The Plant Cell, Vol. 11, 1665–1674, September 1999, www.plantcell.org

The C Terminus of AvrXa10 Can Be Replaced by the Transcriptional Activation Domain of VP16 from the Herpes Simplex Virus

Weiguang Zhu,[1] Bing Yang, Nick Wills, Lowell B. Johnson, and Frank F. White[2]

Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506

The avirulence gene *avrXa10* of *Xanthomonas oryzae* pv *oryzae* directs the elicitation of resistance in a gene-for-gene manner in rice lines carrying the resistance gene *Xa10.* We have localized a transcriptional activator domain in the C terminus of AvrXa10 by using amino acid replacement mutagenesis. One mutant, with replacements at three hydrophobic amino acid residues in the C-terminal domain, was defective for transcriptional activation in yeast and avirulence activity in rice. The activation domain from the herpes virus protein VP16 restored the ability of the bacteria expressing the hybrid protein to elicit a resistance reaction. Elicitation was specific for *Xa10*, and the reaction had the hallmarks of the response to AvrXa10. The results indicate that a domain with the properties of a transcriptional activator plays a critical role in AvrXa10 function. The results also indicate that the protein has the potential to interact with the plant transcriptional program, although a role for the domain in the stability or conformation of the protein in the plant cannot be excluded. In a broader sense, the transcriptional activation domain of *avrXa10* may represent a prokaryotic version of the acidic transcriptional activation domain, which heretofore has been found exclusively in eukaryotes.

INTRODUCTION

The avirulence (*avr*) gene *avrXa10* of *Xanthomonas oryzae* pv *oryzae* is a member of the bacterial *avrBs3* avirulence gene family (Bonas et al., 1989; Hopkins et al., 1992). *avr* genes of phytopathogenic bacteria control the ability of the pathogen to elicit a resistance response. This reaction generally involves the hypersensitive response (HR) in plant cultivars that contain specific, corresponding genes for resistance (*R* genes) (Keen, 1990; Alfano and Collmer, 1997). In addition to programmed cell death, a variety of associated responses occur during an HR, and in toto, the HR and accompanying responses suppress the growth and ingress of the pathogen to healthy tissue (Scheel, 1998). A variety of bacterial Avr proteins, including members of the AvrBs3 family, have been shown to elicit a hypersensitive reaction independent of the bacterium when expressed in the host cell (Gopalan et al., 1996; Leister et al., 1996; Scofield et al., 1996; Tang et al., 1996; Van den Ackerveken et al., 1996). Therefore, the most compelling model for members of the AvrBs3 family, and some other bacterial *avr* gene products, postulates the secretion of the proteins from the bacteria into the cells of the host via a type III secretion system (reviewed in Bonas and Van den Ackerveken, 1997).

Once in the host cells, Avr proteins are perceived in some manner by the host surveillance system and trigger a cascade of events leading to the HR. The perception of the Avr proteins may be by direct protein–protein interaction with the products of the *R* genes. The cloning of an *R* gene corresponding to the AvrBs3 family has not been reported. However, the AvrPto protein, which is not a member of the AvrBs3 family, has been shown to interact with the protein product of *Pto*, which is the *R* gene corresponding to *avrPto*, in a yeast two-hybrid system (Scofield et al., 1996; Tang et al., 1996). The interaction of AvrPto and Pto, which is a serine/threonine kinase, is likely to occur in the cytoplasm and triggers a resistance reaction through an unknown mechanism (Zhou et al., 1995, 1997; Salmeron et al., 1996). In contrast to AvrPto, both AvrBs3 and AvrXa10 have been shown to require the presence of at least one of three nuclear localization signals for avirulence activity (Yang and Gabriel, 1995b; Van den Ackerveken et al., 1996; Zhu et al., 1998). Therefore, if either AvrBs3 or AvrXa10 interacts with the corresponding *R* gene product, as proposed by Van den Ackerveken et al. (1996) for AvrBs3, the signaling, by whatever means, may occur in the nucleus.

AvrXa10 and related proteins are also distinguished by their peculiar structures (Bonas et al., 1989, 1993; Hopkins et al., 1992; De Feyter et al., 1993; Yang et al., 1994). The middle third consists of a repeat domain, which in turn is composed of near-perfect copies of a 34–amino acid sequence. The repeats are nearly identical in sequence at the amino acid level, with some exceptions, particularly at positions 12 and 13 within the repeat. In the case of AvrXa10, the repeat domain has 15.5 copies (Hopkins et al., 1992).

[1] Current address: Molecular Cardiology Division, Southwestern Medical Center, Dallas, TX 75235-8573.

[2] To whom correspondence should be addressed. E-mail fwhite@plantpath.ksu.edu; fax 785-532-5692.

Exchanging the repeat domains changes the specificity of the protein to the specificity of the gene from which the repeat domain was derived or, in the case of rearrangements, to a new specificity (Herbers et al., 1992; Yang et al., 1994; Yang and Gabriel, 1995a; Zhu et al., 1998). Several members of the gene family are also involved in pathogenicity, and the repeat domain also controls the specificity of the pathogenic effects of the genes (Swarup et al., 1991, 1992; Yang et al., 1994, 1996). Therefore, the repeats orchestrate aspects of the specificity of the interaction with host factors.

AvrXa10, AvrXa7, and AvrBs3 all require the C terminus for avirulence activity, although the region does not control specificity of the interaction (Zhu et al., 1998). One possible function for the C terminus was revealed by the fact that AvrXa10 possesses transcriptional activation activity (Zhu et al., 1998). Removal of the C terminus from AvrXa10, although retaining the nuclear localization signals, severely reduced this activity (Zhu et al., 1998). We attempted to determine whether the requirement for avirulence and the activation activity were simply coincidental by localizing further the native activation domain and replacing the domain with a heterologous domain containing similar activation properties but low sequence similarity. We have now localized an activator domain in the C terminus of AvrXa10 and show that the activation domain of VP16 from the animal herpes simplex virus can supply the requirement of this domain for avirulence. The results implicate a role for a eukaryotic-like acidic transcriptional activator domain in the elicitation of resistance and demonstrate the potential of the bacteria to target directly the transcriptional program of the host plant.

