The *Pseudomonas syringae* *avrRpt2* Gene Product Promotes Pathogen Virulence from Inside Plant Cells

Zhongying Chen,¹ Andrew P. Kloeck,¹ Jens Boch,¹ Fumiaki Katagiri,² and Barbara N. Kunkel¹

¹Department of Biology, Washington University, St. Louis, MO 63130, U.S.A.; ²Department of Biological Sciences, University of Maryland-Baltimore County, Baltimore 21250, U.S.A.

Accepted 9 August 2000.

Several bacterial *avr* genes have been shown to contribute to virulence on susceptible plants lacking the corresponding resistance (*R*) gene. The mechanisms by which *avr* genes promote parasitism and disease, however, are not well understood. We investigated the role of the *Pseudomonas syringae* pv. *tomato* *avrRpt2* gene in pathogenesis by studying the interaction of *P. syringae* pv. *tomato* strain PstDC3000 expressing *avrRpt2* with several *Arabidopsis thaliana* lines lacking the corresponding *R* gene, *RPS2*. We found that PstDC3000 expressing *avrRpt2* grew to significantly higher levels and often resulted in the formation of more severe disease symptoms in ecotype No-0 plants carrying a mutant *RPS2* allele, as well as in two Col-0 mutant lines, *cpr5 rps2* and *coil rps2*, that exhibit enhanced resistance. We also generated transgenic *A. thaliana* lines expressing *avrRpt2* and demonstrated, by using several different assays, that expression of *avrRpt2* within the plant also promotes virulence of PstDC3000. Thus, AvrRpt2 appears to promote pathogen virulence from within the plant cell.

Disease resistance in plants is often dependent on the plant's ability to specifically recognize invading pathogens. This mechanism of resistance is controlled at the genetic level and is governed by disease-resistance (*R*) genes that confer on the plant the ability to recognize pathogen strains expressing specific avirulence (*avr*) genes (Baker et al. 1997). Historically, pathogen *avr* genes have been thought to encode or direct the production of molecules that elicit the rapid induction of defense responses on resistant host plants (Alfano and Collmer 1996; Dangl 1994; Leach and White 1996; Vivian and Gibbon 1997). Their prevalence suggests, however, that *avr* genes also must provide a selective advantage for the

pathogen, and it has been proposed that a primary function of bacterial Avr proteins is to promote pathogen growth and disease development on susceptible host plants (Alfano and Collmer 1996; Dangl 1994; Leach and White 1996; Vivian and Gibbon 1997). Accordingly, several bacterial *avr* genes have been demonstrated to contribute to virulence on susceptible plant lines lacking the corresponding *R* gene (Jackson et al. 1999; Kearney and Staskawicz 1990; Lorang et al. 1994; Ritter and Dangl 1995; Swarup et al. 1991). The function of Avr proteins and the mechanisms by which they promote parasitism and disease, however, are not well understood.

Genetic analysis of bacterial plant pathogens has demonstrated that the function of many bacterial *avr* genes depends on the pathogen *hrp/hrc* genes, which are predicted to encode a specialized (Type III) protein secretion apparatus that mediates transfer of bacterial proteins into the cytosol of the host cell (Alfano and Collmer 1997; Galan and Collmer 1999). Additionally, several Avr proteins have been shown to function within plant cells, where they elicit host defense responses on plants carrying a corresponding *R* gene (Bonas and Van den Ackerveken 1997; Duan et al. 1999; Kjemtrup et al. 2000; Leister et al. 1996; McNellis et al. 1998; Stevens et al. 1998; Tobias et al. 1999). These results provide compelling evidence that in many cases bacterial Avr proteins are delivered directly into plant cells, where they trigger defense responses in resistant hosts.

We are interested in learning more about the role of the *Pseudomonas syringae* pv. *tomato* *avrRpt2* gene (Dong et al. 1991; Innes et al. 1993; Whalen et al. 1991) in pathogenesis. *P. syringae* strains expressing *avrRpt2* rapidly induce host defense responses on *Arabidopsis thaliana* plants carrying a functional copy of the corresponding *R* gene, *RPS2*, and are thus unable to multiply to high levels or cause disease in these plants (Kunkel et al. 1993; Yu et al. 1993). Much emphasis has been placed on elucidating the signaling mechanism by which *avrRpt2/RPS2*-mediated pathogen recognition activates these defense responses (Boch et al. 1998; Innes et al. 1993; Leister et al. 1996; McNellis, et al. 1998; Mudgett and Staskawicz 1999; Reuber and Ausubel 1996). The possibility that *avrRpt2* also may contribute to virulence on susceptible plants has not been explored.

One clue as to a possible mechanism by which *avrRpt2* could function to promote pathogenesis is provided by the discovery that *P. syringae* expressing *avrRpt2* can interfere with induction of *avrRpm1/RPM1*-mediated resistance (Ritter and Dangl 1996). These findings suggest that *avrRpt2* can

Z. Chen and A. Kloeck contributed equally to this work and are joint first authors.

Corresponding author: B. N. Kunkel, Department of Biology, Campus Box 1137, Washington University, One Brookings Drive, St. Louis, MO 63130, U.S.A.; Telephone: +1-314- 935-7284; Fax: +1-314-935-4432; E-mail: kunkel@biology.wustl.edu

Current address of J. Boch: Martin-Luther-Universität, D-06120 Halle, Germany.

Current address of F. Katagiri: Novartis Agricultural Discovery Institute, San Diego, CA 92121, U.S.A.

modify host defense signaling pathways in resistant hosts. It has yet to be determined, however, whether *avrRpt2* might function via a similar mechanism to promote virulence in susceptible plants.

Here we show that, in the absence of *RPS2* in the plant, *avrRpt2* acts as a virulence factor that promotes growth of *P. syringae* pv. *tomato* strain DC3000 (*Pst*DC3000) in several different *A. thaliana* ecotypes and mutants that exhibit varying degrees of resistance to *P. syringae*. We also demonstrate that transgenic *A. thaliana* plants expressing *avrRpt2* exhibit enhanced susceptibility to *Pst*DC3000. These results indicate

that *AvrRpt2* functions inside the plant cell to promote bacterial virulence.

RESULTS

Expression of *avrRpt2* in *Pst*DC3000 enhances pathogen virulence in *A. thaliana* lines lacking the corresponding resistance gene, *RPS2*.

To test the hypothesis that *avrRpt2* promotes parasitism and disease in plant hosts lacking a functional *RPS2* gene, we compared the virulence properties of two *P. syringae* pv. *to*-

Fig. 1. Disease symptoms and growth of *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst*DC3000) on *Arabidopsis thaliana* leaf tissue. **A**, Disease symptoms caused by *Pst*DC3000 on *A. thaliana* No-0 *rps2* (top) and Col-0 *rps2* (bottom) plants. Leaves are shown 4 days after inoculation with *Pst*DC3000 (left) and *Pst*DC3000(*avrRpt2*) (right). Plants were inoculated by dipping them into bacterial suspensions (3 to 5×10^8 CFU/cm²) containing the surfactant Silwet L-77. **B**, Growth of *Pst*DC3000 and *Pst*DC3000(*avrRpt2*) in leaf tissue of Col-0 *rps2*, No-0 *rps2*, and No-0 *RPS2* plants. **C**, Growth of *Pst*DC3000 and *Pst*DC3000(*avrRpt2*) in leaf tissue of Col-0 (*CPR5*) *rps2* and *cpr5-2* *rps2* plants. **D**, Growth of *Pst*DC3000 and *Pst*DC3000(*avrRpt2*) in leaf tissue of Col-0 (*COI1*) *rps2* and *coi1-20* *rps2* plants. In **B**, **C**, and **D**, *A. thaliana* plants were inoculated by vacuum infiltration with the indicated *Pst*DC3000 strains at an initial density of 1×10^5 CFU/cm². The concentration of bacteria in the plant leaves was assayed after 0, 2, and 4 days. Data points represent means of three independent determinations $\pm$ standard error of the mean. Experiments presented in **A**, **B**, and **C** were carried out a minimum of three times with similar results. For the experiment in **D**, similar results were obtained in a second independent experiment. The drop in cell number observed on day 4 in No-0 and *coi1-20* plants inoculated with *Pst*DC3000 is reproducible and has been observed in several independent experiments.

