogen interactions involving 'gene-for-gene' recognition.

tomato, *Lycopersicon pimpinellifolium*, and has subsequently been introgressed into many cultivated tomato (*L. esculentum*) cultivars by backcrossing. The *Pto* locus confers resistance specifically to *P. syringae* pv. *tomato* strains that express the avirulence gene *avrPto* (Ronald *et al*. 1992; Martin *et al*. 1993). Interestingly, most tomato cultivars that carry bacterial speck resistance rapidly develop small necrotic lesions when exposed to the organophosphorous insecticide fenthion, and the gene controlling this sensitivity, *Fen*, is located very close to the *Pto* locus on chromosome 5 (Laterrot 1985; Martin *et al*. 1993). Mutagenesis of a bacterial speck-resistant tomato line has identified another gene near *Pto*, named *Prf*, that is required for both *Pto*-mediated resistance and fenthion sensitivity (Salmeron *et al*. 1994, 1996). This finding, along with the observation that overexpression of a *Pto* transgene in tomato plants confers mild sensitivity to fenthion in addition to resistance to *P. syringae* pv. *tomato* strains expressing *avrPto* (Martin *et al*. 1994), suggests that *Pto* and *Fen* share some components in a common signal transduction pathway.

*Pto* and *Fen* were isolated by map-based cloning (Martin *et al*. 1993, 1994) and it was found that they belong to a small clustered gene family consisting of five members. Recently, orthologues of *Pto* and *Fen* have been isolated and characterized from tomato lines that are susceptible to bacterial speck resistance and are insensitive to fenthion sensitivity (Jia *et al*. 1997). Introduction of a *Pto* or *Fen* transgene into a susceptible or fenthion insensitive tomato cultivar results in a marked increase in resistance to *avrPto*-expressing strains of *P. syringae* pv. *tomato* and sensitivity to fenthion, respectively (Martin *et al*. 1993, 1994). *Pto* and *Fen* encode serine–threonine protein kinases that share 80% identity. Expression of *Pto* and *Fen* in *E. coli* and subsequent *in vitro* kinase assays indicated that they are both functional kinases with autophosphorylation activity on serine and threonine residues (Loh & Martin 1995). Therefore, we have hypothesized that a protein phosphorylation cascade is involved in *Pto*-mediated disease resistance and *Fen*-mediated fenthion sensitivity. The Pto protein contains no obvious membrane-spanning or extracellular domain, suggesting an intracellular localization. A potential myristylation site was found at the N-terminus of Pto. Myristylation is often involved in recruiting proteins to the plasma membrane and frequently plays a role in signal transduction pathways in mammalian cells (Boutin 1997). However, recent results indicate that mutation of the invariant glycine residue in the myristylation motif does not impair Pto-mediated resistance to bacterial speck disease (Loh *et al*. 1998). Unlike other *R* gene products, Pto does not contain a region of LRRs.

Isolation of the *Prf* gene revealed that it encodes a large protein with a leucine-zipper, nucleotide-binding site, and a region of LRRs similar to those found in other plant *R* gene products (Salmeron *et al*. 1996). Interestingly, the rice *Xa21* gene product which confers resistance to bacterial blight contains an N-terminal region with similarity to the LRR-type proteins and a C-terminal protein kinase domain with sequence similarity to Pto (Song *et al*. 1995). This interesting structure of the Xa21 protein suggests that LRR-type *R* gene products (e.g. Prf) may physically interact with protein kinases such as Pto to transmit the recognition of a pathogen signal to the plant cell. However, there is currently no evidence to support this and the role of Prf in Pto- or Fen-mediated recognition or signalling remains unclear.

## 3. MOLECULAR BASIS OF GENE-FOR-GENE SPECIFICITY IN THE BACTERIAL SPECK DISEASE RESISTANCE: PHYSICAL INTERACTION BETWEEN AVRPTO AND THE PTO KINASE

Since the early 1980s it had been proposed that the molecular basis of gene-for-gene interactions might be the direct physical interaction of an 'elicitor' molecule produced by the pathogen and a receptor encoded by the plant *R* gene. We (Tang *et al*. 1996), and others (Scofield *et al*. 1996), have recently obtained molecular evidence supporting this hypothesis. By using the yeast two-hybrid system, we observed a physical interaction between Pto and AvrPto. This interaction is highly specific because the related Fen kinase or the product encoded by the susceptible allele *pto*, which is 87% identical to the Pto kinase (Jia *et al*. 1997), failed to interact with AvrPto.