RESULTS

The C Terminus of AvrXa10 Contains a Transcriptional Activation Domain

We wanted to determine whether the requirement of the C terminus for transcriptional activation and avirulence was due to a single domain or separate domains within the region. Our strategy was to correlate the effects of amino acid replacement mutations on transcriptional activation and avirulence. Therefore, a yeast one-hybrid activation assay was developed using the terminal 1.4-kb BamHI fragment of *avrXa10*, which contains the C-terminal 171 bp (Figure 1A), fused to the Gal4 DNA binding domain in pPAS3. We used the level of β-galactosidase activity as measured by the *O*-nitrophenyl-β-D-galactopyranoside substrate in the yeast cells with a *lacZ* reporter gene under the control of Gal4 upstream activation sequences (UAS_G) as the criterion for activation. The detection limit was ~0.5 ± 0.1 units of enzyme activity. The 1.4-kb BamHI fragment directed relatively high levels of transcription activation (39.3 ± 1.4 units), whereas the corresponding 1.4-kb BamHI fragment from *avrXa10*^{TGA1064}, which was truncated 39 codons from the C terminus, had no activation activity (0.3 ± 0.1 units). Activation also was not detected with the 3.1-kb BamHI fragment of *avrXa10* (0.5 ± 0.1 units), which encodes the entire AvrXa10 protein except the C-terminal 171 bp. In agreement with our previous finding, the yeast system was a reliable indicator of activity, and

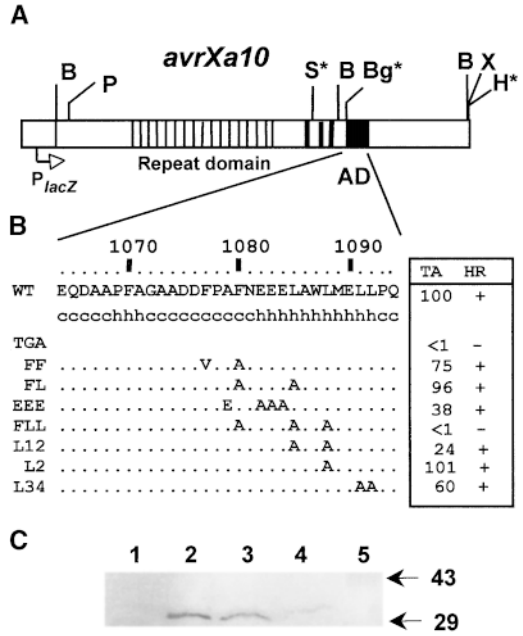

Figure 1. Map of *avrXa10* and C Terminus.

(A) The map shows *avrXa10* as represented in pZWX10-F2H. Black boxes represent nuclear localization motif sequences A, B, and C, and a region of the activation domain (AD). The asterisks denote introduced sites (see text for details). B, BamHI; Bg, BglII; H, HindIII; P, PstI; P_{lacZ}, lacZ promoter; S, SalI; X, XbaI.

(B) Mutations and transcriptional activation activity of mutant domains. Structural prediction according to Garnier et al. (1996) is shown below the wild-type (WT) sequence. The source of activation domain is indicated at left: TGA, AvrXa10^{TGA1064}; FF, AvrXa10$^{FF1077/80}$; FL, AvrXa10$^{FL1080/85}$; EEE, AvrXa10$^{EEE1082/83/84}$; FLL, AvrXa10$^{FLL1080/85/88}$; L12, AvrXa10$^{LL1085/88}$; L2, AvrXa10^{L1088}; and L34, AvrXa10$^{LL1091/92}$. Dots indicate identical residues as given for the wild type. Transcriptional activity (TA) in pPAS3 and ability to elicit an HR in BB10 rice are shown at right. TA scores represent β-galactosidase activity as the percentage of the wild type. (+), HR or avirulence positive; (−), susceptible reaction. The last eight residues for AvrXa10 (SVSVGGTI) are not shown. c, coiled domain; h, helical domain.

(C) Immunoblot of Gal4DB hybrid proteins. Proteins were detected after transfer to nitrocellulose by reaction with anti-Gal4DB monoclonal antibody followed by anti-mouse alkaline phosphatase–conjugated IgG. The source of the activation domain of the Gal4DB hybrid proteins was as follows: lane 1, no Gal4DB; lane 2, AvrXa10^{TGA1064}; lane 3, AvrXa10$^{FLL1080/85/88}$; lane 4, AvrXa10; and lane 5, size standards, which are given in kilodaltons at right.

the primary activation domain resided within the last 39 amino acid residues.

The FLL Mutation Results in the Loss of Transcription and Avirulence Activity

To test which of the residues of the C terminus were critical for transcriptional activation and/or avirulence, amino acid replacements were targeted to some of the hydrophobic and acidic residues. The effects of the mutations on transcriptional activation using the yeast one-hybrid system and on avirulence activity after transfer of the gene to *X. o.* pv *oryzae* strain PXO99^{A}, which has no endogenous copy of *avrXa10*, were then determined. The responses due to replacement derivatives of *avrXa10* are summarized in Figure 1B. Of the derivatives tested, only the triple replacement of the phenylalanine at position 1080 and leucine residues at positions 1085 and 1088 (termed the FLL mutation or *avrXa10*$^{FLL1080/85/88}$) resulted in the loss of activation and hypersensitivity (Figure 1). Two other mutants, EEE and L12, had transcriptional activation activities $<$50% of the wild type (Figure 1). However, both mutants retained the ability to elicit an HR. The eight codons at the end of AvrXa10, which were absent in other members of AvrBs3 (Hopkins et al., 1992) and mutants L12, L2, and L34, were also found not to be required for activation or avirulence (Figure 1B).

To determine whether the loss of transcriptional activation activity by the TGA1064 and FLL mutations was due simply to loss of expression or instability of the Gal4DB hybrid proteins in yeast, an immunoblot analysis using an anti-Gal4DB monoclonal antibody was performed (Figure 1C). Proteins of the expected size for the fusion proteins ($\sim$33 kD) were detected in the yeast with the mutant and wild-type genes (Figure 1C, lanes 2 to 4), whereas no protein was detected in the absence of the vector (Figure 1C, lane 1). Therefore, protein instability or loss of expression does not appear to account for the loss of transcriptional activation. In fact, more protein, as indicated by the intensity of the bands in the immunoblot, was recovered from equal numbers of yeast cells expressing the inactive Gal4DB hybrids as compared with those expressing Gal4DB with the wild-type *avrXa10* activation domain.

