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Research Notes

Sequence and Expression of a Wheat Gene that Encodes a Novel Protein Associated with Pathogen Defense

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Wheat (*Triticum aestivum*) exhibits local acquired resistance to the powdery mildew pathogen *Erysiphe graminis* f. sp. *tritici*. The resistant state can be induced by a preinoculation with the nonhost pathogen *E. g.* f. sp. *hordei*, the barley powdery mildew, and is accompanied by the activation of putative defense genes. Here, we report the sequence of a pathogen-induced gene, *WIR1a*, and a corresponding cDNA, *WIR1*, that encode novel defense-related proteins of 88 and 85 amino acids, respectively. Analysis of the primary structure of these proteins predicts them to be integral membrane proteins with extracytoplasmic C-terminal domains rich in proline and glycine, through which the proteins possibly interact with the cell wall.

In response to pathogen attack, plants can activate defense mechanisms that limit the spread of the pathogen and render the plant resistant to subsequent infections by a wide variety of pathogens. Acquired resistance has been described in many different host plant-pathogen systems (reviewed by Kuc 1982), and the extent of the protection can vary in both space and time between different systems. Although the molecular mechanisms underlying induced resistance are currently not understood, it is hypothesized that the products of a set of host genes that are activated at the onset of acquired resistance play a causal role in the establishment of the resistant state. Because the understanding of acquired resistance at the molecular level would open up attractive possibilities to exploit this phenomenon for crop protection, we decided to study acquired resistance in wheat (*Triticum aestivum* L.).

In winter wheat, induced resistance to the powdery mildew fungus *Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal can be triggered by a previous inoculation with the nonhost pathogen *E. g.* DC. f. sp. *hordei* Ém. Marchal (barley powdery mildew). In this system, acquired resistance is local (i.e., confined to the treated leaves) and lasts from about 12 hr after the inducing treatment to several days (Schweizer *et al.* 1989). Correlated with the onset of the acquired resistance is the activation of a set of host genes. With the aims of establishing a catalogue of putative defense genes and studying the function of the encoded products in disease resistance, several wheat cDNA clones corresponding to transcripts of genes induced by a nonhost pathogen have been cloned (Schweizer *et al.* 1989). We have shown that these wheat genes induced by a nonhost pathogen encode a peroxidase (Rebmann *et al.* 1991a), a thaumatinlike protein (Rebmann *et al.* 1991b), and a glutathione-S-transferase (Dudler *et al.* 1991). Here, we report on the sequences of a pathogen-induced gene, *WIR1a*, and a homologous cDNA, which encode products that belong to a novel class of proteins.

The isolation of the *WIR1* cDNA clone by differential screening of a cDNA library prepared from mRNA of wheat leaves (cultivar Fidel) 14 hr after inoculation with barley powdery mildew spores has been described (Schweizer *et al.* 1989). This cDNA clone was used as a probe for screening a λ EMBL3 genomic wheat (cultivar Cheyenne) library. One positive λ clone (λ WIR1G1) was isolated, and an 842-bp-long *Kpn*I-*Hin*dIII fragment of its insert that hybridized with the cDNA probe was subcloned. This genomic fragment and the *WIR1* cDNA insert were sequenced on both strands. From the comparison and analysis of these sequences, the following conclusions were drawn. The genomic fragment contains a gene, named *WIR1a*, consisting of two exons that encode a putative protein, PWIR1a (88 amino acids with a relative molecular mass of 8,514). The sequences of the gene and the encoded protein are shown in Figure 1A. The intron-exon structure agrees with the similar but not identical *WIR1* cDNA sequence (Fig. 1B). The open reading frame this cDNA contains encodes a protein (PWIR1b) consisting of 85 amino acids of which 87% are identical in the gene-encoded version. As is evident from the alignment of the two sequences (Fig. 1C), all but two of the substitutions are located in the part of the protein encoded by the first exon of the gene. The copy number of genes homologous to *WIR1a* in the wheat genome was estimated by DNA gel blot hybridization with the complete 842-bp *Kpn*I-*Hin*dIII DNA fragment (the sequence of which is shown in Fig. 1A) as a probe. As is evident in Figure 2, several bands are visible in each lane containing DNA from wheat cultivar Fidel digested with *Bam*HI, *Hin*dIII, and *Eco*RI (*Bam*HI cuts once within the *WIR1a* gene, whereas there are no cleavage sites for the latter two enzymes in the sequenced genomic fragment). Thus, the wheat genome appears to contain a number of *WIR1*-related genes.