mato strains: *PstDC3000* and the isogenic *PstDC3000* strain expressing *avrRpt2* [*PstDC3000(avrRpt2)*]. On Columbia (Col-0) plants carrying a mutation at the *RPS2* locus, infection with *PstDC3000(avrRpt2)* did not reproducibly result in more severe disease symptoms or increased pathogen growth when compared with infection with *PstDC3000* (Figs. 1A and B) (Kunkel et al. 1993; Yu et al. 1998). Because *PstDC3000* is very virulent on Col-0, it is possible that the presence of an additional potential virulence factor such as *avrRpt2* does not significantly increase the virulence of this strain on this host. To facilitate detection of a potential role in virulence for *avrRpt2*, we studied the effect of *avrRpt2* in interactions with several less-susceptible *A. thaliana* lines. These included the *A. thaliana* ecotype Nossen (No-0) and two Col-0 mutant lines that exhibit enhanced resistance to infection by *P. syringae*.

We chose to work with No-0 because this ecotype is only moderately susceptible to infection by *PstDC3000* (Kunkel et al. 1993). To investigate the role of *avrRpt2* in virulence, we generated a line of No-0 carrying a mutation at the *RPS2* locus by introgressing the *rps2-201C* mutant allele, which was originally isolated in the Col-0 ecotype (Bent et al. 1994; Kunkel et al. 1993), into No-0. Infection of No-0 *rps2* plants with *PstDC3000* resulted in mild disease symptoms and moderate levels of bacterial growth (Figs. 1A and B). Inoculation with *PstDC3000* did not cause severe disease on No-0 *rps2* plants, yet the response of these plants to *PstDC3000* infection was distinct from that observed in typical resistant interactions. The level of growth achieved by *PstDC3000* on No-0 *rps2* plants was 50- to 100-fold higher than that observed in No-0 *RPS2* plants infected with *PstDC3000(avrRpt2)* (Fig. 1B). Furthermore, inoculation with high levels of *PstDC3000* did not elicit a macroscopic hypersensitive response (HR; Table 1), one of the hallmarks of disease resistance characterized by the rapid, localized collapse of leaf tissue surrounding the site of infiltration (Baker et al. 1997; Hammond-Kosack and Jones 1996; Morel and Dangl 1997). The genetic and molecular bases of this partially susceptible phenotype are not well understood and are currently under investigation.

Inoculation with *PstDC3000* expressing *avrRpt2* resulted in significantly more severe disease symptoms on No-0 *rps2* plants (Fig. 1A). Growth of *PstDC3000(avrRpt2)* in No-0 *rps2* plant tissue also was significantly increased, reaching population levels 50- to 100-fold higher than those observed for *PstDC3000* (Fig. 1B). The severity of disease symptoms and the level of pathogen growth were similar to that observed for Col-0 *rps2* plants inoculated with either strain (Figs. 1A and B). The elevated level of *PstDC3000(avrRpt2)* growth relative to *PstDC3000* was maintained for at least 8 days after inoculation (data not shown). In addition, most plants inoculated with *PstDC3000(avrRpt2)* were severely stunted and died within 2 to 3 weeks after infection, whereas plants inoculated with *PstDC3000* usually recovered. Thus, the expression of *avrRpt2* significantly enhances the virulence of *PstDC3000* on No-0 *rps2* plants by promoting both bacterial growth and disease symptom production.

To determine whether *avrRpt2* virulence activity is specific for the No-0 ecotype or whether *avrRpt2* also promotes growth and/or disease production in other ecotypes, we tested whether *avrRpt2* could enhance virulence of *PstDC3000* in interactions with mutants isolated in the Col-0 genetic background and that exhibit enhanced disease resistance. In these

studies, we used two resistance mutants, previously isolated in our laboratory, that carry the *rps2-201C* mutation. The first was the *cpr5-2* mutant, which exhibits resistance to multiple pathogens as a result of constitutive expression of defense responses (Boch et al. 1998; Bowling et al. 1997). The second mutant, *coi1-20*, carries a newly identified allele of the coronatine-insensitive (*COI1*) locus and exhibits enhanced resistance to virulent *P. syringae* strains, presumably as a result of its insensitivity to the bacterial toxin coronatine (Feys et al. 1994; A. Klock and B. Kunkel, unpublished).

Growth of *PstDC3000* was significantly restricted in *cpr5 rps2* and *coi1 rps2* plants (Figs. 1C and D), and infection with *PstDC3000* did not result in the development of visible disease symptoms on either plant line (Boch et al. 1998, and data not shown). In contrast, *PstDC3000(avrRpt2)* grew to significantly higher levels in both mutants, obtaining final concentrations of 10- to 50-fold higher than those observed for *PstDC3000* (Figs. 1C and D). In these experiments, infection with *PstDC3000(avrRpt2)* did not usually give rise to severe disease symptoms, presumably because expression of *avrRpt2* was not sufficient to promote pathogen growth to the high levels associated with tissue damage and lesion formation. These results demonstrate that expression of *avrRpt2* promotes the growth of *PstDC3000* in plant mutants that exhibit enhanced disease resistance. Furthermore, our findings indicate that *avrRpt2* virulence activity is not specific to interactions between *PstDC3000* and ecotype No-0.

An additional example of *avrRpt2* virulence activity, in which *avrRpt2* promotes the virulence of *P. syringae* strains carrying *avrRpm1* on normally resistant *A. thaliana* plants carrying the corresponding *R* gene *RPM1*, has previously been reported (Ritter and Dangl 1996). In a series of elegant experiments, Ritter and Dangl demonstrated that *avrRpt2* interferes with several *avrRpm1/RPM1*-mediated resistance responses, including induction of the HR, activation of defense gene expression, and restriction of bacterial growth. These results are consistent with our observations and indicate that expression of *avrRpt2* promotes pathogen growth in a variety of *P. syringae*-*A. thaliana* interactions.

Transgenic plants expressing *avrRpt2* exhibit enhanced susceptibility to *PstDC3000*.

Several bacterial Avr proteins, including AvrRpt2, have been shown to function inside plant cells, where they trigger *R* gene-mediated defense responses (Bonas and Van den Ackerveken 1997; Leister et al. 1996; McNellis et al. 1998). Presumably, any virulence activity of these Avr proteins would also be active within plant cells. Consistent with this hypothesis are reports that expression of high levels of some Avr proteins results in the death of the host tissue in plant cells lacking the corresponding *R* gene (Duan et al. 1999; Gopalan et al. 1996; McNellis et al. 1998; Tobias, et al. 1999). It has not been established, however, whether the cell death induced by expression of these Avr proteins reflects a role in virulence.