Hypersensitivity in rice due to AvrXa10 was scored by the appearance of brown discoloration at the site of inoculation within 24 to 72 hr (Figure 2A, leaf 1) and the suppression of bacterial growth in the plant. With *avrXa10*^{TGA1064}, the inoculation sites were void of browning and were chlorotic by 72 hr (Figure 2A, leaf 2), which is characteristic of a susceptible reaction. The FLL1080/85/88 derivative was similar in phenotype to *avrXa10*^{TGA1064} (Figure 2A, leaf 3), whereas other replacements produced an HR and had leaf phenotypes similar to *avrXa10* (data not shown). The population of bacteria with the wild-type *avrXa10* in the plant remained below $\sim 1 \times 10^7$ colony-forming units (CFU) per leaf in the 4-day period (Figure 2B, curve 1). The bacterial populations with

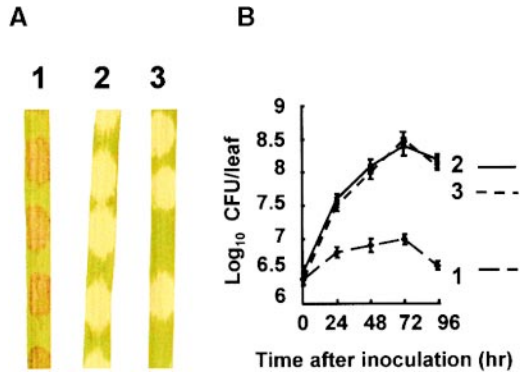

Figure 2. Phenotypic Effect of *avrXa10*$^{FLL1080/85/88}$.

(A) Derivatives of *avrXa10* were transferred to strain PXO99^{A}, and leaves from BB10 were infiltrated with bacterial suspensions and photographed at 72 hr after inoculation. Dark brown discoloration is indicative of resistance reaction (leaf 1). Yellow coloration is indicative of susceptible reaction and loss of avirulence activity (leaves 2 and 3). Reactions on the leaves were the result of inoculations with PXO99^{A} containing the following *avr* genes: leaf 1, *avrXa10*; leaf 2, *avrXa10*^{TGA1064}; and leaf 3, *avrXa10*$^{FLL1080/85/88}$.

(B) Populations of PXO99^{A} with *avrXa10* or derivatives in leaves of BB10. Curves represent the population of bacteria with the following genes: 1, *avrXa10*; 2, *avrXa10*^{TGA1064}; and 3, *avrXa10*$^{FLL1080/85/88}$. Populations are expressed as CFU per leaf. Error bars indicate $\pm$SD.

avrXa10^{TGA1064} or *avrXa10*$^{FLL1080/85/88}$ reached the maximum levels of $\sim 5 \times 10^8$ CFU within the 4-day period (Figure 2B, curves 2 and 3, respectively). Thus, the loss or severe reduction in transcriptional activation in yeast due to the FLL mutation was concomitant with loss of avirulence activity.

Expression of the FLL Mutant of *avrXa10* in Plant Cells Does Not Restore Avirulence Activity

The FLL mutation in *avrXa10* might interfere with the ability of the bacterium to transfer the protein to the plant cell and therefore not be directly responsible for loss of avirulence activity in the host cell. To test this possibility, the *avrXa10* gene and the FLL derivative were expressed transiently in rice leaf cells with (BB10) or without (IR24) *Xa10.* Elicitor activity was measured by the relative level of expression of a constitutive reporter gene that was delivered simultaneously with the *avr* gene. The expectation was that cells containing an active *avr* gene and the corresponding *R* gene would be severely limited in the ability to express the reporter gene due to the death of the cell and associated deterioration of the transcription machinery.

Bombardment of leaves with pYB100 (which contains the cauliflower mosaic virus 35S promoter [35S] fused to *avrXa10*) or pYB110 (35S–*avrXa10*$^{FLL1080/85/88}$) led to the detection of numerous β-glucuronidase (GUS)–positive foci

after staining (Figures 3A to 3D). However, the number of GUS-positive foci on BB10 leaves that were bombarded with pYB100 was 75% lower than was the number of foci on leaves of the susceptible line IR24 bombarded with the same plasmid at the same time (Figure 3E). The numbers of GUS-positive foci did not differ in BB10 and IR24 leaves bombarded with 35S–*avrXa10*$^{FLL1080/85/88}$ (pYB110) and were similar to the number obtained using 35S–*avrXa10* in IR24 (Figure 3E). Although we have not been able to detect

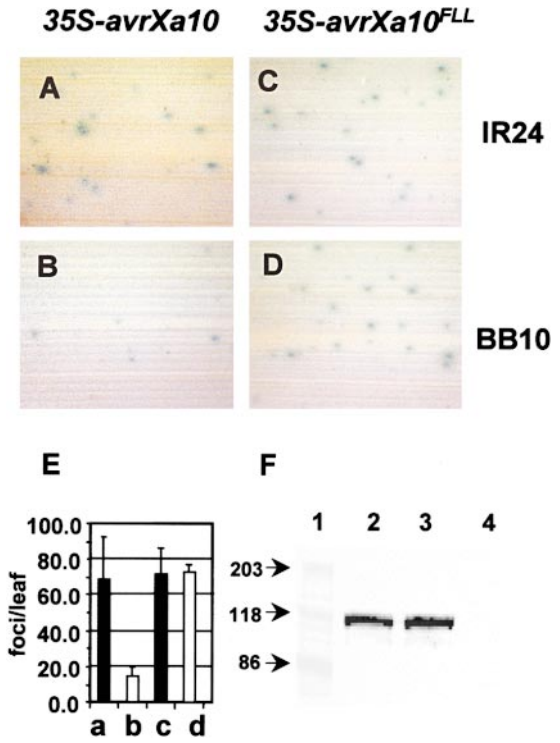

Figure 3. Effect of 35S–*avrXa10*$^{FLL1080/85/88}$ in Rice Tissue after Particle Bombardment.

(A) to **(D)** Leaf sections of IR24 (**[A]** and **[C]**) and BB10 (**[B]** and **[D]**) were bombarded with plasmids pYB100 and pYB110 containing 35S–*avrXa10* and 35S–*avrXa10*$^{FLL1080/85/88}$, respectively, and stained with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide after 36 hr. Blue foci represent leaf cells that express GUS from the ubiquitin (*Ubi*)–*uidA* gene present in both plasmids.

(E) Average number of GUS-positive foci per leaf section after bombardment with particles containing 35S–*avrXa10* (a and b) or 35S–*avrXa10*$^{FLL1080/85/88}$ (c and d). Error bars indicate ±SD. Black bars, IR24; open bars, BB10.

(F) AvrXa10$^{FLL1080/85/88}$ levels in *X. oryzae* pv *oryzae*. Protein was extracted from PXO99^{A} containing the indicated gene and subjected to immunoblot analysis using polyvalent antisera directed against AvrXa10. Lane 1, molecular mass standards (size indicated in kilodaltons); lane 2, *avrXa10*$^{FLL1080/85/88}$; lane 3, *avrXa10*; and lane 4, vector only.

Figure 4. Sequence of the C Terminus of AvrXa10 and Derivatives Containing VP16 Transcriptional Activation Domain.

Boxed residues indicate the original AvrXa10^{TGA1064} sequence. LPQ at the end of mVP16 and mVP16/FL/AA is derived from the last three residues of wild-type AvrXa10. Dots represent residues from wild-type AvrXa10. WT, wild-type sequence; VP16, 77–amino acid fusion; mVP16, 20–amino acid fusion; and mVP16/FL/AA, 20–amino acid fusion with phenylalanine and leucine at positions 479 and 483, respectively, replaced with alanine residues (triangles).

