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

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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).
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 C terminus of AvrXa10, thus implicating a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10. The results implicate a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10, thus implicating a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10. The results implicate a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10, thus implicating a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10, thus implicating a role for a eukaryotic-like acidic transcriptional activator domain in the C terminus of AvrXa10."
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 PXO99a, 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 avrXa10FLL1080/1085/1088) 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 (~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 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 avrXa10TGA1064, 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 FLL1080/1085/1088 derivative was similar in phenotype to avrXa10TGA1064 (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 ~1 x 10^7 colony-forming units (CFU) per leaf in the 4-day period (Figure 2B, curve 1). The bacterial populations with avrXa10TGA1064 or avrXa10FLL1080/1085/1088 reached the maximum levels of ~5 x 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–avrXa10FLL1080/1085/1088) led to the detection of numerous β-glucuronidase (GUS)-positive foci.

Figure 2. Phenotypic Effect of avrXa10FLL1080/1085/1088.

(A) Derivatives of avrXa10 were transferred to strain PXO99a, 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 PXO99a containing the following avr genes: leaf 1, avrXa10; leaf 2, avrXa10TGA1064; and leaf 3, avrXa10FLL1080/1085/1088.

(B) Populations of PXO99a with avrXa10 or derivatives in leaves of BB10. Curves represent the population of bacteria with the following genes: 1, avrXa10; 2, avrXa10TGA1064; and 3, avrXa10FLL1080/1085/1088. Populations are expressed as CFU per leaf. Error bars indicate ± SD.
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–avrXa10FLL1080/85/88 (pYB110) and were similar to the number obtained using 35S–avrXa10 in IR24 (Figure 3E). Although we have not been able to detect AvrXa10 in the plant cells, we extracted protein from PXO99A to determine whether AvrXa10FLL1080/85/88 was produced in significantly lower quantities in the bacterium. No differences were observed in the amount of protein from bacteria containing avrXa10 or avrXa10FLL1080/85/88 (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 avrXa10TGA1064 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 avrXa10VP16. Strains containing avrXa10VP16 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 avrXa10VP16 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\textsuperscript{mVP16}, or avrXa10\textsuperscript{mVP16} were inoculated on BB10 (Figure 5B, curves 1, 3, and 4, respectively), although growth suppression in the plant due to avrXa10\textsuperscript{mVP16} was less than for avrXa10 and avrXa10\textsuperscript{mVP16}. No growth suppression in BB10 was observed for PXO99\textsuperscript{A} containing avrXa10\textsuperscript{TGA1064} or the vector alone (Figure 5B, curves 2 and 7, respectively) or in IR24 for PXO99\textsuperscript{A} containing either avrXa10\textsuperscript{mVP16} (data not shown) or avrXa10\textsuperscript{mVP16} (Figure 5B, curve 5).

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

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

Experiments were performed to measure other parameters of the resistance response due to avrXa10\textsuperscript{mVP16}. 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\textsuperscript{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\textsuperscript{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\textsubscript{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...
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 hTAFII31 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–avrXa10GUS 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 AvrXa10GUS 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 Feyer and Gabriel, 1991; Hopkins et al., 1992; Swarup et al., 1992; De Feyer 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. J ohnson, 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 preserved 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 PXO99A, 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 (PlacZ) in pBlueScript KS+ and new SalI and HindIII sites. The yeast strain H7c (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 EEE1002/83/84 mutation was introduced at positions 1082, 1083, and 1084 with oligonucleotide 5′-CATGGGAGCGGCGGCGCTGTT-GAATTCCGGGAAATC-3′ and created a new EcoRI site in the region. The FF1077/80 mutation was created using the oligonucleotide 5′-GAGCTCTCCTCTTGTGGCTAGCCGCGGACGTCCGCGCTGCCG-3′ and incorporated a unique NheI site into avrXa10. The FL1080/85 mutation was introduced with oligonucleotide 5′-GTTTATCAACCATGCGCCTCCTTTGCTGGCCTGGGAAC-3′, and the FLL1080/85/88 mutation was introduced with the oligonucleotide 5′-CAATGCTCTATGCGCCATGCGCGCCTCTTTGCTGGCCTGCCGGAAATC-3′. In both of the latter changes, the SstI site within the activation domain region was lost. The LL1085/88 (L12), LL1088 (L2), and LL1091/92 (L34) variants were created by using PCR. LL1085/88 was generated with the ADMUT primer and 5′-TCA-GAGCTCTCAGGAGAATAGCTCCATGCCAATACATCC-3′, which changes the leucines at positions 1085 and 1088 to alanine residues. LL1088 was generated using 5′-TCAAGAAGCT-TCACCTGAGCAATAGCTCCATGCCAATACATCC-3′, which changes the leucine residue at position 1088 to an alanine residue. LL1091/92 was generated with oligonucleotide 5′-TCA-GAGCTCTCAGGAGGCTGCTCCATGCCAATACATCC-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'-AGAGGGTGTCACGACCCACAAAGCAGAGGAGG-3'. The PCR products were digested with SalI and HindIII and ligated into SalI-HindIII-digested pZWX10-F2H.

The VP16 activation domain from p5T T1193-CRF2, which contained the terminal 241 bp of VP16 on a BglII-BamHI fragment, was cloned into the BglII-digested avrXa10GTA2G264 in pZW17 to create pZWX10-VP16 and the gene avrXa10D53. The minimal VP16 activation domain, as delimited by Uesugi et al. (1997), was created by PCR amplification of p5T T1193-CRF2 by using the reverse primer 5'-CCCTGGGAATTGACGAACTCCCGCAGTGAAGCTTCAGTG-3' and the forward primer 5'-CAACACAGATCTTGCCGACTCCGAGT-TTGAGC-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 pZWX10-F2H and was tested for avirulence activity on 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 avrXa10FL, creating pYB110 with 35S-avrXa10FL.

DNA from PXO99A (Young et al., 1994) and reacted with polyvalent antisera directed against AvrXa10 that are present in PXO99A (Young et al., 1994). Although the antisera detects homologs of AvrXa10 that are present in PXO99A (Young et al., 1994), these proteins are not detected at the level of loading and exposure.

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 polyclonal antisera directed against AvrXa10 (Young et al., 1994).

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