To explore the possibility that AvrRpt2 functions within plant cells to promote virulence, we studied the interaction between *PstDC3000* and transgenic *A. thaliana* plants expressing *avrRpt2*. Two different *avrRpt2* transformation constructs were made. The first was generated by placing the *avrRpt2* coding region under the transcriptional control of the

Cauliflower mosaic virus (CaMV) 35S promoter (p35S-*avrRpt2*) and then introduced into No-0 *rps2-201C* plants by *Agrobacterium*-mediated transformation (Clough and Bent 1998). The second construct was generated by placing *avrRpt2* under the control of the *A. thaliana* *RPS2* regulatory region (pRPS2-*avrRpt2*) and then used to transform Col-0 *rps2-101C* plants (Leister et al. 1996). Several lines homozygous for single-locus insertions of the transgenes were isolated in each case and then selected for further characterization. Transgenic No-0 *rps2* lines homozygous for the empty transformation vector also were generated as controls. Expression of *avrRpt2* in several of the transgenic lines was verified by RNA gel blot analysis, as well as by testing for expression of the *avrRpt2* gene by monitoring for *RPS2*-dependent (e.g., HR-induced) seedling death in F₁ plants generated by crossing the transgenic lines to plants carrying a functional *RPS2* allele (data not shown). Expression of *avrRpt2* in the transgenic plants we obtained did not appear to be detrimental as the transgenic plants were healthy and green. Several of the transgenic lines were slightly smaller than the untransformed parental plants or plants carrying the vector only, an observation that may reflect one or more physiological alterations caused by the *avrRpt2* transgene (data not shown). The potential effects of *avrRpt2* expression on plant physiology are under investigation.

The transgenic lines expressing *avrRpt2* were more susceptible to infection by *Pst*DC3000 than were the control transgenic lines or the parental lines (Figs. 2A and B). The increased susceptibility in the No-0 *rps2* plants expressing *avrRpt2* was apparent in the development of significantly more severe disease symptoms (Fig. 2A) and in the higher concentration of bacteria present by the fourth day after inoculation (Fig. 2C). Although *Pst*DC3000(*avrRpt2*) was not detectably more virulent than *Pst*DC3000 on Col-0 *rps2* plants, transgenic Col-0 *rps2* plants expressing *avrRpt2* developed more severe disease symptoms after infection with *Pst*DC3000 than did the untransformed Col-0 *rps2* plants (Fig. 2B). However, as the level of growth of *Pst*DC3000 is already very high in Col-plants, we did not observe a significant increase in bacterial growth in the transgenic Col-0 *rps2* plants expressing *avrRpt2* (data not shown).

The ability of the *avrRpt2* transgene to promote pathogen virulence was further tested by crossing the pRPS2-*avrRpt2* construct from a Col-0 *rps2* transgenic line into *cpr5 rps2* plants. The resulting *cpr5 rps2* lines expressing *avrRpt2* supported levels of bacterial growth that were significantly higher than those of the parental *cpr5 rps2* line (Fig. 2D). Thus, expression of *avrRpt2* in transgenic plants provides *avrRpt2* virulence activity in trans to *Pst*DC3000, a result indicating that AvrRpt2 functions inside the plant cell to promote symptom production and pathogen growth and/or persistence within the leaf tissue.

Expression of *avrRpt2* in transgenic plants inhibits *RPM1*-mediated resistance responses.

As an independent assay for *avrRpt2* virulence activity inside plant cells, we tested whether expression of *avrRpt2* in transgenic *A. thaliana* plants could interfere with *avrRpm1/RPM1*-mediated resistance. Col-0 *rps2* and No-0 *rps2* plants, both of which naturally carry functional alleles of *RPM1*, are resistant to *Pst*DC3000 strains expressing

avrRpm1 [*Pst*DC3000(*avrRpm1*)] and exhibit typical resistance responses after infection by this strain. These responses include restriction of bacterial growth (Fig. 3), absence of disease symptoms (data not shown), and macroscopic tissue collapse, indicative of the HR when high doses of *Pst*DC3000(*avrRpm1*) are infiltrated into the leaf (Table 1) (Debener et al. 1991).

In No-0 *rps2* and Col-0 *rps2* transgenic plants expressing *avrRpt2*, we observed strong inhibition of *avrRpm1/RPM1*-mediated resistance responses. *Pst*DC3000(*avrRpm1*) grew to high levels in transgenic leaf tissue (Fig. 3) and caused severe disease symptoms (data not shown). In addition, inoculation with high doses of *Pst*DC3000(*avrRpm1*) failed to trigger an HR in these plants (Table 1). Thus, the interference of *avrRpt2* with expression of *avrRpm1/RPM1*-mediated resistance responses also appears to occur inside the plant cell. These results are consistent with previous observations that this interference occurs outside of the bacterial cell (Ritter and Dangl 1996) and support the prediction that some Avr proteins such as AvrRpt2 may act inside the plant cell to suppress host defense responses (Alfano and Collmer 1996; Vivian and Gibbon 1997).

Expression of *avrRpt2* in transgenic plants does not inhibit *RPS4* and *RPS5* mediated resistance.

To address whether AvrRpt2 interferes with other *R* gene-mediated resistance responses, we monitored the ability of *avrRpt2* transgenic plants to mount defense responses upon infection by *Pst*DC3000 expressing one of three additional *avr* genes: *avrB*, *avrRps4*, and *avrPphB*. Recognition of bacteria expressing *avrB* also is governed by the *RPM1* gene (Bisgrove et al. 1994), whereas resistance to strains expressing *avrRps4* and *avrPphB* is mediated by *RPS4* and *RPS5*, respectively (Hinsch and Staskawicz 1996; Simonich and Innes 1995). As with the *avrRpm1*-mediated HR, transgenic plants expressing *avrRpt2* failed to mount an HR upon inoculation with *Pst*DC3000(*avrB*) (Table 1), indicating that *avrRpt2* also interferes with the *avrB*-mediated HR. These plants also exhibited significant disease symptoms upon infection with *Pst*DC3000(*avrB*) (data not shown). In contrast, we observed that transgenic plants expressing *avrRpt2* retained the ability to exhibit a normal HR when inoculated with *Pst*DC3000(*avrRps4*) or *Pst*DC3000(*avrPphB*) (Table 1). We

Table 1. *avr* gene-mediated hypersensitive responses (HRs) in transgenic *Arabidopsis thaliana* plants expressing *avrRpt2*^a

<i>Pseudomonas syringae</i> pv. <i>tomato</i> strain	<i>A. thaliana</i> line			
	No-0 <i>rps2</i> control	No-0 <i>rps2</i> <i>avrRpt2</i> transgenic ^b	Col-0 <i>rps2</i> control	Col-0 <i>rps2</i> <i>avrRpt2</i> transgenic ^c
<i>Pst</i>	–	–	–	–
<i>Pst</i> (<i>avrRpm1</i>)	+	–	+	–
<i>Pst</i> (<i>avrB</i>)	+	–	+	–
<i>Pst</i> (<i>avrPphB</i>)	–	–	+	+
<i>Pst</i> (<i>avrRps4</i>)	+	+	–	–

^a Tissue collapse indicative of the HR was scored visually 16 to 20 h after infiltration with bacterial suspensions of approximately 5×10^7 CFU/cm². Each experiment consisted of at least six inoculations for nontransgenic plants and a minimum of 15 inoculations for *avrRpt2*-expressing transgenic plants. – = No tissue collapse; + = HR visible.

^b No-0 *rps2* transgenic plants carrying the p35S-*avrRpt2* construct.