AvrXa10 in the plant cells, we extracted protein from PXO99^{A} to determine whether AvrXa10$^{FLL1080/85/88}$ was produced in significantly lower quantities in the bacterium. No differences were observed in the amount of protein from bacteria containing *avrXa10* or *avrXa10*$^{FLL1080/85/88}$ (Figure 3F). Therefore, the FLL mutant of *avrXa10* is defective in avirulence activity, regardless of the delivery method to the host cell.

Replacement of a Mutant AvrXa10 Transcriptional Activation Domain with a Heterologous Domain Restores Avirulence Activity

If *avrXa10* requires a transcriptional activation domain for avirulence, then replacement of the truncated C-terminal coding region with the domain of another transcription factor might restore avirulence activity. The BglII site, immediately upstream of the stop codon in the *avrXa10*^{TGA1064} sequence (see Figure 1), was used to introduce a DNA fragment encoding the activation domain of VP16 from herpes simplex virus. The initial construct incorporated 77 amino acid residues from the C terminus of VP16 (Figure 4, VP16) resulting in the chimeric gene termed *avrXa10*^{VP16}. Strains containing *avrXa10*^{VP16} caused a browning reaction at the site of inoculation, although the response was weaker in terms of the degree of brown discoloration compared with the reaction to bacteria containing *avrXa10* (Figure 5A, leaves 3 and 1, respectively).

The weak resistance reaction of strains containing *avrXa10*^{VP16} led to concern that the 77–amino acid coding region may interfere with the avirulence activity due to the size or composition of the domain. Therefore, a second hy-

brid gene was constructed by incorporating the minimal subdomain of VP16 that is required for transcriptional activation (Figure 4, mVP16; Uesugi et al., 1997). The smaller hybrid gene resulted in an HR that was similar to the response to *avrXa10* (Figure 5A, leaf 4). The HR was dependent on the presence of *Xa10* because the HR reaction was observed only on BB10 plants and not on IR24 (Figure 5A, leaf 5). Bacterial growth in the plant was suppressed when bacteria containing *avrXa10*, *avrXa10*^{VP16}, or *avrXa10*^{mVP16} were inoculated on BB10 (Figure 5B, curves 1, 3, and 4, respectively), although growth suppression in the plant due to *avrXa10*^{VP16} was less than for *avrXa10* and *avrXa10*^{mVP16}. No growth suppression in BB10 was observed for PXO99^{A} containing *avrXa10*^{TGA1064} or the vector alone (Figure 5B, curves 2 and 7, respectively) or in IR24 for PXO99^{A} containing either *avrXa10*^{VP16} (data not shown) or *avrXa10*^{mVP16} (Figure 5B, curve 5).

The F479 and L483 residues of VP16 have been proposed on the basis of analysis by nuclear magnetic resonance to make nonpolar contacts with the human transcription cofactor hTAF$_{II}$31 (Uesugi et al., 1997). A version of *avrXa10*^{mVP16} was constructed in which these two residues were substituted with alanine residues (Figure 4, mVP/FL/AA). When introduced to PXO99^{A}, no avirulence activity (Figure 5A, leaf 6) or suppression of growth in the plant was observed by strains carrying *avrXa10*$^{mVP/FL/AA}$ on inoculated leaves of BB10 rice (Figure 5B, curve 6).

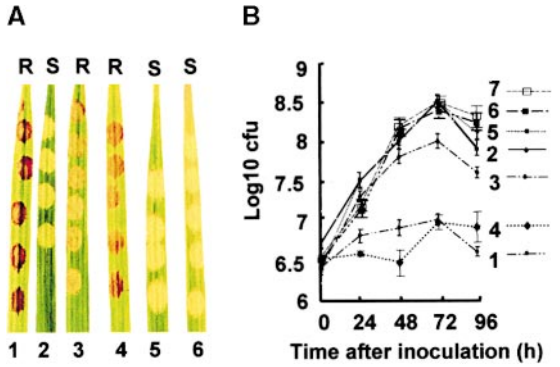

Figure 5. Effect of VP16 Transcription Activation Domain on AvrXa10 Activity.

(A) Leaf reactions to inoculation with PXO99^{A} with the following combinations of *avrXa10* derivatives and rice cultivars: leaf 1, *avrXa10* and BB10; leaf 2, *avrXa10*^{TGA1064} and BB10; leaf 3, *avrXa10*^{VP16} and BB10; leaf 4, *avrXa10*^{mVP16} and BB10; leaf 5, *avrXa10*^{mVP16} and IR24; and leaf 6, *avrXa10*$^{mVP16/FL/AA}$ and BB10. Letters above leaves indicate the scoring of the reaction: R, resistant reaction; S, susceptible reaction. Note that the weak reaction for leaf 3 was scored as R.

(B) Populations of PXO99^{A} containing modifications of *avrXa10* in rice leaves. Numbers refer to inoculations as indicated for **(A)**. Curve 7 represents population from inoculations of IRBB10 with PXO99^{A} (pH M1), which has only the vector and no copy of *avrXa10.* Error bars indicate ±SD. h, hr.

The *avrXa10*^{mVP16} Gene Induces a Typical HR Response

Experiments were performed to measure other parameters of the resistance response due to *avrXa10*^{VP16}. Resistance reactions in rice to bacterial infection have been correlated with the induction of specific gene transcripts for peroxidase (Chittoor et al., 1997). In addition, induction of pathogenesis-related proteins and callose deposition at the inoculation site occurs in the HR of a variety of plant species (Dietrich et al., 1994; Morris et al., 1998). Inoculation of bacteria containing *avrXa10*^{mVP16} induced transcription of the peroxidase gene *POX22.3* and the pathogenesis-related protein gene *PR-1* after only 16 hr in rice plants containing the *Xa10* gene (Figure 6A). Callose deposition, as revealed by aniline blue staining, was also observed only at the inoculation site when bacteria containing either *avrXa10*^{mVP16} or the *avrXa10* hybrid gene were inoculated on plants with *Xa10* (Figures 6C and 6D, respectively). In the absence of *Xa10*, fluorescence resulting from callose deposition was observed only at the border of the inoculation site, where the syringe occasionally caused wounding of the plant cells (Figure 6E). Thus, the response of the plant containing *Xa10* to the hybrid protein with the VP16 transcriptional activation domain was indistinguishable from the response to wild-type AvrXa10.

