Research Note

Cloning and Characterization of a Bean Udp-Glucosyltransferase cDNA Expressed During Plant–Bacterial Interactions

T. A. Sullivan, J. L. Jakobek, and P. B. Lindgren

Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616, U.S.A.

Accepted 13 September 2000.

A cDNA clone, which corresponds to an RNA transcript that accumulates in bean during the hypersensitive reaction, was isolated and designated Hra25 (for hypersensitive reaction associated). Hra25 is predicted to encode a UDP-glucosyltransferase. Northern analysis was used to study Hra25 transcript accumulation in bean in response to incompatible and compatible strains of *Pseudomonas syringae*, an Hrp[−] mutant, and wounding. Our data suggest that the Hra25 transcript is activated in response to specific *avr*-derived signals as well as non-*avr*, general signals.

The molecular mechanisms in plants that are requisite for pathogen recognition and activation of defense signal transduction are still largely undefined (Rushton and Somssich 1998; Scheel 1998). It is generally recognized that avirulence (*avr*) gene-resistance (*R*) gene signal transduction pathways play important roles in the activation of plant defense responses during many incompatible plant–pathogen interactions, resulting in resistance (Leach and White 1996; Martin 1999). Signal-transduction pathways, independent of *avr-R* gene pathways, also have been shown to be involved with defense regulation. For instance, Hrp[−] mutants of *Pseudomonas syringae* pv. *tabaci* lack intact type III secretion systems, cannot transport *avr* and virulence gene products into plant cells, and are unable to elicit a hypersensitive reaction (HR) or disease (Lindgren 1997). These mutants, however, do induce defense transcript accumulation and phytoalexin synthesis (Jakobek and Lindgren 1993). Numerous non-*avr*-derived elicitors also have been isolated from pathogens that activate plant defense responses (Hahn 1996). For example, eubacterial flagellins are signals that activate defense responses in *Arabidopsis* and tomato (Felix et al. 1999; Gomez-Gomez et al. 1999). Thus, there are general signal-transduction pathways for activating defense responses in

plants, which are distinct from *avr-R* gene pathways.

During compatible plant–pathogen interactions resulting in disease, various defense responses are induced, although they may be delayed compared with incompatible interactions (Dixon and Lamb 1990). Although little is known about defense induction during compatible interactions, the induction of these responses presumably involve pathways independent of *avr-R* gene pathways. Compatible plant–bacterial interactions are further complicated by certain pathogenic bacteria that can suppress plant defense responses (Jakobek et al. 1993). Thus, it appears that subsets of defense responses may be regulated very differently during compatible and incompatible interactions.

The identification and study of non-*avr* plant defense signal transduction pathways provide new and useful insights into understanding and enhancing disease resistance. To further elucidate the molecular pathways needed for plant defense induction, we sought to identify novel pathogen-induced genes and study their expression during various plant–bacterial interactions. A cDNA library specific to bean tissue undergoing an HR to *P. syringae* pv. *tabaci* was screened with a combination of differential and cold-plaque screening as previously described (Jakobek et al. 1999). During this process, we identified a cDNA clone, designated Hra25 (for hypersensitive reaction associated), which corresponds to a transcript expressed during the HR (GenBank accession no. AF303396).

The predicted Hra25 open reading frame encodes a protein of 462 amino acids. BLAST searches (Altschul et al. 1990) demonstrated that the predicted Hra25 product shares homology with UDP-glucosyltransferases, a family of enzymes that catalyze the conjugation of glucose from sugar nucleotides to various substrates (Narimatsu 1994; O'Reilly 1995). The predicted Hra25 product contains a conserved UDP-glucosyltransferase signature sequence spanning amino acids 342 to 385 (data not shown) (Erps et al. 1994; Hundle et al. 1992). Although Hra25 transcript accumulated in bean in response to an incompatible pathogen, the function of the Hra25 protein in defense remains to be ascertained.