^c Col-0 *rps2* transgenic plants carrying the pRPS2-*avrRpt2* construct.

were also unable to detect interference between *avrRpt2* and either *avrRps4* or *avrPphB* in experiments in which nontransgenic plants were inoculated with *P. syringae* carrying both *avr* genes (data not shown).

Although Col-0 plants carry a functional *RPS4* gene (Hinsch and Staskawicz 1996), in our experiments we found that Col-0 *rps2* plants failed to exhibit rapid tissue collapse

indicative of an HR when inoculated with *PstDC3000*-(*avrRps4*) (Table 1). This result may not be surprising, given that *RPS4*-mediated resistance in Col-0 plants is associated with a less-robust HR than that observed in other ecotypes (W. Gassman, M. Hinsch, and B. Staskawicz, *personal communication*). We also found that No-0 and No-0 *rps2* plants do not possess a functional *RPS5* gene because they developed disease symptoms when inoculated with *PstDC3000*(*avrPphB*) and exhibited no HR when challenged with high levels of *PstDC3000*(*avrPphB*) (Table 1 and data not shown).

The results from the HR experiments indicate that, among the gene-for-gene interactions we have assayed, *AvrRpt2* interferes only with HRs induced upon *RPM1*-mediated pathogen recognition. These results also indicate that the transgenic plants expressing *avrRpt2* retain the ability to exhibit an HR when challenged with certain *PstDC3000* strains and are thus not compromised in their ability to mount this defense response.

To determine whether the HRs induced in the transgenic plants expressing *avrRpt2* are indicative of induction of pathogen resistance, we monitored the growth of *PstDC3000*-(*avrRps4*) and *PstDC3000*(*avrPphB*) in transgenic Col-0 *rps2* plants expressing *avrRpt2*. The growth of *PstDC3000* expressing either *avr* gene was significantly lower than that observed for the virulent *PstDC3000* strain (Fig. 4A). Thus, expression of *avrRpt2* in transgenic plants does not prevent the induction of either *avrRps4*/*RPS4*- or *avrPphB*/*RPS5*-mediated resistance.

Interestingly, we did observe that the level of growth of *PstDC3000*(*avrRps4*) and *PstDC3000*(*avrPphB*) was reproducibly higher in the transgenic plants expressing *avrRpt2* than in the nontransgenic control plants (Fig. 4B). In addition, the transgenic plants did sometimes develop mild disease symptoms, consisting of chlorosis and a few isolated water-soaked lesions, upon infection with these strains (data not shown). Thus, even in these resistant interactions, in which the restriction of bacterial growth (and elicitation of the HR in the case of Col-0 *rps2* transgenic plants) indicates that the *avr/R* gene-mediated resistance responses are triggered, expression of *avrRpt2* promotes pathogen virulence within the resistant host tissue.

DISCUSSION

avrRpt2 acts as a virulence factor in *PstDC3000*.

We have shown that in the absence of a functional copy of *RPS2*, *avrRpt2* functions to promote virulence of *PstDC3000* on a variety of different *A. thaliana* plant lines. This includes promoting pathogen growth and symptom production in the partially susceptible ecotype No-0, as well as enhancing the growth of *PstDC3000* in two Col-0 mutants that exhibit resistance to *P. syringae* (*cpr5-2* and *coil-20*). Our findings extend the previous discovery made by Ritter and Dangel that expression of *avrRpt2* in *P. syringae* interferes with *avrRpm1*/*RPM1*-mediated resistance in *A. thaliana* (Ritter and Dangel 1996). Thus, *avrRpt2* can be added to the growing list of pathogen *avr* genes that also function as virulence (*vir*) factors on susceptible host plants lacking the corresponding *R* gene (Dangel 1994; Jackson et al. 1999; Leach and White 1996; Shan et al. 2000).

Like many other *avr/vir* genes described to date (Lorang et al. 1994; Lorang and Keen 1995; Shan et al. 2000), the effect

Fig. 2. Disease symptoms and growth of *Pseudomonas syringae* pv. *tomato* strain DC3000 (*PstDC3000*) on transgenic plants expressing *avrRpt2*. **A**, *PstDC3000* disease symptoms 4 days postinoculation on No-0 *rps2* plants transgenic for the empty transformation vector pMON10098 (left) or No-0 *rps2* carrying the p35S-*avrRpt2* construct (right). **B**, *PstDC3000* disease symptoms 5 days postinoculation on Col-0 *rps2* plants (left) or Col-0 *rps2* carrying the pRPS2-*avrRpt2* construct (right). **C**, Growth of *PstDC3000* in two independent No-0 *rps2* transgenic lines carrying the p35S-*avrRpt2* construct (squares and triangles) or in No-0 *rps2* plants transgenic for pMON10098 (circles). **D**, Growth of *PstDC3000* in *cpr5-2 rps2* (circles) and *cpr5-2 rps2* plants carrying the pRPS2-*avrRpt2* construct (squares). For comparison, growth of *PstDC3000* on Col-0 (*CPR5 rps2*) (triangles) also is shown. For assessment of disease symptom production plants were inoculated by dipping them into bacterial suspensions (3 to 5×10^8 CFU/cm²) containing the surfactant Silwet L-77. **C and D**, *Arabidopsis thaliana* plants were inoculated by vacuum infiltration with the indicated *PstDC3000* strains at an initial density of 1×10^5 CFU/cm². Data points represent means of three independent determinations $\pm$ standard error of the mean. Similar results were obtained in a second independent experiment.

of *avrRpt2* can be very subtle, contributing only quantitatively to the virulence of *PstDC3000* on some host plants (e.g., Col-0 *rps2*; Figs. 1A and B). One possible explanation for these results is that virulence is provided by a large number of redundant factors (Kjemtrup et al. 2000) and that, in many cases, adding (or removing) a single virulence factor from a very aggressive pathogen strain may result in little or no alteration in virulence on its preferred host. We found that our ability to detect the virulence activity of *avrRpt2* was facilitated by studying the interaction of *PstDC3000(avrRpt2)* with different *A. thaliana* ecotypes or mutants that exhibited varying degrees of resistance to infection by *PstDC3000*. Thus, because *PstDC3000* is not fully virulent on these lines, any increase in virulence provided by *avrRpt2* can be more readily detected. This approach also may prove to be useful for researchers who wish to investigate the function of other *avr* genes or potential virulence factors in pathogenesis.

AvrRpt2 functions inside plant cells to promote virulence of *PstDC3000*.

To determine whether AvrRpt2 functions within the plant to promote bacterial virulence, we generated transgenic *A. thaliana* lines expressing *avrRpt2*. In these experiments, we demonstrated that expression of *avrRpt2* in the plant conferred enhanced susceptibility to infection by *PstDC3000* in several different interactions. No-0 *rps2* and Col-0 *rps2* transgenic plants expressing *avrRpt2* exhibited more severe disease symptoms upon infection with *PstDC3000*. In No-0 *rps2* plants, this was also associated with reproducibly higher levels of pathogen growth in the transgenic plant tissue. Likewise, expression of *avrRpt2* in transgenic plants promoted the growth of *PstDC3000* on *cpr5 rps2* mutant plants that exhibit enhanced resistance to *P. syringae* (Fig. 2C), as well as the growth of avirulent *PstDC3000* strains expressing *avrRpm1*, *avrRps4*, or *avrPphB* (Figs. 3 and 4). Because the *avrRpt2*

transgene encodes a cytoplasmic protein that lacks a secretion signal (Bent et al. 1994; Leister et al. 1996; Mindrinos et al. 1994), it appears that AvrRpt2 functions within plant cells to promote pathogenesis on susceptible plants lacking *RPS2*. This is the first example that we know of in which an *avr* gene product has been shown to promote pathogen virulence from within the plant.