There are three lines of evidence that indicate that this interaction occurs *in vivo* and plays a crucial role in disease resistance. First, when expressed transiently via *Agrobacterium* in plant cells in the absence of the *Pseudomonas* bacteria, the AvrPto protein activated the HR. This is consistent with other reports that at least some bacterial avirulence proteins are probably delivered

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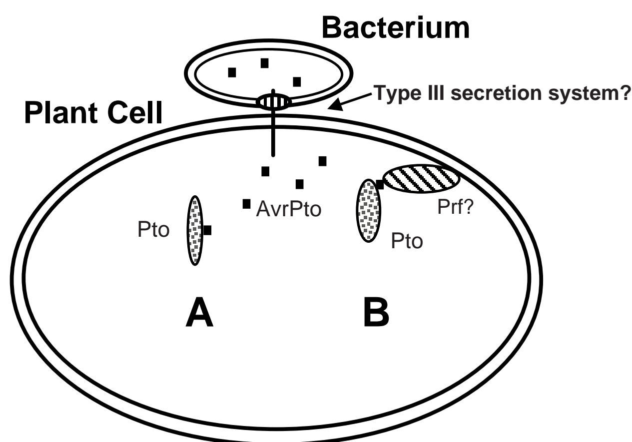


Figure 2. A model for delivery of the AvrPto protein from *Pseudomonas syringae* pv. *tomato* and its recognition in the plant cell by the Pto kinase.

directly into plant cells via a bacterial type III secretion system (figure 2; Gopalan *et al*. 1996; Leister *et al*. 1996; Van Ackerveken *et al*. 1996). Second, removal of portions of the AvrPto protein that are required for Pto interaction rendered the truncated AvrPto non-functional *in planta*. However, deletions of the C-terminal sequence that are not essential for Pto interaction in the yeast did not prevent the resistance outcome in the plant. Finally, domain-swapping analysis between Pto and the closely related Fen kinase has delineated a small region in Pto that is required for AvrPto interaction. Chimeric proteins containing this region were able to interact with AvrPto when expressed in the yeast two-hybrid system and conferred resistance to bacterial speck disease when expressed in stable transgenic plants (Tang *et al*. 1996). In contrast, replacement of this region with the corresponding sequence of Fen resulted in the inability of the chimeric proteins to interact with AvrPto and in the loss of disease resistance in transgenic plants. These results demonstrate that physical association between Pto and AvrPto plays a decisive role in the initial signal recognition and disease resistance.

The physical association of the Pto and AvrPto proteins supports a receptor–ligand model for recognition in this plant–pathogen interaction, thereby providing a molecular explanation for the gene-for-gene specificity in bacterial speck disease resistance. During pathogen attack, the bacterial Hrp secretion system is believed to deliver AvrPto into the plant cell where it interacts with the Pto kinase. The question remains, of course, as to how the physical interaction of AvrPto to Pto activates the disease resistance response. We are currently investigating two models for this process. First, it is possible that binding of AvrPto to Pto may effect a conformational change in the Pto kinase thus enhancing its phosphorylation activity directed at downstream components (see figure 2, model A). Alternatively, it is plausible that AvrPto mediates the interaction between Pto and other plant proteins in a receptor complex, perhaps including Prf, and this interaction serves to activate Pto (figure 2, model B). How universal is this intracellular recognition in plant–pathogen interactions? Several *R* gene products conferring resistance to bacteria, haustoria-forming fungal, and intracellular viruses are apparently cytoplasmic, and the Avr proteins or race-specific elicitors of some of these pathogens are known to function inside the plant cell. In these cases, it seems plausible that a direct interaction of an *R* gene product and its cognate Avr protein within the plant cell might occur. However, some *R* gene products, such as Cf-9 and Xa21, are predicted to have extracellular domains. In these systems, extracellular recognition events might be more likely although such interactions have not yet been demonstrated. The recognition event between a plant and pathogen is believed to initiate several signalling pathways leading to concerted defence responses that ultimately restrict growth and spread of the invading pathogen. Our attempts to elucidate signalling mechanisms that are activated upon AvrPto–Pto recognition and activation of defence responses by these pathways is the focus of § 4.