Studies were completed to determine the temporal relationship between Hra25 transcript accumulation and the development of an HR in response to incompatible *P. syringae* strains. A visible HR occurs in bean 10 to 12 h after infiltration with

Corresponding author: P. B. Lindgren; E-mail: pete_lindgren@ncsu.edu

Current address of T. A. Sullivan: Paradigm Genetics, 104 Alexander Dr., P.O. Box 14528, Research Triangle Park, NC 27709-4538, U.S.A.

Nucleotide and/or amino acid sequence data can be found at GenBank accession number AF303396 for Hra25.

incompatible *P. syringae* pv. *tabaci* ATCC Pt11528. Northern analysis demonstrated that Hra25 transcript was detected in leaf tissue 6 h after infiltration, and levels remained relatively high as an HR developed (Fig. 1). Incompatible *P. syringae* pv. *tomato* JL1065 (Whalen et al. 1991) also induces an HR in bean similar to Pt11528. Northern analysis demonstrated that the pattern of Hra25 transcript accumulation and levels of transcript detected in bean after infiltration with JL1065 (Fig. 2) were very similar to those observed after infiltration with Pt11528. Therefore, Hra25 transcript accumulation in bean correlated with the production of the HR in response to incompatible *P. syringae* strains.

Certain plant defense responses are suppressed during compatible bacterial interactions. For instance, *P. syringae* pv. *phaseolicola*, the causal agent of halo blight in bean, produces a suppressor that inhibits the accumulation of transcripts for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and phytoalexin synthesis (Jakobek et al. 1993). When we examined Hra25 induction in bean after infiltration with *P. syringae* pv. *phaseolicola* NPS3121 (Lindgren et al. 1986), we found that the Hra25 transcript accumulated by 24 h (Fig. 2). This compares with 6 h after infiltration with Pt11528 (Fig. 1). In addition, the levels of Hra25 transcript detected in response to NPS3121 were lower compared with the levels observed in plants infiltrated with Pt11528. Although the levels and timing of transcript accumulation differed during the compatible versus incompatible interactions, we did not observe an inhibition of Hra25 transcript accumulation by NPS3121 similar to that previously observed for PAL, CHS, and CHI transcripts (Jakobek et al. 1993). The fact that transcript accumulated in response to NPS3121 suggests the induction of Hra25 by this bacterium occurs by a signaling process distinct from *avr-R* gene pathways.

Specific *avr* genes have been used as experimental tools to elucidate the molecular pathways involved with the activation

of many defense responses such as the HR (Hammond-Kosack and Jones 1996). The avirulence gene *avrRpt2* from *P. syringae* pv. *tomato* JL1065 interacts with a resistance gene in bean to elicit genotype-specific resistance and an HR (Innes et al. 1993). When bean is infiltrated with NPS3121 containing *avrRpt2* on plasmid pLH12 (Innes et al. 1993), the usual compatible interaction is changed into an incompatible interaction, and a rapid HR develops. When bean was infiltrated with NPS3121(*avrRpt2*), Hra25 transcript accumulated at 8 h and an HR was induced approximately 12 h after infiltration (Fig. 2). In addition, the levels of Hra25 transcript detected in plants infiltrated with NPS3121(*avrRpt2*) were higher than in plants infiltrated with NPS3121 (Fig. 2). In fact, the pattern and level of Hra25 transcript accumulation induced by NPS3121-(*avrRpt2*) more closely resembled the incompatible response to *P. syringae* pv. *tomato* JL1065, the strain from which *avrRpt2* was isolated, than the wild-type compatible response. Thus, Hra25 transcript accumulation correlated with the HR induced by the avirulence gene *avrRpt2*.

Previously we demonstrated that certain defense responses were induced in bean in response to various non-*avr*, general stimuli that do not induce the HR or disease (Jakobek and Lindgren 1993). For instance, transcripts for PAL, CHS, CHI, and CHT accumulated and phytoalexins were synthesized in bean in response to infiltration with *P. syringae* pv. *tabaci* Hrp⁻ mutants. When bean was infiltrated with Pt11528::Hrp1, the levels of Hra25 transcript were significantly lower than those detected in plants infiltrated with the wild-type strain and appeared similar to levels detected in water-infiltrated control plants (Fig. 3).