In the majority of the interactions between *PstDC3000* strains and transgenic plants expressing *avrRpt2* that we studied, we observed a moderate increase in pathogen growth (approximately 10- to 50-fold) within the transgenic plants. These findings suggest that, in most cases, the expression of *avrRpt2* enables *PstDC3000* to partially suppress or overcome the plant defense responses that prevent bacterial growth and/or symptom production. In the case of *RPM1*-mediated resistance, however, the virulence effect of *avrRpt2* was much more dramatic. In No-0 *rps2* and Col-0 *rps2* transgenic plants, the expression of *avrRpt2* appeared to completely inhibit both the *RPM1*-mediated HR and restriction of pathogen growth (Table 1 and Fig. 3). These results suggest that, in this particular case, AvrRpt2 acts inside the plant cell to promote virulence by inhibiting a very early step in the *RPM1*-dependent defense signaling pathway. Interestingly, among the known *avr/R* gene interactions we have assayed to date, AvrRpt2 appears to interfere specifically with *RPM1*-dependent pathogen recognition events because transgenic plants expressing *avrRpt2* retained the ability to mount both an HR and an effective resistance response when challenged with *PstDC3000* expressing *avrRps4* or *avrPphB* (Table 1 and Fig. 4).

What are the possible mechanisms by which *avrRpt2* functions to promote virulence of *PstDC3000*? As discussed, we have observed that *PstDC3000(avrRpt2)* grows to significantly higher levels than *PstDC3000* in No-0 *rps2*, *cpr5 rps2*, and *col1 rps2* plants. In all three lines, the plants exhibit en-

Fig. 3. *avrRpt2* expressed in transgenic plants interferes with *RPM1*-mediated disease resistance. **A**, Growth of *Pseudomonas syringae* pv. tomato strain DC3000 expressing *avrRpm1* [*PstDC3000(avrRpm1)*] in No-0 *rps2* plants carrying the p35S-*avrRpt2* construct (filled squares) in No-0 *rps2* plants transgenic for the empty transformation vector pMON10098 (filled circles) or in No-0 *rps2* plants (open circles). Similar results were obtained in a second independent experiment. **B**, Growth of *PstDC3000(avrRpm1)* in transgenic Col-0 *rps2* plants carrying the pRPS2-*avrRpt2* construct (closed squares) or in untransformed Col-0 *rps2* plants (open circles). For comparison, growth of *PstDC3000* in transgenic Col-0 *rps2* plants carrying pRPS2-*avrRpt2* also is shown (open squares). Plants were inoculated by vacuum infiltration with the indicated *PstDC3000* strains at an initial density of 1×10^5 CFU/cm². Data points represent means of three independent determinations $\pm$ standard error of the mean.

hanced disease resistance to *Pst*DC3000 in the absence of any known *avr/R* gene interaction. In *cpr5 rps2* and *coi1 rps2* mutants, resistance is attributed to the partially deregulated expression of defense responses in the absence of *R*-gene mediated pathogen recognition (Boch et al. 1998; Bowling et al. 1997; A. Kloeck and B. Kunkel, *unpublished*). In addition, although *avrRpt2* does not appear to interfere with *RPS4* or *RPS5*-mediated induction of defense responses, we observed enhanced growth of *Pst*DC3000 carrying *avrRps4* or *avrPphB* in transgenic plants expressing *avrRpt2* (Fig. 4B). Thus, the ability of *avrRpt2* to promote pathogen virulence cannot be attributed solely to interference with resistance mediated by known *avr/R* gene interactions. This mechanism can explain the apparently complete inhibition of *RPM1*-mediated resistant responses, but not the more subtle increase in virulence observed in the other interactions we investigated.

We hypothesize that *avrRpt2* acts to promote virulence via two different mechanisms that could function simultaneously. The first, which involves interference between *avrRpt2* and activation of *R* gene-mediated resistance responses, appears to be specific to *RPM1* and results in complete inhibition of resistance. This mechanism may be similar to what has been proposed for *P. syringae* pv. *phaseolicola* *virPphA*, a gene that may promote virulence by inhibiting the induction of resistance responses in a cultivar (e.g., *R* gene)-specific manner. Interestingly, *virPphA* also has been demonstrated to function as an *avr* gene in soybean (Jackson et al. 1999).

The second mechanism by which we propose that *avrRpt2* promotes virulence results in an increase in growth of *Pst*DC3000 on several different *A. thaliana* lines. This mechanism may involve the modification or inhibition of a common component of the defense response, rather than interfering specifically with known *R*-gene-mediated resistance. Thus, although *RPS4*- and *RPS5*-mediated resistance responses appear to be properly induced in the presence of *AvrRpt2*, the transgenic plants are not able to mount a fully effective defense that results in complete restriction of pathogen growth. This second mechanism also could account for the enhanced bacterial growth observed in No-0 *rps2* and the Col-0 *cpr5 rps2* and *coi1 rps2* mutants because resistance in these lines is attributed to the enhanced expression of defense responses in the absence of *R*-gene-mediated pathogen recognition (Boch et al. 1998; Bowling et al. 1997; A. Kloeck, M. Lim, and B. Kunkel, *unpublished*).

It is possible that these two virulence mechanisms could be mediated by modification of a single signaling component that is required for specific *R*-gene-mediated resistance and is involved in a common defense response pathway. In support of this hypothesis is the finding that some defense-signaling genes in *A. thaliana* may be involved in multiple aspects of defense. For example, the *EDS1* gene is required both for resistance mediated by a subset of *R*-genes and for the limitation of growth of some virulent pathogens (Aarts et al. 1998). These findings suggest that *EDS1* may encode a signaling component that is important for activation of *R*-gene-specific resistance as well as for the function of common defense responses. *AvrRpt2* could promote virulence by interacting with a component of defense response signaling that functions in a similar manner.

Although we find the above hypothesis very attractive, we cannot rule out the possibility that *avrRpt2* may promote the

overall virulence of *Pst*DC3000 via a mechanism that does not involve modification of host defense responses. Rather, *AvrRpt2* may function by altering host physiology or metabolism in some other manner, perhaps by facilitating the production or release of nutrients for the bacterium, thereby rendering the host tissue more suitable for pathogen growth (Alfano and Collmer 1996).

We currently favor the hypothesis that *AvrRpt2* promotes the overall virulence of *Pst*DC3000 by modifying a common component of the host defense response. We have demonstrated that infection of No-0 *rps2* plants with *Pst*DC3000 results in the production of mild disease symptoms and intermediate levels of pathogen growth (Fig. 1). In preliminary experiments, we have observed that the moderate disease in No-0 plants is associated with the induction of a weak defense response upon infection with *Pst*DC3000, including induction of pathogenesis-related gene expression and the accumulation of autofluorescent cell wall-bound phenolics, whereas infection with *Pst*DC3000(*avrRpt2*) is not (A. Kloeck, M. Lim, and B. Kunkel, *unpublished*). These findings suggest that infection with *Pst*DC3000(*avrRpt2*) results in the inhibition of these (and presumably other) host defense responses, thus providing the pathogen with a window of opportunity to grow and cause damage to host tissue. Although the mechanism by which *avrRpt2* might accomplish this is not clear, one possibility is that *AvrRpt2* may interact directly with a component of a defense-signaling pathway in the host in a manner similar to what has been observed for some virulence factors of mammalian pathogens (Finlay and Cossart 1997). We are now in the process of investigating the mechanism(s) by which *AvrRpt2* functions to promote bacterial virulence in plants.