DISCUSSION

AvrXa10 contains a region in the C terminus that is strikingly similar to the general class of acidic transcriptional activation domains. Nearly 50% of the 30 terminal residues of AvrXa10 are acidic or large hydrophobic amino acids. The transcriptional activation potential of the protein AvrXa10 was first demonstrated by creating fusions of AvrXa10 to the Gal4 DNA binding domain (Zhu et al., 1998). The hybrid AvrXa10 was capable of activating transcription of reporter genes with a *UAS*$_G$ binding motif both in yeast and Arabidopsis (Zhu et al., 1998). Although the C terminus was required for avirulence and high transcriptional activation, the actual position of the activation domain was not known. Using amino acid replacements and the yeast one-hybrid system, we were able to pinpoint more precisely a domain near the extreme C terminus that is sufficient for transcriptional activation activity in yeast and required for avirulence. We did not identify any mutation that eliminated activation in yeast without also eliminating avirulence. The domain from VP16 has little sequence identity with the C terminus of AvrXa10 other than the general juxtaposition of acidic and bulky amino acid residues, yet this domain was capable of replacing the endogenous domain. Furthermore, activation

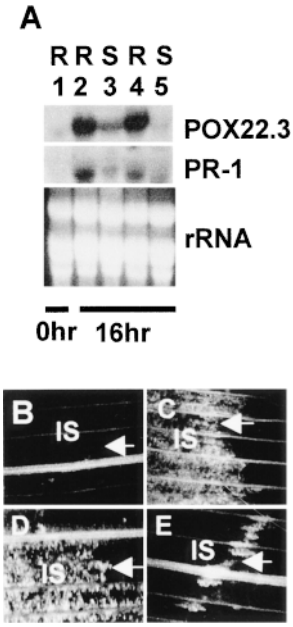

Figure 6. Expression of Defense-Associated Responses in Rice Leaves with *avrXa10*^{mVP16}.

(A) Autoradiograph of mRNA for peroxidase and pathogenesis-related protein PR-1 in BB10 or IR24 leaves after inoculation with PXO99^{A} containing *avrXa10* or *avrXa10*^{mVP16}. RNA was isolated at 0 and 16 hr. Lane 1 contains *avrXa10*^{mVP16} and BB10; lane 2, *avrXa10* and BB10; lane 3, *avrXa10* and IR24; lane 4, *avrXa10*^{mVP16}; and lane 5, *avrXa10*^{mVP16} and IR24. Ethidium bromide–stained rRNA is shown at bottom for loading comparison between lanes. R, resistant interaction; S, susceptible interaction.

(B) to **(E)** Aniline blue–stained rice leaf tissue 24 hr after inoculation with PXO99^{A} containing the following genes: **(B)** *avrXa10*^{TGA1064} on BB10; **(C)** *avrXa10*^{mVP16} on BB10; **(D)** *avrXa10* on BB10; and **(E)** *avrXa10*^{mVP16} on IR24. Callose appears white. Arrows indicate border of inoculated area, and IS indicates inoculation site. Note in **(E)** border cells at the inoculation site that were wounded in the inoculation process. Leaves were stained at 24 hr after inoculation.

domains are thought to function in recruitment of transcription cofactors by a combination of charge attraction and noncovalent contacts with various factors (reviewed in Ptashne and Gann, 1997). We mutated two of the residues of the VP16 domain that are thought to be critical for the noncovalent contact with hTAF$_{II}$31 and found that these residues were also required for avirulence activity of the hybrid protein. Therefore, the domain in AvrXa10 behaves similarly to the general aspects of other acidic activation domains on the basis of broad specificity and structural similarity and, in the case of AvrXa10, is required for avirulence.

The VP16 and p53 activation domains have been determined to form a helical conformation upon binding of transcription cofactors (Uesugi et al., 1997). However, the significance of such a helical conformation has been called into question, at least for the Gal4 activation domain (Ansari et al., 1998), and the structural requirement for activation remains controversial. Activation domains may simply provide relatively nonspecific contact sites, which are rather flexible with regard to overall protein structure. Single amino acid changes commonly do not disrupt activation. Similarly, we have not ascribed a critical requirement for a single amino acid and did not observe severe reductions in either avirulence or activation unless three amino acids were altered. Structure prediction analysis suggests that the stretch of amino acids from positions 1079 to 1092 in AvrXa10 could form a helix in solution, and other regions could possibly form helical conformations upon binding a plant factor. The FLL mutation may have affected contact sites or altered the overall conformation of the region that is required for avirulence and activation.

Despite the tight requirement for activation as measured in the yeast system, we do not assume that the structural requirements of the domain are the same for activity in plants and yeast, and we expect that some mutations may affect avirulence without eliminating transcriptional activation in yeast. Some of the mutations appeared to have quantitative effects on transcriptional activity, yet avirulence activity was still apparent, and a threshold may exist below which avirulence activity is lost. Similarly, mutations in the activation domain may have different effects on different *avr* and *R* gene combinations.

A fourfold difference was observed between the expression of a *GUS* reporter gene in resistant and susceptible leaf cells when 35S–*avrXa10* was delivered by particle bombardment. The bombardment method was based on experiments with *avrRpt2* and leaf cells of Arabidopsis containing the *R* gene *RPS2* (Leister et al., 1996). In the latter experiment, reductions in GUS activity were observed upon bombardment of resistant leaf tissue with *avrRpt2* when under the control of the 35S promoter. Bombardment of plant leaves with 35S–*avrXa10*$^{FLL1080/85/88}$ showed no difference in *GUS* expression between resistant and susceptible tissues. We did not measure the amount of protein delivered or synthesized within the plant cell, and one alternative explanation is that the FLL mutation leads to protein instability within the plant cell. However, levels of AvrXa10$^{FLL1080/85/88}$ in the bacterium were similar to those of AvrXa10 and therefore protein instability does not appear to be responsible for the lack of activity. We interpret the results as evidence that AvrXa10, similar to AvrBs3 and other members of the family, is secreted to the host cell and that the FLL mutation is defective in elicitor activity, regardless of the delivery method.

Deletion of the C terminus of AvrXa10, AvrXa7, and AvrBs3 resulted in the loss of avirulence activity of each protein (Zhu et al., 1998). However, not all members of the *avrBs3* family require the C terminus of the protein product for avirulence and therefore are not likely to require the transcriptional activation domain for avirulence activity. The

gene *avrBs3-2*, another member of *avrBs3* family, has avirulence activity on tomato. This gene was initially cloned without the C terminus, and the C-terminal portion can be deleted up to the third repeat and still have avirulence activity (Canteros et al., 1991; Bonas et al., 1993). Therefore, the protein product can elicit resistance without the nuclear localization signal regions and the transcription activation domain and would appear to not require these functions.