## 4. PTO-MEDIATED SIGNAL TRANSDUCTION AND SUBSEQUENT DEFENCE RESPONSES

### (a) *Pto-dependent signalling pathway leading to an oxidative burst*

An early localized plant defence response to pathogen attack is the production of potentially cytotoxic reactive oxygen species (ROS) termed the 'oxidative burst'. ROS may play several roles in plant defence responses including direct killing of the pathogen, cell wall strengthening and perhaps signalling (for review, see Lamb & Dixon 1997). Plants are believed to have a mechanism for production of $O_2^-$ that is mediated by an NADPH oxidase, analogous to that in mammalian neutrophils (Segal & Abo 1993; Groom *et al*. 1996). Evidence for such an oxidase includes the observations that diphenyleneiodonium, an inhibitor of flavin-containing oxidases such as NADPH oxidase and xanthine oxidase, prevents the production of ROS during the plant's response to pathogen attack, and antibodies to mammalian NADPH oxidase components cross-react with plant proteins of similar sizes (Desikan *et al*. 1996; Low & Merida 1996).

A Pto-mediated signalling pathway leading to the oxidative burst has been characterized by Chandra and co-workers (1996). In these experiments, the rate of $H_2O_2$ production was monitored in two transgenic tomato (+ or − Pto) cell suspension cultures when challenged with a virulent or avirulent (*avrPto*-expressing) strain of *P. syringae* pv. *tomato*. There were two phases of oxidative burst present in *Pto*-expressing transgenic culture cells challenged with *P. syringae* pv. *tomato* pathogen expressing *avrPto*. The first phase, occurring within minutes of pathogen addition, appeared nonspecific because the compatible interaction also yielded the response. The second phase, beginning 1–3 h after the initial phase, was specific to the incompatible interaction, indicating that recognition of AvrPto by Pto initiated a signalling pathway leading to the oxidative burst. Furthermore, this Pto-mediated signalling pathway may be different from those initiated by other types of stress–defence-related signals, because the absence of Pto did not impair development of the oxidative burst in response to other elicitors (Chandra *et al*. 1996). There are two similar phases of oxidative burst for incompatible

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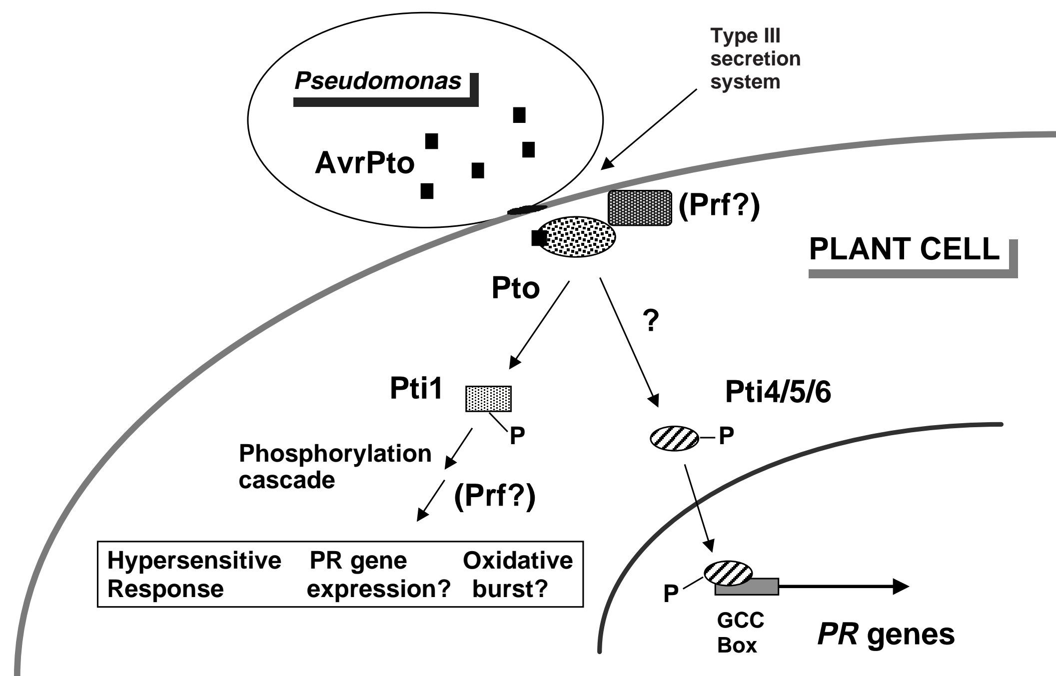