Fig. 1. Hra25 (for hypersensitive reaction associated) transcript accumulation in bean after infiltration with incompatible *Pseudomonas syringae* pv. *tabaci* Pt11528 or H₂O. Growth of *Phaseolus vulgaris* cv. Red Kidney and the procedures used to inoculate plants were previously described (Jakobek and Lindgren 1993). Total RNA was isolated from bean leaf tissue as previously described (Jakobek et al. 1993). RNA samples (10 µg) were fractionated on a 1% agarose-formaldehyde gel in 1 × 3-(*N*-morpholino)-propanesulfonic acid (MOPS) buffer (Ausubel et al. 1990) and blotted to Nytran N hybridization membrane (Schleicher and Schuell, Keene, NH, U.S.A.). The membrane was prehybridized and hybridized at 65°C, following the manufacturer's protocols. The Hra25 insert was labeled with α-³²P-dCTP by random hexamer labeling (Feinberg and Vogelstein 1983) and used as a hybridization probe. The filter was hybridized for 18 h and then washed, following the manufacturer's protocols. The filter was subsequently hybridized to a bean cDNA clone, designated H1, which is complementary to a gene with an unknown function that is constitutively expressed (Lawton and Lamb 1987) and used as a control to demonstrate equal RNA loading.

Fig. 2. Hra25 (for hypersensitive reaction associated) transcript accumulation in bean in response to avirulence gene *avrRpt2*. Bean plants were infiltrated with *Pseudomonas syringae* pv. *tomato* JL1065, *P. syringae* pv. *phaseolicola* NPS3121 containing *avrRpt2* (3121+avr), *P. syringae* pv. *phaseolicola* NPS3121, *P. syringae* pv. *tabaci* Pt11528 (Wt), or H₂O. Total RNA was isolated from plants at various times after treatment and hybridized to ³²P-labeled Hra25 and H1.

Fig. 3. Hra25 (for hypersensitive reaction associated) transcript accumulation in bean after infiltration with Hrp⁻ mutant *Pseudomonas syringae* pv. *tabaci* Pt11528::Hrp1 or H₂O. *P. syringae* pv. *tabaci* Pt11528 was included as a control (Wt). Total RNA was isolated at various times after infiltration and hybridized to ³²P-labeled Hra25 and H1.

Fig. 4. Hra25 (for hypersensitive reaction associated) transcript accumulation in bean after wounding. Leaves were mechanically wounded six to eight times on each half of the primary leaves by crushing with forceps, taking care to avoid crushing the midvein of the leaves. Untreated plants and plants infiltrated with *Pseudomonas syringae* pv. *tabaci* Pt11528 (Wt) were included as controls. Total RNA was isolated at various times after treatment and hybridized to ³²P-labeled Hra25 and H1, as described in Fig. 1.

Wounding also induces certain defense transcripts and phytoalexins (Bowles 1993; Lawton and Lamb 1987), and Hra25 transcript was observed in wounded bean leaves (Fig. 4). The relative levels of Hra25 transcript detected in wounded plants were lower compared with those detected in plants infiltrated with incompatible *P. syringae* strains, and transcript levels appeared to be higher than in plants infiltrated with Pt11528::Hrp1.

Although the function is not clearly understood, the data presented here contribute to our understanding of the regulation of bean Hra25, encoding a putative UDP-glucosyltransferase, which is expressed during pathogen interactions. These Northern analyses indicate that Hra25 is regulated by at least two signaling pathways. The rapid and strong induction of Hra25, by both of the incompatible bacterial pathogens tested as well as by *avrRpt2*, suggests that one signaling pathway is triggered by *avr-R* gene interactions.