A number of models can be constructed regarding the requirement for the C terminus of these Avr proteins in avirulence. The C terminus of AvrXa10 may be required for transcriptional activation of host genes, possibly the *R* gene (*Xa10*) directly, by the Avr protein acting as an autonomous transcription factor. AvrBs3 family members have not been demonstrated to have DNA binding activity, nor are recognizable DNA binding motifs present in the sequence. The protein may have an unknown DNA binding component or may only function in a complex with one or more host factors. We are currently investigating the capacity of the protein to bind DNA. Alternatively, the protein may function as a transcription factor, but recognition by the *R* gene product only requires the proper conformation in a protein–protein interaction model and localization to the nucleus.

Members of the *avrBs3* family have been shown to have effects in susceptible interactions, and the prevalence of the genes in a variety of species provides evidence for a broad role in virulence within the genus *Xanthomonas* (Bonas et al., 1989, 1993; Canteros et al., 1991; De Feyter and Gabriel, 1991; Hopkins et al., 1992; Swarup et al., 1992; De Feyter et al., 1993). The *pthA* gene, which is a homolog of *avrXa10*, controls the ability of the bacteria to grow within and induce hyperplastic cankers on citrus (Swarup et al., 1991, 1992). Preliminary reports indicate that the canker-inducing ability of *pthA* is dependent on nuclear localization signal motifs (Gabriel et al., 1996). These observations hint at a requirement of the activation domain for the pathogenicity effects of the proteins as well as a role in avirulence. However, we do not know the role of the domain in pathogenicity at this time.

We do know that the ability of the VP16 to complement the domain of AvrXa10 is not specific for AvrXa10. Our recent work indicates that the domain also complements the C-terminal requirements of AvrXa7 for avirulence (B. Yang, W. Zhu, L.B. Johnson, and F.F. White, unpublished results). This latter gene is interesting because *avrXa7* has effects on pathogenicity in some strains, and we are currently examining the role of the domain in pathogenicity. Clearly, given the broad range of plant species that are susceptible to the effects of this protein family, the bacteria have likely targeted a conserved signal transduction component of the host. In turn, the host plant has apparently co-opted the system in the recognition and resistance response to the pathogen. Conclusive evidence regarding the role of AvrXa10 and related members of the AvrBs3 family as transcription factors awaits the identification of putative promoters in the host plants.

METHODS

Strains, Plasmids, and General Methods

Standard cultural methods were used for *Escherichia coli* (Ausubel et al., 1988). Unless otherwise noted, bacterial and gene manipulations were as previously reported (Zhu et al., 1998). All strains of *Xanthomonas oryzae* pv *oryzae* were derived from PXO99^{A}, which lacks an endogenous copy of *avrXa10* (Hopkins et al., 1992). The plasmid pCH4 contained *avrXa10* with the endogenous promoter in pBluescript KS+ (Stratagene, La Jolla, CA). A second version, pZWX10-F2H, contained the *avrXa10* coding sequence fused to the *lacZ* promoter (P_{lacZ}) in pBluescript KS+ and new SalI and HindIII sites. The yeast strain Hf7c (Clontech Laboratories, Palo Alto, CA) contained both *His3* and *LacZ* genes with GAL4-regulated promoters. The plasmid pPAS3 contained the DNA binding domain sequence of *GAL4* under the control of the alcohol dehydrogenase (*ADH1*) promoter and a polylinker at the C terminus for generating fusions to the *GAL4* coding sequence (Estruch et al., 1994). The plasmid was maintained in yeast by selection for leucine auxotrophy and in *E. coli* by selection for resistance to carbenicillin. Methods for yeast manipulations were as described previously (Guthrie and Fink, 1991). Sequencing was performed at the Iowa State University Sequencing Facility.

Mutagenesis

Mutants were constructed according to Kunkel et al. (1987) or by polymerase chain reaction (PCR; Scharf et al., 1986). The nucleotide sequence of *avrXa10* has been published (GenBank accession number J03710; Hopkins et al., 1992). All changes were sequenced, and in cases in which avirulence activity was lost, the repeat and 5′ domains were swapped with a wild-type carboxyl coding sequence and tested for the ability to elicit a hypersensitive response (HR) to ensure that other regions of the genes were not altered during the mutagenesis process.

The TGA1064 mutation has a BglII site (phase 2) immediately before a stop codon at position 1064 in the predicted amino acid sequence and was previously described (Zhu et al., 1998). The EEE1082/83/84 mutation was introduced at positions 1082, 1083, and 1084 with oligonucleotide 5′-CCATGCGAGCGCCGCTGCGTTGAATTCCGGGAAATC-3′ and created a new EcoRI site in the region. The FF1077/80 mutation was created using the oligonucleotide 5′-GAGCTCCTCTTCGTTGCTAGCCGGGACGTCATCCGCTGCC-3′ and incorporated a unique NheI site into *avrXa10.* The FL1080/85 mutation was introduced with oligonucleotide 5′-GCTCCATCAACCATGCGGCCTCCTCTTCGTTGGCTGCCGGGAAACT-3′, and the FLL1080/85/88 mutation was introduced with the oligonucleotide 5′-CAATGCTCCATCGCCCATGCGGCCTCCTCTTCGTTGGCTGCCGGGAAATC-3′. In both of the latter changes, the SstI site within the activation domain region was destroyed. The LL1085/88 (L12), L1088 (L2), and LL1091/92 (L34) variants were created by using PCR. LL1085/88 was generated with the ADMUT primer and 5′-TCAGAAGCTTCACTGAGGCAATAGCTCCATTGCCCATGCTGCCTCCTCTTC-3′, which changes the leucines at positions 1085 and 1088 to alanine residues. L1088 was generated using 5′-TCAGAAGCTTCACTGAGGCAATAGCTCCATCGCCCATGCGAGCTCC-3′, which changes the leucine residue at position 1088 to an alanine residue. LL1091/92 was generated with oligonucleotide 5′-TCAGAAGCTTCACTGAGGTGCTGCCTCCATCAACCATGCGAGCTCC-3′

and replaces leucine residues at positions 1091 and 1092 with alanine residues. The latter three primers also introduced a stop codon after codon 1094 and removed the additional eight codons found in *avrXa10* in comparison with *avrBs3.* The upstream primer (ADMUT) for all was 5′-AGAAGGGTCGACCCAGCCCAATGCACGAGGGAG-3′. The PCR products were digested with SalI and HindIII and ligated into SalI-HindIII–digested pZWX10-F2H.