The transcription initiation site on the *WIR1a* gene was determined by an S1-mapping experiment (performed as described in Dudler and Travers 1984) with a single-

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stranded antisense DNA probe containing the first 212 nucleotides of the gene (Fig. 1). As shown in Figure 3, three probe fragments with apparent sizes of 121, 122, and 123 nucleotides were protected by RNA extracted from induced leaves (cultivar Cheyenne, 14 hr after inoculation with *E. g.* f. sp. *hordei*) to roughly equal extents. This either implies multiple transcription start sites at nucleotides 88, 89, and 90 (i.e., 24–26 bp downstream of the putative TATA box of the *WIR1a* gene) (Fig. 1A) or may reflect transcripts from different *WIR1* genes. In contrast, no signal was detected with RNA extracted from uninoculated control plants. These data are compatible with the *WIR1a* gene being pathogen-induced.

The time course of *WIR1* mRNA accumulation after inoculation with *E. g.* f. sp. *hordei* (incompatible interaction) and *E. g.* f. sp. *tritici* (compatible interaction) was determined by Northern blot analysis (Fig. 4). In both cases, *WIR1*-specific mRNA had accumulated to the maximal level by 14 hr after the inoculation and remained constant in abundance for the rest of the measured period (46 hr). The apparent difference in intensity between the signals resulting from the incompatible and the compatible interactions was not consistently observed and most likely reflects variations in spore density during the inoculation procedure rather than a difference in the inducing capacity of the two pathogens. In contrast to pathogen infection, wounding (by Carborundum treatment of the leaves) did not induce *WIR1* mRNA accumulation (not shown). The time course of *WIR1*-specific mRNA accumulation after infection was the same as the one observed for a thaumatin-like protein (Rebmann *et al.* 1991) and a peroxidase (unpublished observations), suggesting a coordinate regulation of the genes encoding these putative defense proteins. In contrast to this set of genes, a gene encoding a glutathione-S-transferase isozyme is activated earlier; homologous transcripts reached a steady-state concentration 2 hr after inoculation, as compared with 14 hr for the former three mRNAs (Dudler *et al.* 1991; unpublished observations).

The PWIR1a and PWIR1b protein sequences were compared with the entries in the EMBL (release 29) and SWISS-PROT (release 20) sequence data bases. No signifi-