Hra25 was also induced by non-*avr*, general stimuli that were generated by the compatible bacterial pathogen and by wounding. Other defense genes we have previously tested also were induced by general stimuli, including Hrp⁻ mutants. Hra25 differs in one interesting aspect, however, as it is not significantly induced by an Hrp⁻ mutant (Jakobek and Lindgren 1993; Jakobek et al. 1999). The fact that Hra25 was not induced by the Hrp⁻ mutant indicates that its regulation may represent another scenario for signaling pathways, leading to defense gene activation. Continued research into the activation of Hra25 and other defense-related genes will help elucidate the intricate molecular pathways required for defense activation and lead to an understanding of how each gene contributes to disease resistance.

LITERATURE CITED

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment tool. *J. Mol. Biol.* 215:403-410.
Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds. 1990. *Current Protocols in Mo-*

- lecular Biology*. Wiley-Interscience, New York.
Bowles, D. J. 1993. Local and systemic signals in the wound response. *Semin. Cell Biol.* 4:103-111.
Dixon, R. A., and Lamb, C. J. 1990. Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:339-367.
Erps, L. T., Ritter, J. K., Hersh, J. H., Blossom, D., Martin, N. C., and Owens, I. S. 1994. Identification of two single base substitutions in the UGT1 gene locus which abolish bilirubin uridine diphosphate glucuronosyltransferase activity in vitro. *J. Clin. Invest.* 93:564-570.
Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
Felix, G., Duran, J. D., Volko, S., and Boller, T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* 18:265-276.
Gomez-Gomez, L., Felix, G., and Boller, T. 1999. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *Plant J.* 18:277-284.
Hahn, M. G. 1996. Microbial elicitors and their receptors in plants. *Annu. Rev. Phytopathol.* 34:387-412.
Hammond-Kosack, K. E., and Jones, J. D. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8:1773-1791.
Hundle, B. S., O'Brien, D. A., Alberti, M., Beyer, P., and Hearst, J. E. 1992. Functional expression of zeaxanthin glucosyltransferase from *Erwinia herbicola* and a proposed uridine diphosphate binding site. *Proc. Natl. Acad. Sci. USA* 89:9321-9325.
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.* 175:4859-4869.
Jakobek, J. L., and Lindgren, P. B. 1993. Generalized induction of defense responses in bean is not correlated with the induction of the hypersensitive reaction. *Plant Cell* 5:49-56.
Jakobek, J. L., Smith, J. A., and Lindgren, P. B. 1993. Suppression of bean defense responses by *Pseudomonas syringae*. *Plant Cell* 5:57-63.
Jakobek, J. L., Smith-Becker, J. A., and Lindgren, P. B. 1999. A bean cDNA expressed during a hypersensitive reaction encodes a putative calcium-binding protein. *Mol. Plant-Microbe Interact.* 12:712-719.
Lawton, M. A., and Lamb, C. J. 1987. Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. *Mol. Cell. Biol.* 7:335-341.
Leach, J. E., and White, F. F. 1996. Bacterial avirulence genes. *Annu. Rev. Phytopathol.* 34:153-179.
Lindgren, P. B. 1997. The role of *hrp* genes during plant-bacterial interactions. *Annu. Rev. Phytopathol.* 35:129-152.
Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. A gene cluster of *Pseudomonas syringae* pv. *phaseolicola* controls pathogenicity on bean and hypersensitivity on non-host plants. *J. Bacteriol.* 168:512-522.
Martin, G. B. 1999. Functional analysis of plant disease resistance genes and their downstream effectors. *Curr. Opin. Plant Biol.* 2:273-279.
Narimatsu, H. 1994. Recent progress in molecular cloning of glucosyltransferase genes of eukaryotes. *Microbiol. Immunol.* 38:489-504.
O'Reilly, D. R. 1995. Baculovirus-encoded ecdysteroid UDP-glucosyltransferases. *Insect Biochem. Mol. Biol.* 25:541-550.
Rushton, P. J., and Somssich, I. E. 1998. Transcriptional control of plant genes responsive to pathogens. *Curr. Opin. Plant Biol.* 1:311-315.
Scheel, D. 1998. Resistance response physiology and signal transduction. *Curr. Opin. Plant Biol.* 1:305-310.
Whalen, M. C., Innes, R. W., Bent, A. F., and Staskawicz, B. J. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell* 3:49-59.