Figure 3. A comprehensive model for the role of Pto in pathogen recognition and signal transduction.

interactions which have been observed in previous work with different plant–pathogen systems (Orlandi *et al.* 1992; Levine *et al.* 1994).

The mechanism by which the *R* gene product might activate the oxidative burst during the incompatible interaction remains unclear. In mammalian systems, activation of the oxidative burst is associated with the translocation of p47-phox, p67-phox, and rac2 components of the NADPH oxidase to the membranes for assembly. Phosphorylation of p47-phox is necessary for this assembly process (Segal & Abo 1993). Evidence has been reported for the translocation of similar components from the cytosol to the plasma membrane in tomato cells after treatment with race-specific elicitors of *Cladosporium fulvum* (Xing *et al.* 1997). Hence, in the case of Pto-mediated the oxidative burst pathway, it is possible that the Pto kinase or a downstream component such as Pti1 kinase (see following paragraphs) may phosphorylate components of the NADPH oxidase enzyme complex and lead to the second phase of the oxidative burst observed in the incompatible interaction.

### (b) *Pti1: a substrate of Pto with a role in the hypersensitive response*

Elucidation of the Pto signalling pathway would benefit from the identification of proteins that physically interact with this kinase. Towards this goal we used a LexA-based yeast two-hybrid system to directly clone genes encoding proteins which interact physically with the Pto kinase (Gyuris *et al.* 1993; Golemis *et al.* 1994; `http://xanadu. mgh.harvard.edu`). We screened approximately $2 \times 10^6$ cDNAs derived from tomato leaves inoculated with *P. syringae* pv. *tomato* using the two-hybrid system and isolated ten distinct classes of cDNA clones encoding Pto-interacting (Pti) proteins. Sequence analysis of the ten *Pti* clones revealed homology, in some cases, with genes of known function (Zhou *et al.* 1995, 1997; J. Zhou, unpublished data).

We initially focused on *Pti1* because sequence analysis indicated that it shared homology with various protein kinases (Zhou *et al.* 1995). Analysis of glutathione-S-transferase (GST)-Pti1 fusion protein expressed in *E. coli* showed that *Pti1* encodes an active protein kinase with autophosphorylation specificity on serine and threonine residues (Zhou *et al.* 1995). Cross-phosphorylation assays revealed that Pto specifically phosphorylates Pti1 and that Pti1 doesn't phosphorylate Pto. Furthermore, the Fen kinase was unable to phosphorylate Pti1 and was not phosphorylated by Pti1. Although the product of the recessive *pto* also phosphorylated Pti1 (Jia *et al.* 1997), qualitative differences in the phosphorylation pattern of Pti1 by Pto and pto have been observed (G. Sessa, M. D'Ascenzo and G. Martin, unpublished data). Collectively, these results indicate that Pti1 is a specific substrate for Pto and acts as a downstream component of Pto in a phosphorylation cascade (see figure 3). To examine the functionality of Pti1 in disease resistance, transgenic tobacco plants overexpressing the Pti1 cDNA were generated. These *Pti1*-transgene plants showed an enhanced HR in leaves when challenged with *P. syringae* pv. *tabaci* expressing the *avrPto* (Zhou *et al.* 1995). Therefore, it is likely that Pti1 kinase is involved in the Pto-mediated HR. It remains uncertain whether Pti1 plays a role in other defence responses (figure 3).

A clearer understanding of the exact role of Pti1 in the formation of the HR will come from identification of proteins that act downstream of Pti1. Pti1 may serve as a component in a Pto-mediated phosphorylation cascade leading to the HR and defence gene expression (figure 3). However, it is also possible that Pti1 may phosphorylate components of the NADPH oxidase complex, thereby activating the oxidative burst machinery for generation of

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ROS. ROS may then activate signalling events leading to the HR or the elevated level of ROS alone may result in cell death. We are currently attempting to identify proteins that interact with Ptil or that are specific substrates for this kinase.