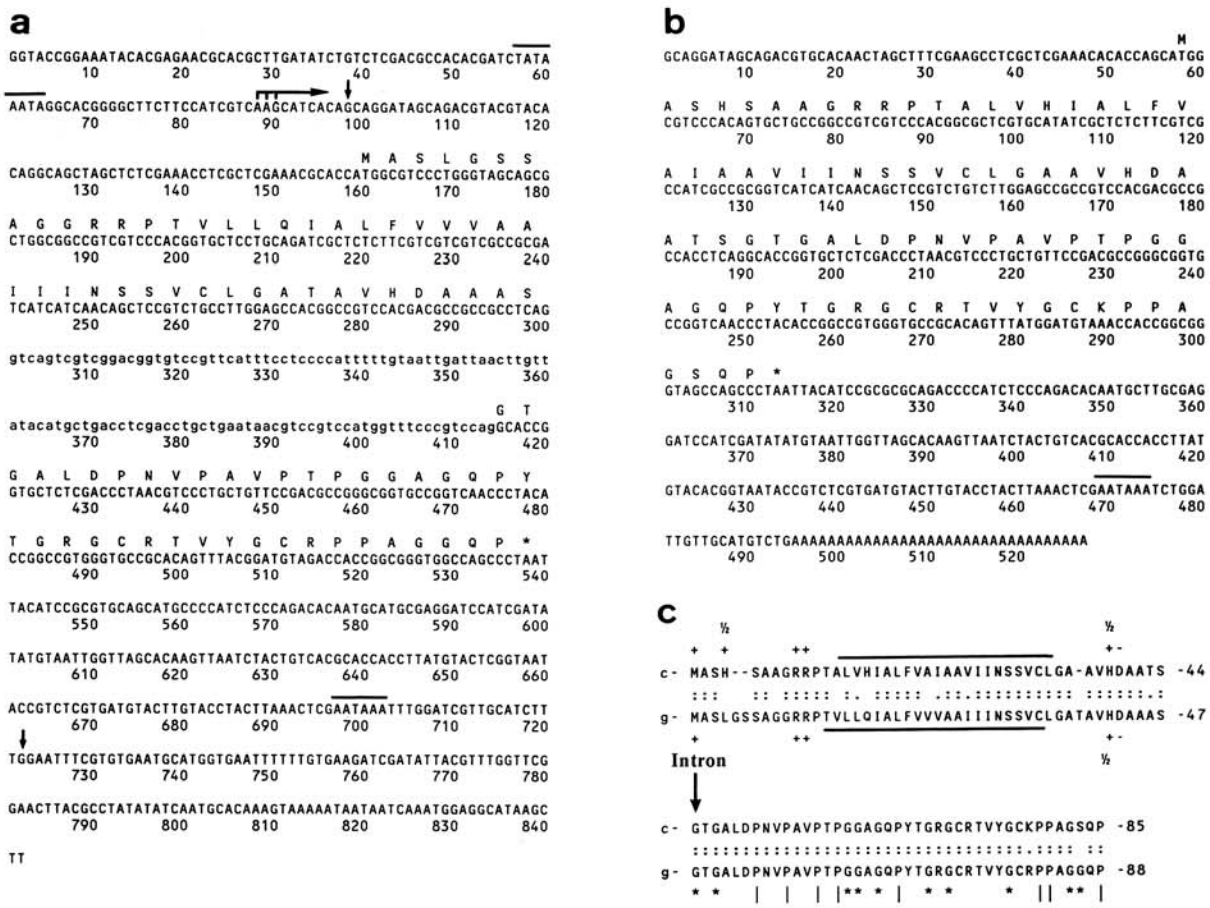

Fig. 1. Nucleotide and deduced amino acid sequences of **A,** the *WIR1a* gene (accession number M95500) and **B,** the *WIR1* cDNA (accession number M94959). The amino acid sequences are given in the single-letter code. Asterisks denote stop codons. The nucleotide sequence of the intron is given in lowercase letters. The TATA box and the AAUAAA polyadenylation signal are overlined. The horizontal arrow indicates the transcription initiation site as determined by S1 mapping, and the vertical arrows mark the position in the gene of the 5′ and 3′ ends of the cDNA. **C,** Alignment of the proteins encoded by the cDNA (c) and the gene (g). Colons denote identical amino acids and periods of conserved substitutions in the two sequences. Hyphens indicate gaps introduced to optimize the alignment. The vertical arrow marks the position of the intron in the gene relative to the protein sequence. The putative membrane-spanning segments are marked by solid lines. The + and − signs indicate charged amino acids (histidine is given half a positive charge) around the putative transmembrane segment. Asterisks indicate glycine residues, and vertical bars indicate proline residues.

cant similarities were found. However, the hydropathy profile (not shown) of the PWIR1 proteins show that the proteins consist of a hydrophobic N-terminal half and a polar C-terminal half that are encoded by separate exons in the *WIR1a* gene. Analysis of the amino acid sequence with the algorithms of Kyte and Doolittle (1982), Rao and Argos (1986), and Eisenberg *et al.* (1984) all predict a membrane-spanning helix in the N-terminal half (Fig. 1B), suggesting that the protein is membrane-associated. The polar C-terminal part encoded by the second exon exhibits a remarkably asymmetric amino acid composition. It consists of 24% glycine (10 out of 41 residues; marked by asterisks in Fig. 1C) and 20% proline (eight out of 41; marked by vertical bars in Fig. 1C).