MATERIALS AND METHODS

Bacterial strains and plasmids.

The bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 and the *avrRpt2*, *avrB*, *avrRpm1*, *avrRps4*, and *avrPphB* (formerly *avrPph3*) avirulence genes have been described previously (Debener et al. 1991; Hinsch and Staskawicz 1996; Innes et al. 1993; Jenner et al. 1989; Staskawicz et al. 1987; Whalen et al. 1991). The *avrRpt2*, *avrB*, *avrRpm1*, *avrRps4*, and *avrPphB* genes were introduced into *Pst*DC3000 on plasmids pV288 (Whalen et al. 1991), pVB01 (Kunkel et al. 1993), K48 (Debener et al. 1991), pV316-1A (Hinsch and Staskawicz 1996), and pPPY424 (Fillingham et al. 1992), respectively, by triparental mating using the helper plasmid pRK2013 (Figurski and Helinski 1979). *Pst*DC3000 strains not expressing cloned *avr* genes carried control plasmids pLAFR3 or pVSP61 (Staskawicz et al. 1987; Whalen et al. 1991).

Plant material, growth conditions, and inoculation procedures.

A. thaliana ecotypes Columbia (Col-0) and Nossen (No-0) were used in this study. The susceptible *rps2-201C* and *rps2-101C* mutants and the *cpr5-2 rps2-201C* mutant line that exhibits enhanced disease resistance have been described previously (Boch et al. 1998; Kunkel et al. 1993; Yu et al. 1993). The No-0 *rps2-201C* line was generated by crossing Col-0 *rps2-201C* plants with wild-type No-0 *RPS2* plants. F₂ progeny from this cross homozygous for *rps2-201C* were used in

four additional rounds of crossing to No-0 *RPS2*. Prior to performing each successive cross, plants carrying the *rps2-201C* allele were identified by using PG11, a closely linked molecular marker (Bent et al. 1994). The heterozygous plants resulting from the fifth backcross to No-0 *RPS2* were allowed to self-pollinate. F₂ progeny homozygous for *rps2-201C* were identified by assaying for the absence of *RPS2*-mediated resistance to *Pst*DC3000(*avrRpt2*). The *coi1-20 rps2-201C* mutant line was isolated in a screen for mutants with enhanced resistance to *Pst*DC3000(*avrRpt2*) and was shown by genetic complementation tests to carry a mutation at the *COI1* locus (Feys et al. 1994; A. Klok and B. Kunkel, unpublished).

A. thaliana plants were maintained in growth chambers under an 8 h photoperiod at 24°C and 75% humidity. Mass inoculation of plants was carried out by dipping entire leaf rosettes into bacterial suspensions containing the surfactant Silwet L-77, as previously described (Kunkel et al. 1993). Disease symptoms were scored 4 days following inoculation. Bacterial growth within leaf tissue was monitored, as described by Whalen et al. (1991). Pipette infiltrations to assay for the HR were carried out with *Pst*DC3000 strains suspended in 10 mM MgCl₂ to a density of approximately 2 to 5 × 10⁷ CFU/ml (Kunkel et al. 1993). Leaves were scored for tissue collapse 16 to 20 h after inoculation.

Generation of *avrRpt2* transformation constructs.

The p35S-*avrRpt2* transformation construct was generated by placing the *avrRpt2* coding region under the control of the CaMV 35S promoter in the pMON10098 transformation vector (Klee et al. 1991). The *avrRpt2* coding region was engineered to have *Xba*I and *Nde*I restriction recognition sites at its 5' end and an *Xho*I site at its 3' end, using the polymerase chain reaction (PCR). Two primers, 5'-GCTCTAGACATATGAAAATTGCTCCAGTTG-3' (ZYC1) and 5'-CCGCTCGAGGCGGTAGAGCATTGCG-3' (ZYC2; restriction sites in bold and *avrRpt2*-homologous sequences underlined) were used to amplify the *avrRpt2* coding region using pRSR0 (Innes et al. 1993) DNA as the template. The resulting product was digested with *Nde*I and *Xho*I and cloned into pET-21b (Novagen, Madison, WI, U.S.A.) to generate plasmid pET21-*avrRpt2*, which carries an in-frame fusion of AvrRpt2 to a 6XHis tag at its carboxyl terminus. The resulting fusion was further engineered using PCR to have an *Xba*I site at the 5' end and a *Bam*HI site at the 3' end of the *avrRpt2*-6XHis coding region. This was accomplished by using pET21-*avrRpt2* DNA as the template, ZYC1 as the forward primer, and 5'-ACCGGATCCAGCCGGATCTCAGTGG-3' (ZYC3, *Bam*HI restriction site in bold and pET-21b-homologous sequences underlined) as the reverse primer. The resulting DNA product was digested with *Xba*I and *Bam*HI and cloned into Bluescript KS(+). One resulting clone was used as a source of DNA to subclone the *Xba*I-*Bam*HI fragment into pMON10098. To verify that the 6XHis tag did not affect AvrRpt2 avirulence and virulence activities, the *avrRpt2*-6XHis fusion was subcloned into the broad host-range vector pUCA12 (Kranz et al. 1997), under the control of wild-type *avrRpt2* regulatory sequences. The resulting clone, pUCA12::*avrRpt2*-6XHis, was introduced into *Pst*DC3000 by triparental mating and used to inoculate No-0 *RPS2* and No-0 *rps2-201C* plants. Like *Pst*DC3000 expressing the wild-type

avrRpt2 gene, *Pst*DC3000 carrying pUCA12::*avrRpt2*-6XHis elicited a normal HR on No-0 *RPS2* plants and exhibited enhanced virulence on No-0 *rps2-201C* plants.

The pRPS2-*avrRpt2* fusion was generated by subcloning the approximately 1.4-kb region of genomic DNA upstream of the *RPS2* coding region from pBI1.R2 (Mindrinos et al. 1994) into pBI121 (Clontech, Palo Alto, CA) such that the *RPS2* upstream region and a multiple cloning site replaced the

Fig. 4. *RPS4*- or *RPS5*-mediated resistance responses are induced in transgenic plants expressing *avrRpt2*. **A**, Levels of *Pseudomonas syringae* pv. *tomato* strain DC3000, *Pst*DC3000 expressing *avrRps4*, and *Pst*DC-3000 expressing *avrPphB* 4 days after inoculation in Col-0 *rps2* plants carrying the pRPS2-*avrRpt2* construct. **B**, Levels of *Pst*DC3000(*avrRps4*) and *Pst*-DC3000(*avrPphB*) 4 days after inoculation in untransformed Col-0 *rps2* plants (white bars) and Col-0 *rps2* plants carrying the pRPS2-*avrRpt2* construct (black bars). Each bar represents the mean and standard error of three independent replicate samples. The plants were inoculated by vacuum infiltration with the indicated *Pst*DC3000 strains at an initial density of 1 × 10⁵ CFU/cm². In each case the level of bacteria present in leaf tissue immediately after infection was approximately 5 × 10² CFU/cm². The data in A and B were obtained from different experiments. Similar results were obtained in two additional independent experiments.