### (c) *Pti4/5/6: transcription factors and their role in PR gene activation*

It has long been recognized that transcriptional activation of a battery of plant defence-related genes is associated with pathogen invasion (reviewed by Bowles 1990; Lamb *et al*. 1989; Dixon & Lamb 1990). Such defence genes include those encoding pathogenesis-related proteins (PRs; reviewed by Cutt *et al*. 1992), hydroxy-proline rich glycoproteins, and enzymes for phytoalexin biosynthesis such as phenylalanine ammonia lyase (PAL) and chalcone synthase (Showalter *et al*. 1985; Bell *et al*. 1986). Although the role of some of these proteins in plant disease resistance has yet to be established, in some cases their enzymatic functions (glucanases, chitinases) indicate they are well-suited for defence against pathogens (Mauch *et al*. 1988; Niderman *et al*. 1995). Several studies involving overexpression of *PR* transgenes indicate that these genes do play a role in disease resistance (Broglie *et al*. 1991; Alexander *et al*. 1993; Liu *et al*. 1994).

Transcription activation of defence-related genes has been investigated in several genetically defined plant–pathogen interactions. In many cases, defence-related genes are induced in both compatible and incompatible interactions. However, critical differences in expression are often observed and may have a causal role in the establishment of the resistance phenotype. For example, the expression of defence genes is often more rapid and profound in a resistant plant challenged with an avirulent pathogen (Van Kan *et al*. 1992; Ashfield *et al*. 1994). We have found that transcripts of several *PR* genes accumulate earlier in tobacco plants inoculated with an avirulent *P. syringae* pv. *tabaci* strain expressing *avrPto* than in those inoculated with a virulent strain lacking *avrPto* (Zhou *et al*. 1997). This accelerated *PR* gene expression is apparently mediated by the Pto-mediated signalling pathway which is known to be conserved in tobacco plants (Thilmony *et al*. 1995). In addition, further acceleration of *PR* gene expression is seen in the tobacco plants overexpressing a *Pto* transgene (Zhou *et al*. 1997). Acceleration of *PR* gene expression is also observed after Pto–AvrPto recognition in tomato when comparing compatible and incompatible interactions (Y. Jia & G. B. Martin, unpublished data).

Zhou and co-workers (1997) identified three genes by using the yeast two-hybrid system that encode putative transcription factors named Pti4, Pti5, and Pti6 (hereafter referred to as Pti4/5/6). Sequence analysis revealed that Pti4/5/6 contain a DNA-binding domain, a region of acidic residues possibly involved in transcriptional activation, and nuclear localization sequences. Interaction of the Pto kinase with these proteins is specific because the closely related Fen and Ptil kinases didn't interact with Pti4/5/6, nor did a Pto kinase-deficient protein or the product of an allele of *Pto* (*pto*; Jia *et al*. 1997). This latter observation is consistent with the fact that tomato plants carrying *pto* are susceptible to the *avrPto*-expressing *P. syringae* pv. *tomato* pathogens.

Database comparisons revealed that Pti4/5/6 bear a striking similarity to ethylene responsive element-binding proteins (EREBPs) (Ohme-Takagi & Shinshi 1995). The EREBPs have been demonstrated to bind a *cis*-element (GCCGCC) conferring ethylene responsiveness to the β-1,3 glucanase gene *gln2* of tobacco. As this sequence has been found in the promoters of many *PR* genes, it has been referred to as the PR box (Zhou *et al*. 1997). Thus, the identification of Pti4/5/6 as Pto-interacting proteins suggests that Pto may regulate *PR* gene expression in tomato by activation of specific transcription factors (figure 3). The sequence similarity and PR box-binding properties among Pti4/5/6 and EREBPs strongly implicates these proteins as functional homologues. This is supported by the observation that EREBP-2 interacts with Pto kinase in a yeast two-hybrid system (Zhou *et al*. 1997).

