MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 3, No. 2, pp. 72-77, 1990

# Structural Analysis and Activation by Fungal Infection of a Gene Encoding a Pathogenesis-Related Protein in Potato

**Janet L. Taylor, Karl-Heinrich Fritzemeier, Isolde Häuser, Erich Kombrink, Frauke Rohwer, Martin Schröder, Günter Strittmatter, and Klaus Hahlbrock**

Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, D-5000 Köln 30, Federal Republic of Germany.
Received 7 August 1989. Accepted 20 October 1989.

**The structure, genomic organization, and temporal pattern of activation of a gene encoding a pathogenesis-related protein (PR1) in potato (*Solanum tuberosum*) have been analyzed. The gene is rapidly activated in leaves from the potato cultivar Datura, containing the resistance gene R1, in both compatible and incompatible interactions with appropriate races of the late-blight fungus *Phytophthora infestans*. Activation is also observed in leaves treated with fungal elicitor. The gene occurs in multiple, very similar copies and encodes a polypeptide ($M_r$ = 25,054; pI = 5.5) that is classified as a PR protein by several criteria. Small fragments with great sequence similarity to portions of the two exons were found closely linked to the expressed gene, which altogether represents a simple case of genome organization in potato. The coding sequence of the *prp1* gene and the deduced amino acid sequence are strikingly similar to the corresponding sequences of a 26-kDa heat shock protein from soybean.**

*Additional keywords*: disease resistance, *in situ* RNA hybridization, pseudogenes.

The operational term "pathogenesis related" (PR) is commonly applied to a characteristic group of proteins accumulating in pathogen-infected or elicitor-treated plant tissue. Typically, but not invariably, PR proteins have low molecular weights and extreme isoelectric points (Van Loon 1985). Several such proteins, in potato (Kombrink *et al.* 1988) as well as in other plants (Legrand *et al.* 1987; Kauffmann *et al.* 1987), have recently been identified as acidic and basic chitinases and 1,3-β-glucanases. Beyond that, however, the biochemical functions of PR proteins are currently unknown.

After inoculation of potato leaves with *Phytophthora infestans* (Mont.) de Bary, chitinases and 1,3-β-glucanases accumulate slowly over several days (Kombrink *et al.* 1988). Some other functionally unidentified PR proteins accumulate much more rapidly. Among these are several acidic proteins with apparent molecular weights in the range of about 14,000–30,000. These proteins are detectable as translation products of mRNA isolated within a few hours after fungal infection or elicitor treatment (Fritzemeier *et al.* 1987).

The most rapidly activated genes studied so far in potato leaves infected with *P. infestans* are those encoding two enzymes of general phenylpropanoid metabolism, phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL) (Fritzemeier *et al.* 1987; Cuypers *et al.* 1988). The number of PAL genes in potato is unusually high, whereas there are only two 4CL genes, each consisting however of several exons (M. Becker-Andre, K.-H. Fritzemeier, I. Häuser, H.-J. Joos, G. Strittmatter, and K. Hahlbrock, unpublished results). Here we report on a gene, encoding a typical pathogenesis-related (PR) protein, that has a relatively simple exon/intron structure and genomic organization. This gene is activated with time courses very similar to those of PAL and 4CL in both compatible and incompatible interactions of potato leaves with *P. infestans*. It bears striking similarity with a gene encoding a heat shock protein in soybean.

## MATERIALS AND METHODS

**Enzymes.** Enzymes for DNA and RNA manipulation were purchased from Boehringer (Mannheim, Federal Republic of Germany), Gibco-BRL (Gaithersburg, MD), or Pharmacia (Uppsala, Sweden), and were used according to the instructions of the manufacturers. The AMV reverse transcriptase used for cDNA synthesis was purchased from Life Sciences (Greenwich, CT). Radioactive isotopes and rabbit reticulocyte lysate were obtained from Amersham Buchler (Braunschweig, Federal Republic of Germany).

**Plant material.** Plants of the potato cultivar Datura (*Solanum tuberosum* L., carrying resistance gene R1) were grown in vermiculite and watered with Hoagland's solution for 6 wk during a 16-hr day (20° C)/8-hr night (17° C) cycle (Rohwer *et al.* 1987).