The VP16 activation domain from pSJT1193-CRF2, which contained the terminal 241 bp of VP16 on a BglII-BamHI fragment, was cloned into the BglII-digested *avrXa10*^{TGA1064} in pZW17 to create pZWX10-VP16 and the gene *avrXa10*^{VP16}. The minimal VP16 activational domain, as delimited by Uesugi et al. (1997), was created by PCR amplification of pSJT1193-CRF2 by using the reverse primer 5′-CCTTGGAATTGACGAACTCCCGCAGTGAAGCTTCAGTG-3′ and the forward primer 5′-CAACACAGATCTTGGCCGACTTCGAGTTTGAGC-3′. The product also retained the last three codons of *avrXa10.* The product was digested with BglII and HindIII and ligated to BglII-HindIII–digested pZW17 to create *avrXa10*^{mVP16} in pZWX10mVP16. The phenylalanine and leucine residues at positions 479 and 483, respectively, in *avrXa10*^{mVP16} were replaced with alanine residues by PCR, using the reverse primer 5′-CACTGAAGCTTCACTGCGGGAGTTCGTCAATTCCAGCGGCATCGGTAGCCATCTGCTC-3′ to create *avrXa10*$^{mVP16/FL/AA}$.

Transcriptional Activation Assays

The plasmid pPAS3 has a BamHI site in the same frame (phase 0) as the C-terminal BamHI and promoter proximal BamHI sites in the coding sequence of *avrXa10* (Hopkins et al., 1992; Estruch et al., 1994). The activation domain was cloned as a 1.4-kb BamHI fragment from *avrXa10* and derivatives in pCH4. The 3.1-kb BamHI fragment from *avrXa10* in pCH4 was also cloned into pPAS3. Assays for β-galactosidase activity in Hf7c were performed in triplicate as previously described (Zhu et al., 1998).

RNA Gel Blot and Tissue Staining

RNA gel blot analysis of defense response genes *POX22.3* and *PR-1* was performed as described previously (Chittoor et al., 1997). The probe for *PR-1* was a maize cDNA (Morris et al., 1998). The *POX22.3* 3′ untranslated region was generated by PCR as described previously (Chittoor et al., 1997). Callose was stained with aniline blue, according to Dietrich et al. (1994).

Avirulence Testing

Mutant C-terminal coding domains were cloned as SalI-HindIII fragments into pZWX10-F2H and were tested for avirulence activity on BB10 and IR24, as previously described (Hopkins et al., 1992). BB10 and IR24 are near-isogenic rice lines. BB10 contains the gene *Xa10* for resistance to bacterial blight.

Rice Leaf Tissue Bombardment

Genes for *avrXa10* and *avrXa10*$^{FLL1080/85/88}$ were placed under the control of the 35S promoter and cloned in tandem with the structural gene for β-glucuronidase (GUS) under the control of the maize ubiquitin promoter. The 331-bp KpnI-PstI fragment from *avrXa10* was amplified with the T7 primer and AvrKBM-5 (5′-GGGGTACCAGGAGATCTGCCATGGACCCCATTCGTTCG-3′), which introduced KpnI and BglII sites and a Kozak consensus sequence (Kozak, 1989) at the beginning of *avrXa10*, and the 84-bp KpnI-BglII PCR was cloned into *avrXa10.* The gene was then cloned downstream of the 35S promoter in a modified version of pRTL2 using BglII (Schardl et al., 1987). The *avrXa10* gene with the 35S promoter and termination sequence (35S–*avrXa10*) was then cloned as a HindIII fragment into pACH15, which contains a ubiquitin-promoted *uidA* gene (Christensen and Quail, 1996), to give plasmid pYB100. The FLL1080/85/88 version of pYB100 was created by replacing the PstI-XbaI fragment of 35S–*avrXa10* with the corresponding fragment of *avrXa10*$^{FLL1080/85/88}$, creating pYB110 with 35S–*avrXa10*^{FLL}.

DNA from pYB100 and pYB110 was prepared and absorbed onto tungsten particles according to Finer and McMullen (1991). Five leaf sections of 1.5 cm were taken from 10-day-old IR24 and BB10 rice seedlings and placed adjacent to each other for each bombardment. Leaves were bombarded using a custom-made particle inflow gun (Finer et al., 1992). Thirty-six hours after bombardment, the leaves were stained with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, as described by Jefferson (1987). Blue foci were counted under the dissecting microscope. The bombardment with each plasmid was performed three times, and the average number of foci for the three experiments was determined.

Protein Analysis

Proteins were extracted from yeast and reacted with an anti-Gal4DB monoclonal antibody, as specified by the antibody supplier (Clontech Laboratories). Protein size standards were purchased from Gibco BRL (Gaithersburg, MD). Anti-mouse alkaline phosphatase–conjugated IgG was purchased from Sigma (St. Louis, MO). Proteins were extracted from bacteria as described previously (Zhu et al., 1998) and reacted with polyvalent antisera directed against AvrXa10 (Young et al., 1994). Although the antisera detects homologs of AvrXa10 that are present in PXO99^{A} (Young et al., 1994), these proteins are not detected at the level of loading and exposure.

ACKNOWLEDGMENTS

We thank Diana Pavlisko for preparation of the manuscript, Dr. Juan Estruch for the plasmid pPAS3, and Dr. Steve Triezenberg for pSJT1193-CRF2. Work was supported from funds provided by the U.S. Department of Agriculture agreements 94-37303-0548 and 98-353303-6446 and the Kansas Agricultural Experiment Station. W.Z. was supported in part by a grant from the Rockefeller Foundation. This paper is contribution No. 99-337-J from the Kansas Agricultural Experiment Station.

Received March 17, 1999; accepted June 11, 1999.

REFERENCES

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.

Ansari, A.Z., Reece, R.J., and Ptashne, M. (1998). A transcriptional activating region with two contrasting modes of protein interaction. Proc. Natl. Acad. Sci. USA **95,** 13543–13548.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., and Smith, J.A. (1988). Current Protocols in Molecular Biology. (New York: John Wiley and Sons).

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.

Bonas, U., Stall, R.E., and Staskawicz, B.J. (1989). Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria.* Mol. Gen. Genet. **218,** 127–136.

Bonas, U., Conrads-Strauch, J., and Balbo, I. (1993). Resistance in tomato to *Xanthomonas campestris* pv. *vesicatoria* is determined by alleles of the pepper-specific avirulence gene *avrBs3.* Mol. Gen. Genet. **238,** 261–269.

Canteros, B., Minsavage, G., Bonas, U., Pring, D., and Stall, R. (1991). A gene from *Xanthomonas campestris* pv. *vesicatoria* that determines avirulence in tomato is related to *avrBs3.* Mol. Plant-Microbe Interact. **4,** 628–632.

Chittoor, J.M., Leach, J.E., and White, F.F. (1997). Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae.* Mol. Plant-Microbe Interact. **10,** 861–871.

Christensen, A.H., and Quail, P.H. (1996). Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. Transgenic Res. **5,** 213–218.