There are several questions that remain to be answered about the possible role of Pti4/5/6 in Pto-mediated disease resistance. For example, it is not clear whether Pti4/5/6 are functionally redundant in the activation of *PR* genes or whether Pti4/5/6 regulate different sets of *PR* genes. Equally unclear is the mechanism by which the Pto kinase interacts with and possibly activates Pti4/5/6. Preliminary data indicates that the Pto domain mediating interaction with Pti4/5/6 is different from the domain required for interaction with AvrPto (R. Frederick and G. Martin, unpublished data). Although phosphorylation of Pti4/5/6 by Pto has not been established, transcription factors are often regulated by phosphorylation that can either enhance or inhibit their DNA binding activity to the cognate *cis*-elements and thereby regulate gene expression (Hunter 1992). It is also possible that phosphorylation of Pti4/5/6 by Pto kinase may enhance the transactivation potential of these transcription factors. Phosphorylation of transcription factors to enhance their transactivation potential is not uncommon in mammalian cells (Karin 1996). Finally, Pto may play a role in localizing Pti4/5/6 to the nucleus as observed for other NLS-containing proteins (for review, see Jean & Hübner 1996). Studies to elucidate *in vivo* localization of Pti4/5/6 of tomato after bacterial pathogen treatment are in progress.

### (d) *Cross-talk between ethylene and Pto-mediated pathways*

The interaction of Pto and Pti4/5/6 and the striking similarities among EREBPs and Pti4/5/6 suggests that there may be cross-talk between the ethylene and Pto-mediated disease resistance pathways. Although ethylene is unlikely to be a component in the signalling chain leading to disease resistance, it is conceivable that during pathogen attack some branches of the Pto-mediated and ethylene pathways may converge at similar transcription factors. In addition to serving as an ethylene-responsive element, the PR box may also be involved in the pathogen-activated transcription of *PR* genes.

Finally, the Pto kinase may regulate ethylene levels by modulating expression of genes involved in ethylene biosynthesis. At least one such gene in tomato, *ACC2* encoding ACC oxidase that converts ACC to ethylene, contains a PR box in its promoter (Barry *et al*. 1996). Thus, transcription factors likes Pti4/5/6 may bind to the promoter of this gene to increase production of ethylene

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in tomato when Pto–AvrPto interact. Interestingly, a transcript in tomato that hybridizes to a *Petunia* ACO probe was shown to be induced more rapidly in an incompatible interaction than a compatible one (Y. Jia and G. B. Martin, unpublished data). Furthermore, transgenic plants overexpressing the Pto gene are often epinastic, a typical characteristic associated with high ethylene levels. All these observations imply that signalling pathways of Pto and ethylene may overlap in activation of the defence response during pathogen infection.

## 5. PERSPECTIVE

Clearly, a great deal remains to be learned about the mechanisms that contribute to specific recognition of AvrPto by the Pto kinase, transduction of this information, and subsequent activation of defence responses. However, much has been revealed by identifying proteins that physically interact with Pto and focusing on their biological significance in the plant cell. The identification of Pto-interacting proteins such as Pti1 and Pti4/5/6 has provided important information in delineating Pto-mediated signalling pathways involved in defence responses. Further biochemical characterization will be required to elucidate precisely how interaction with Pto activates downstream components, and attempts to reveal the biological relevance of such interactions *in vivo* will be the main challenge in the future. It remains unclear whether Pto phosphorylates AvrPto or if Pto phosphorylation activity is affected by interaction with AvrPto. In addition, the autophosphorylation sites of Pto, Pti1 and Fen and phosphorylation sites of Pti1 by Pto have yet to be characterized. Furthermore, little is known about the role of Prf in the Pto- and Fen-mediated signalling pathways. Recent data indicates that some defence responses mediated by the Fen kinase in response to fenthion are similar to those mediated by Pto in disease resistance (Chandra *et al*. 1996; D. Halterman and G. B. Martin, unpublished data). Further characterization of Prf may uncover some common and perhaps some unique components in the Pto and Fen signalling pathways. Finally, activation of transcription factors is the final stage in signalling transduction events leading to regulation of gene expression. Further study of the interaction of Pto with Pti4/5/6 will not only help elucidate the biochemical basis of transcription factor activitation but may also open the way for novel methods to improve disease resistance in plants.

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