CaMV 35S promoter and the GUS gene of pBI121. The *avrRpt2* coding region from pKExavrRpt2 (Leister et al. 1996) was cloned into the multiple cloning site of the resulting plasmid to generate pBI1.Rpro11-*avrRpt2*, which contains the *avrRpt2* gene under control of the *RPS2* upstream region.

Plant transformations.

No-0 *rps2-201C* and Col-0 *rps2-101C* plants were transformed by the *Agrobacterium* sp. floral dipping procedure as described by Clough and Bent (1998), with the exception that *A. tumefaciens* strain ABI was used to deliver the p35S-*avrRpt2* construct (Klee et al. 1991). Primary transformants (T₁) were selected on 0.5× MS media (Murashige and Skoog 1962) plates containing 50 µg of kanamycin (Sigma, St. Louis, MO, U.S.A.) per ml. Several kanamycin-resistant seedlings for each construct were selected and allowed to self-fertilize to generate T₂ progeny. The number of insertion sites present in each transformation event was determined by monitoring the segregation of kanamycin resistance in the T₂ progeny. Homozygous T₂ plants from lines carrying a single insertion were identified by progeny testing. For each construct, several lines determined to be homozygous for insertions at a single locus were selected for further analysis.

The expression and *avr* activity of the pRPS2-*avrRpt2* and p35S-*avrRpt2* constructs in the transgenic plants were assayed by crossing the transgenic plant lines to wild-type plants carrying a functional *RPS2* gene. The F₁ progeny from these crosses were germinated on 0.5× MS plates containing kanamycin to assay for survival. The progeny derived from crosses with several different transgenic lines died at 2 to 3 weeks of age, presumably as a result of the induction of a systemic HR triggered by the *avrRpt2/RPS2* interaction. F₁ plants from control crosses in which the transgenic plants were crossed to plants carrying a mutant *RPS2* allele did not exhibit this lethal phenotype. The transgenic lines that gave rise to F₁ progeny that died in these experiments were chosen for further experimentation. Expression of *avrRpt2* in these transgenic plants was verified by RNA blot analysis (Sambrook et al. 1989). In No-0 *rps2-201C* p35S-*avrRpt2* transgenic plants, expression of *avrRpt2* also was monitored by protein gel blot analysis (Sambrook et al. 1989) using anti-AvrRpt2 antibodies (McNellis et al. 1998). In these transgenic lines, expression of *avrRpt2* resulted in the accumulation of an approximately 27 kDa AvrRpt2 protein, which is significantly smaller than that observed for the full-length fusion protein (approximately 36 kDa). This result is consistent with previous reports that the AvrRpt2 protein is subject to N-terminal processing in plant cells. This proteolytic processing has been observed in transgenic plants expressing *avrRpt2* and in protein extracts from *A. thaliana* plants infected with *PstDC-3000(avrRpt2)* (McNellis et al. 1998; Mudgett and Staskawicz 1999).

The *avrRpt2* transgene was introduced into the *cpr5 rps2* mutant line by crossing with Col-0 *rps2* transgenic plants carrying the pRPS2-*avrRpt2* construct. The resulting F₁ plants were allowed to self-fertilize, and the F₂ seeds were collected and plated onto 0.5× MS plates containing kanamycin to select for plants carrying pRPS2-*avrRpt2*. The resulting kanamycin-resistant plants were then screened to identify plants homozygous for the *cpr5-2* mutation by assaying for the appearance of spontaneous necrotic lesions and enhanced resis-

tance to *PstDC3000* (Boch et al. 1998). Kanamycin-resistant plants that were homozygous for *cpr5-2* were allowed to self-fertilize, and the resulting progeny was assayed for kanamycin resistance to identify lines that were homozygous for the pRPS2-*avrRpt2* transgene.

ACKNOWLEDGMENTS

We thank J. Glazebrook and R. Zentella for helpful discussion and G. Kalinowski and V. Joardar for helpful comments on the manuscript. We also are grateful to M. B. Mudgett and B. Staskawicz for providing us with anti-AvrRpt2 antisera. This work was supported by NIH grant GM52536 and a David and Lucille Packard Fellowship for Science and Engineering awarded to B. Kunkel and NSF grant MCB-9604830 awarded to F. Katagiri. A. Kloeck was a DOE-Energy Biosciences Research Fellow of the Life Sciences Research Foundation. Funds to cover publication costs were provided by the Jean Lowenhaupt Botany Fund.

LITERATURE CITED

- Aarts, N., Metz, M., Holub, E., Staskawicz, B. J., Daniels, M. M., and Parker, J. E. 1998. Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two *R* gene-mediated signaling pathways in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 95:10306-10311.
- Alfano, J. R., and Collmer, A. 1996. Bacterial pathogens in plants: Life up against the wall. *Plant Cell* 8:1683-1698.
- Alfano, J. R., and Collmer, A. 1997. The Type III (Hrp) secretion pathway of plant pathogenic bacteria: Trafficking harpins, avr proteins, and death. *J. Bacteriol.* 179:5655-5662.
- Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S. P. 1997. Signaling in plant-microbe interactions. *Science* 276:726-733.
- Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B. J. 1994. *RPS2* of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. *Science* 265:1856-1860.
- Bisgrove, S. R., Simonich, M. T., Smith, N. M., Sattler, A., and Innes, R. W. 1994. A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. *Plant Cell* 6:927-933.
- Boch, J., Verbsky, M. L., Robertson, T. L., Larkin, J. C., and Kunkel, B. N. 1998. Analysis of resistance gene-mediated defense responses in *Arabidopsis thaliana* plants carrying a mutation in *CPR5*. *Mol. Plant-Microbe Interact.* 11:1196-1206.
- Bonas, U., and Van den Ackerveken, G. 1997. Recognition of bacterial avirulence proteins occurs inside the plant cell: A general phenomenon in resistance to bacterial diseases? *Plant J.* 12:1-7.
- Bowling, S. A., Clarke, J. D., Liu, Y., Klessig, D. F., and Dong, X. 1997. The *cpr5* mutant of Arabidopsis expresses both *NPR1*-dependent and *NPR1*-independent resistance. *Plant Cell* 9:1573-1584.
- Clough, S. J., and Bent, A. F. 1998. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16:735-743.
- Dangl, J. L. 1994. The enigmatic avirulence genes of phytopathogenic bacteria. Pages 99-118 in: *Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals*. Springer-Verlag, Berlin.
- Debener, T., Lehnackers, H., Arnold, M., and Dangl, J. 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. *Plant J.* 1:289-302.
- Dong, X., Mindrinos, M., Davis, K. R., and Ausubel, F. M. 1991. Induction of *Arabidopsis thaliana* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. *Plant Cell* 3:61-72.
- Duan, Y. P., Castañeda, A., Zhao, G., Erdos, G., and Gabriel, D. W. 1999. Expression of a single, host-specific, bacterial pathogenicity gene in plant cells elicits division, enlargement, and cell death. *Mol. Plant-Microbe Interact.* 12:556-560.
- Feys, B. J., Benedetti, C. E., Penfold, C. N., and Turner, J. G. 1994. Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell* 6:751-759.