Because no sequence similarities of PWIR1 to known proteins could be detected, we can only speculate about possible functions of PWIR1 at present. Analysis of its primary sequence and the distribution of charged amino acids around the putative membrane-spanning segment predicts it to be a class II integral membrane protein (von Heijne and Gavel 1988), that is, a protein with an N terminus facing the cytoplasm and an extracytoplasmic C-terminal domain. This topology is also suggested by the method of Hartmann *et al.* (1989), which predicts the transmembrane topology of known proteins with high confidence by the charge difference between 10- and 15-amino acid segments flanking the first transmembrane sequence. A negative charge difference between the C-terminal and the N-terminal flanking segments [Δ(C-N) $<$ 0] indicates an N terminus facing the cytoplasm and an extracytoplasmic C terminus, whereas a positive value implies the opposite

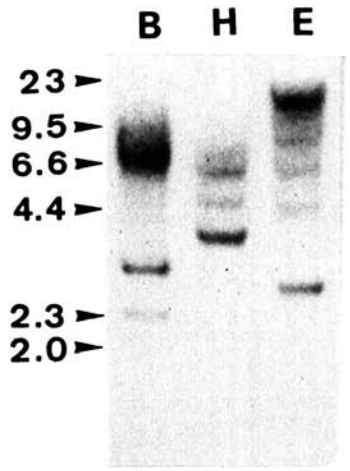

Fig. 2. DNA gel blot hybridization of 10 μg of wheat DNA digested with *Bam*HI (B), *Hin*dIII (H), and *Eco*RI (R). The probe was the 842-bp *Kpn*I-*Hin*dIII fragment containing the *WIR1a* gene. The positions of molecular size markers are indicated on the left.

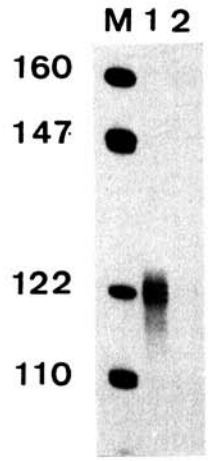

Fig. 3. S1 mapping of the *WIR1a* transcript. Ten micrograms of total RNA from infected (lane 1) and uninfected (lane 2) plants was hybridized with a single-stranded antisense probe containing the first 212 nucleotides of the *WIR1a* gene (Fig. 1A). The sizes of marker fragments (lane M) in nucleotides are indicated on the left.

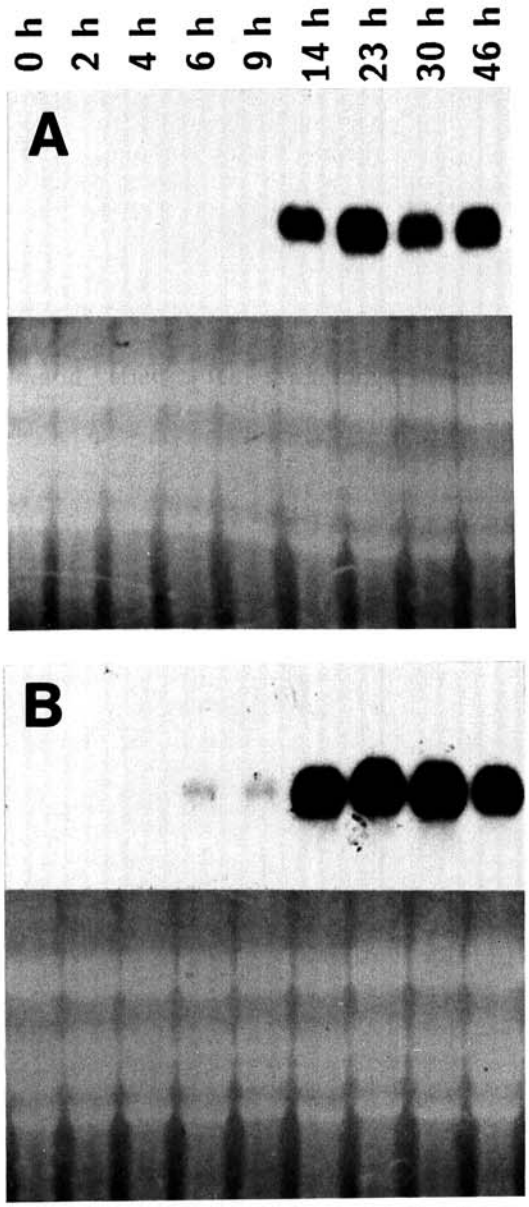

Fig. 4. Time course of *WIR1* mRNA accumulation in response to inoculation with spores of **A,** *Erysiphe graminis* f. sp. *tritici* and **B,** *E. g.* f. sp. *hordei*. The autoradiograms of the RNA gel blots (upper panels) and the corresponding gels stained with ethidium bromide (lower panels) are shown. The *WIR1* cDNA insert was used as a hybridization probe. Ten micrograms of total RNA extracted from leaves at the indicated time points after inoculation was loaded per lane.