De Feyter, R., and Gabriel, D.W. (1991). At least six avirulence genes are clustered on a 90-kilobase plasmid in *Xanthomonas campestris* pv. *malvacearum.* Mol. Plant-Microbe Interact. **4,** 423–432.

De Feyter, R., Yang, Y., and Gabriel, D.W. (1993). Gene-for-genes interactions between cotton *R* genes and *Xanthomonas campestris* pv. *malvacearum avr* genes. Mol. Plant-Microbe Interact. **6,** 225–237.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A., and Dangl, J.L. (1994). *Arabidopsis* mutants simulating disease resistance response. Cell **77,** 565–577.

Estruch, J.J., Crossland, L., and Goff, S.A. (1994). Plant activating sequences: Positively charged peptides are functional as transcriptional activation domains. Nucleic Acids Res. **22,** 3983–3989.

Finer, J.J., and McMullen, M.D. (1991). Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. In Vitro Cell. Dev. Biol. **27P,** 175–182.

Finer, J.J., Vain, P., Jones, M.W., and McMullen, M.D. (1992). Development of the particle inflow gun for DNA delivery to plant cells. Plant Cell Rep. **11,** 323–328.

Gabriel, D.W., Yuan, Q., Yang, Y., and Chakrabarty, P.K. (1996). Role of nuclear localizing signal sequences in three disease phenotypes determined by the *Xanthomonas avr/pth* gene family. In Biology of Plant–Microbe Interactions, G. Stacey, B. Mullin, and P.M. Gresshoff, eds (St Paul, MN: International Society for Molecular Plant–Microbe Interactions), pp. 197–202.

Garnier, J., Gibrat, J.F., and Robson, B. (1996). GOR method for predicting protein secondary structure from amino acid sequence. Methods Enzymol. **266,** 540–553.

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.

Guthrie, C., and Fink, G.R. (1991). Guide to Yeast Genetics and Molecular Biology. (San Diego, CA: Academic Press).

Herbers, K., Conrads-Strauch, J., and Bonas, U. (1992). Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. Nature **356,** 172–174.

Hopkins, C.M., White, F.F., Choi, S.H., Guo, A., and Leach, J.E. (1992). A family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae.* Mol. Plant-Microbe Interact. **5,** 451–459.

Jefferson, R.H. (1987). Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep. **5,** 387–405.

Keen, N.T. (1990). Gene-for-gene complimentary in plant–pathogen interactions. Annu. Rev. Genet. **24,** 447–463.

Kozak, M. (1989). The scanning model for translation: An update. J. Cell Biol. **108,** 229–241.

Kunkel, T.A., Roberts, J.D., and Zakour, R.A. (1987). Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154,** 367–382.

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.

Morris, S.W., Vernooij, B., Titatarn, S., Starrett, M., Thomas, S., Wiltse, C.C., Frederiksen, R.A., Bhandhufalck, A., Hulbert, S., and Uknes, S. (1998). Induced resistance responses in maize. Mol. Plant-Microbe Interact. **11,** 643–658.

Ptashne, M., and Gann, A. (1997). Transcriptional activation by recruitment. Nature **386,** 569–577.

Salmeron, J.M., Oldroyd, G.E.D., Tommens, C.M.T., Scofield, S.R., Kim, H., Lavelle, D.T., Dahlbeck, D., and Staskawicz, B.J. (1996). Tomato Prf is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the Pto kinase gene cluster. Cell **86,** 123–133.

Schardl, C.L., Byrd, A.D., Benzion, G., Altschuler, M.A., Hildebrand, D.F., and Hunt, A.G. (1987). Design and construction of a versatile system for the expression of foreign genes in plants. Gene **61,** 1–11.

Scharf, S.J., Horn, G.T., and Erlich, H.A. (1986). Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science **233,** 1076–1078.

Scheel, D. (1998). Resistance response physiology and signal transduction. Curr. Opin. Plant Biol. **1,** 305–310.

Scofield, S.R., Tobias, C.M., Rathjen, J.P., Chang, J.H., Lavelle, D.T., Michelmore, R.W., and Staskawicz, B.J. (1996). Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. Science **274,** 2063–2065.

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 cankerlike lesions on citrus. Phytopathology **81,** 802–809.

Swarup, S., Yang, Y., Kingsley, M.T., and Gabriel, D.W. (1992). A *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on nonhosts. Mol. Plant-Microbe Interact. **5,** 204–213.

Tang, X., Frederick, R.D., Zhou, J., Halterman, D.A., Jia, Y., and Martin, G.B. (1996). Initiation of plant disease resistance by physical interaction of *AvrPto* and *Pto* kinase. Science **274,** 2060–2063.

Uesugi, M., Nyanguile, O., Lu, H., Levine, A.J., and Verdine, G.L. (1997). Induced alpha helix in the VP16 activation domain upon binding to a human TAF. Science **277,** 1310–1313.

Van den Ackerveken, G., Marois, E., and Bonas, U. (1996). Recognition of the bacterial avirulence protein *AvrBs3* occurs inside the host plant cell. Cell **87,** 1307–1316.

Yang, Y., and Gabriel, D.W. (1995a). Intragenic recombination of a single plant pathogen gene provides a mechanism for the evolution of new host specificities. J. Bacteriol. **177,** 4963–4968.

Yang, Y., and Gabriel, D.W. (1995b). *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. Mol. Plant-Microbe Interact. **8,** 627–631.

Yang, Y., De Feyter, R., and Gabriel, D.W. (1994). Host-specific symptoms and increased release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from leaves are determined by the 102-bp tandem repeats of *pthA* and *avrb6*, respectively. Mol. Plant-Microbe Interact. **7,** 345–355.

Yang, Y., Yuan, Q., and Gabriel, D.W. (1996). Watersoaking function(s) of XcmH1005 are redundantly encoded by members of the *Xanthomonas avr/pth* gene family. Mol. Plant-Microbe Interact. **9,** 105–113.

Young, S.A., White, F.F., Hopkins, C.M., and Leach, J.E. (1994). AVRXa10 protein is in the cytoplasm of *Xanthomonas oryzae* pv. *oryzae.* Mol. Plant-Microbe Interact. **7,** 799–804.

Zhou, J., Loh, Y.-T., Bressan, R.A., and Martin, G.B. (1995). The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by *Pto* and is involved in the hypersensitive response. Cell **83,** 925–935.

Zhou, J., Tang, X., and Martin, G.B. (1997). The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a *cis*-element of pathogenesis-related genes. EMBO J. **16,** 3207–3218.

Zhu, W., Yang, B., Chittoor, J.M., Johnson, L.B., and White, F.F. (1998). AvrXA10 contains an acidic transcriptional activation domain in the functionally conserved C terminus. Mol. Plant-Microbe Interact. **11,** 824–832.