- Figurski, D., and Helinski, D. R. 1979. Replication of an origin-containing derivative of plasmid RK2 is dependent on a plasmid function provided in trans. *Proc. Natl. Acad. Sci. USA* 76:1648-1652.
- Fillingham, A. J., Wood, J. R., Crute, I. R., Mansfield, J. W., Taylor, J. D., and Vivian, A. 1992. Avirulence genes from *Pseudomonas syringae* pathovars *phaseolicola* and *pisi* confer specificity towards both host and non-host species. *Physiol. Mol. Plant Pathol.* 40:1-15.
- Finlay, B. B., and Cossart, P. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. *Science* 276:718-725.
- Galan, J. E., and Collmer, A. 1999. Type III secretion machines: Bacterial devices for protein delivery into host cells. *Science* 284:1322-1328.
- Gopalan, S., Bauer, D. W., Alfano, J. R., Loniello, A. O., He, S. Y., and Collmer, A. 1996. Expression of the *Pseudomonas syringae* avirulence protein AvrB in plant cells alleviates its dependence on the hypersensitive response and pathogenicity (Hrp) secretion system in eliciting genotype-specific hypersensitive cell death. *Plant Cell* 8:1095-1105.
- Hammond-Kosack, K. E., and Jones, J. D. G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8:1773-1791.
- Hinsch, M., and Staskawicz, B. 1996. Identification of a new Arabidopsis disease resistance locus, *RPS4*, and cloning of the corresponding avirulence gene, *avrRps4*, from *Pseudomonas syringae* pv. *pisi*. *Mol. Plant-Microbe Interact.* 9:55-61.
- Innes, R. W., Bent, A. F., Kunkel, B. N., Bisgrove, S. R., and Staskawicz, B. J. 1993. Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. *J. Bacteriol.* 175:4859-69.
- Jackson, R. W., Athanassopoulos, E., Tsiamis, G., Mansfield, J. W., Sema, A., Arnold, D. L., Gibbon, M. J., Murillo, J., Taylor, J. D., and Vivian, A. 1999. Identification of a pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pv. *phaseolicola*. *Proc. Natl. Acad. Sci. USA* 96:10875-10880.
- Jenner, C., Hitchin, E., Brown, I., Walters, K., and Mansfield, J. 1989. A gene for avirulence from *Pseudomonas syringae* pv. *phaseolicola*. *Mol. Plant-Microbe Interact.* 4:553-562.
- Kearney, B., and Staskawicz, B. J. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. *Nature* 346:385-386.
- Kjemtrup, S., Nimchuk, Z., and Dangel, J. L. 2000. Effector proteins of phytopathogenic bacteria: Bifunctional signals in virulence and host recognition. *Curr. Opin. Microbiol.* 3:73-78.
- Klee, H. J., Hayford, M. B., Kretzmer, K. A., Barry, G. F., and Kishore, G. M. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell* 3:1187-1193.
- Kranz, R. G., Gabbert, K. K., and Madigan, M. T. 1997. Positive selection systems for discovery of novel polyester biosynthesis genes based on fatty acid detoxification. *Appl. Environ. Microbiol.* 63:3010-3013.
- Kunkel, B. N., Bent, A. F., Dahlbeck, D., Innes, R. W., and Staskawicz, B. J. 1993. *RPS2*, an Arabidopsis disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Plant Cell* 5:865-75.
- Leach, J. E., and White, F. F. 1996. Bacterial avirulence genes. *Annu. Rev. Phytopathol.* 34:153-179.
- Leister, R. T., Ausubel, F. M., and Katagiri, F. 1996. Molecular recognition of pathogen attack occurs inside of plant cells in plant disease resistance specified by the Arabidopsis genes *RPS2* and *RPM1*. *Proc. Natl. Acad. Sci. USA* 93:15497-15502.
- Lorang, J. M., and Keen, N. T. 1995. Characterization of *avrE* from *Pseudomonas syringae* pv. *tomato*: A *hrp*-linked avirulence locus consisting of at least two transcriptional units. *Mol. Plant-Microbe Interact.* 8:49-57.
- Lorang, J. M., Shen, H., Kobayashi, D., Cooksey, D., and Keen, N. T. 1994. *avrA* and *avrE* in *Pseudomonas syringae* pv. *tomato* PT23 play a role in virulence on tomato plants. *Mol. Plant-Microbe Interact.* 7:508-515.
- McNellis, T. W., Mudgett, M. B., Li, K., Aoyama, T., Horvath, D., Chua, N. H., and Staskawicz, B. J. 1998. Glucocorticoid-inducible expression of a bacterial avirulence gene in transgenic Arabidopsis induced hypersensitive cell death. *Plant J.* 14:247-357.
- Mindrinis, M., Katagiri, F., Yu, G., and Ausubel, F. M. 1994. The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide binding site and leucine-rich repeats. *Cell* 78:1089-1099.
- Morel, J. B., and Dangel, J. L. 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ.* 4:671-683.
- Mudgett, M. B., and Staskawicz, B. J. 1999. Characterization of the *Pseudomonas syringae* pv. *tomato* AvrRpt2 protein: Demonstration of secretion and processing during bacterial pathogenesis. *Mol. Microbiol.* 32:927-941.
- Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
- Reuber, T. L., and Ausubel, F. M. 1996. Isolation of Arabidopsis genes that differentiate between resistance responses mediated by the *RPS2* and *RPM1* disease resistance genes. *Plant Cell* 8:241-249.
- Ritter, C., and Dangel, J. L. 1995. The *avrRpm1* gene of *Pseudomonas syringae* pv. *maculicola* is required for virulence on Arabidopsis. *Mol. Plant-Microbe Interact.* 8:444-453.
- Ritter, C., and Dangel, J. L. 1996. Interference between two specific pathogen recognition events mediated by distinct plant disease resistance genes. *Plant Cell.* 8:251-257.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Shan, L., He, P., Zhou, J., and Tang, X. 2000. A cluster of mutations disrupt the avirulence but not the virulence function of AvrPto. *Mol. Plant-Microbe Interact.* 13:592-598.
- Simonich, M. T., and Innes, R. W. 1995. A disease resistance gene in Arabidopsis with specificity for the *avrPph3* gene of *Pseudomonas syringae* pv. *phaseolicola*. *Mol. Plant-Microbe Interact.* 8:637-640.
- Staskawicz, B., Dahlbeck, D., Keen, N., and Napoli, C. 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. *J. Bacteriol.* 169:5789-5794.
- Stevens, C., Bennet, M. A., Athanassopoulos, E., Tsiamis, G., Taylor, J. D., and Mansfield, J. W. 1998. Sequence variations in alleles of the avirulence gene *avrPphE.R2* from *Pseudomonas syringae* pv. *phaseolicola* lead to loss of recognition of the AvrPphE protein within bean cells and a gain in cultivar-specific virulence. *Mol. Microbiol.* 29:165-177.
- Swarup, S., De Feyter, R., Brlansky, R. H., and Gabriel, D. W. 1991. A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit canker like lesions on citrus. *Phytopathology* 81:802-809.
- Tobias, C. M., Oldroyd, G. E. D., Chang, J. H., and Staskawicz, B. J. 1999. Plants expressing the *Pto* disease resistance gene confer resistance to recombinant PVX containing the avirulence gene *AvrPto*. *Plant J.* 17:41-50.
- Vivian, A., and Gibbon, M. J. 1997. Avirulence genes in plant-pathogenic bacteria: Signals or weapons? *Microbiology* 143: 693-704.
- Whalen, M., Innes, R., Bent, A., and Staskawicz, B. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis thaliana* and a bacterial gene determining avirulence on both *Arabidopsis* and soybean. *Plant Cell* 3: 49-59.
- Yu, G. L., Katagiri, F. and Ausubel, F. M. 1993. *Arabidopsis* mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Mol. Plant-Microbe Interact.* 6: 434-443.
- Yu, I., Parker, J. and Bent, A. F. 1998. Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proc. Natl. Acad. Sci. USA* 95: 7819-7824.