orientation. Because for PWIR1a and PWIR1b this charge difference has a negative value (Fig. 1C), the C terminus is predicted to be extracytoplasmic. The relatively high proline and glycine content (together 45%) of the C-terminal half is reminiscent of certain classes of cell wall proteins, namely the proline-, hydroxyproline-, and glycine-rich proteins (for a review see Varner and Lin 1989). Although PWIR1 contains no repetitive sequence elements, a feature that is characteristic for the latter proteins, the richness in glycine and proline may nevertheless indicate a structural rather than a catalytic function. On the basis of this analysis, we speculate that PWIR1 is an integral membrane protein with an extracytoplasmic C-terminal domain that possibly interacts with the cell wall via its (possibly hydroxylated) proline or tyrosine residues. Thus, PWIR1 may increase the adhesion of the membrane to the cell wall in case of pathogen attack. An increase in the strength of attachment between the plasmalemma and the cell wall has indeed been observed as a result of pathogen infection in a similar system by Lee-Stadelmann *et al.* (1984). When barley coleoptile epidermal tissue was inoculated with *E. g.* f. sp. *hordei*, these authors observed what they called concave plasmolysis of the infected cells that was detectable from 24 hr after inoculation in compatible and incompatible interactions. This phenomenon was interpreted as the result of an increased adhesion of the membrane to the cell wall. It is intriguing to hypothesize that a barley homologue of PWIR1 may be involved in this process. Such barley homologues do indeed exist because we have recently found that barley mRNA cross-hybridizing with the wheat *WIR1* cDNA probe accumulates after infection with barley and wheat powdery mildew with a similar time course as in the wheat system (unpublished). Clearly, to test these speculations, PWIR1-specific antibodies are needed. We hope that such antibodies will allow the localization of the protein in the cell and, thus, help clarify the function of these novel putative defense proteins.

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LITERATURE CITED

Dudler, R., and Travers, A. A. 1984. Upstream elements necessary for optimal function of the hsp 70 promoter in transformed flies. Cell 38:391-398.

Dudler, R., Hertig, C., Rebmann, G., Bull, J., and Mauch, F. 1991. A pathogen-induced wheat gene encodes a protein homologous to glutathione-S-transferases. Mol. Plant-Microbe Interact. 4:14-18.

Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. 1984. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol. 179:125-142.

Hartmann, E., Rapoport, T. A., and Lodish, H. F. 1989. Predicting the orientation of eukaryotic membrane-spanning proteins. Proc. Natl. Acad. Sci. USA 86:5786-5790.

Kuc, J. 1982. Induced immunity to plant disease. BioScience 32:854-860.

Kyte, J., and Doolittle, R. F. 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-132.

Lee-Stadelmann, O. Y., Bushnell, W. R., and Stadelmann, E. J. 1984. Changes of plasmolysis form in epidermal cells of *Hordeum vulgare* infected by *Erysiphe graminis*: Evidence for increased membrane-wall adhesion. Can. J. Bot. 62:1714-1723.

Rao, M. J. K., and Argos, P. 1986. A conformational preference parameter to predict helices in intergral membrane proteins. Biochim. Biophys. Acta 869:197-214.

Rebmann, G., Hertig, C., Bull, J., Mauch, F., and Dudler, R. 1991a. Cloning and sequencing of cDNAs encoding a pathogen-induced putative peroxidase of wheat (*Triticum aestivum* L.). Plant Mol. Biol. 16:329-331.

Rebmann, G., Mauch, F., and Dudler, R. 1991b. Sequence of a wheat cDNA encoding a pathogen-induced thaumatin-like protein. Plant Mol. Biol. 17:283-285.

Schweizer, P., Hunziker, W., and Mosinger, E. 1989. cDNA cloning, *in vitro* transcription and partial sequence analysis of mRNAs from winter wheat (*Triticum aestivum* L.) with induced resistance to *Erysiphe graminis* f. sp. *tritici*. Plant Mol. Biol. 12:643-654.

Varner, J. E., and Lin, L.-S. 1989. Plant cell wall architecture. Cell 56: 231-239.

von Heijne, G., and Gavel, Y. 1988. Topogenic signals in integral membrane proteins. Eur. J. Biochem. 174:671-678.