Current address of J. L. Taylor: Plant Biotechnology Institute, 110 Gymnasium Road, Saskatoon, Saskatchewan, S7N OW9, Canada.
Current address of K.-H. Fritzemeier: Schering AG, Müllerstr. 170-178, 1000 Berlin 65, Federal Republic of Germany.
Current address of I. Häuser: Bayer AG, 5090 Leverkusen, Federal Republic of Germany.
Current address of F. Rohwer: Weenderstr. 93, 3400 Göttingen, Federal Republic of West Germany.
Address correspondence to K. Hahlbrock: Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, Carl-von-Linne-Weg 10, D-5000 Köln 30, Federal Republic of Germany.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank as accession number J03679.

© 1990 The American Phytopathological Society

**Treatment of plant material.** Zoospores from *P. infestans* races 1 and 4 as well as culture filtrate elicitor were prepared and used for inoculation of potato leaves as described previously (Fritzemeier *et al.* 1987; Cuypers *et al.* 1988).

**Construction and screening of a cDNA library.** Leaves were harvested 4 hr after spraying with a spore suspension of *P. infestans* race 4 at a concentration of $10^5$–$10^6$ spores per milliliter. Total RNA was isolated from the leaves (Dunsmuir *et al.* 1988), and poly(A)$^+$ RNA was prepared by chromatography on oligo(dT) cellulose (Fritzemeier *et al.* 1987). The synthesis of cDNA was performed according to published procedures (Lapeyre and Amalric 1985). The resulting DNA was cloned into lambda gt10 and packaged (Gigapack, Stratagene, La Jolla, CA). Differential hybridization was conducted with $^{32}$P-labeled, single-stranded cDNA derived from mRNA of leaves sprayed with either race 4 spore suspension or water. The inserts of cDNA clones specifically induced in infected leaves were subcloned into the vector pUC9. All cloning operations were performed according to standard procedures (Maniatis *et al.* 1982).

**Blot hybridization.** For RNA blot analysis, 20 μg of total RNA was separated on denaturing 1.2% (w/v) agarose-formaldehyde gels and transferred to GeneScreenPlus membranes (Du Pont, Wilmington, DE); the membranes were hybridized to $^{32}$P-labeled cDNA. Single bands were obtained in each case. Steady-state mRNA levels at various time points after inoculation were determined by cDNA hybridization to 5–10 μg of total RNA on nitrocellulose membranes using a Hybri-Slot Manifold apparatus (Gibco-BRL).

Genomic blots consisted of 20 μg of DNA from cultivar Datura leaves, digested to completion with *Eco*RI and run on 0.8% (w/v) agarose gels. DNA was blotted to nylon membranes and hybridized with a $^{32}$P-labeled genomic restriction fragment.

***In situ* RNA hybridization.** The method described elsewhere (Schmelzer *et al.* 1988; Cuypers *et al.* 1988) was used with the following minor modifications: $^{35}$S-labeled antisense RNA ($1.2 \times 10^9$ dpm/μg) was applied at a final concentration of 0.1 ng/μl hybridization solution.

**Hybrid-select translation.** Ten micrograms of plasmid containing the cDNA insert was bound to nitrocellulose membranes and hybridized to 300 ng poly(A)$^+$ RNA. The bound RNA was eluted and translated *in vitro* in rabbit reticulocyte lysate containing $^{35}$S-methionine (Fritzemeier *et al.* 1987; Somssich *et al.* 1986). Two-dimensional gel electrophoresis was performed according to standard procedures (Dunsmuir *et al.* 1988).

**Runoff transcription.** Transcriptionally active nuclei were isolated from potato leaves treated with either culture-filtrate elicitor or water. The runoff transcription reaction contained 200 μCi of $^{32}$P-labeled UTP (410 Ci/mmol) and 200 μg of nuclear DNA. The labeled RNA was extracted and purified for hybridization to 1 μg of the plasmid cDNA clone on slot blots (Fritzemeier *et al.* 1987; Somssich *et al.* 1986).

**DNA sequencing.** Templates consisted of 300- to 400-base pair (bp) fragments obtained from the genomic or cDNA clones by restriction enzyme or exonuclease III digestion. Plasmid DNA was isolated and purified on a CsCl gradient (Maniatis *et al.* 1982). The plasmids were denatured and used in standard dideoxy sequencing reactions (Sanger *et al.* 1977).

**Primer extension.** Reactions contained 10 μg of total RNA annealed to 0.1 pmol end-labeled primer using γ-[$^{32}$P]ATP with a specific activity of 5,000 Ci/mol. The oligonucleotide sequence was 5′-GGCTAAAAGGAC-TATAC-3′.

## RESULTS

**Differential screening of a cDNA library.** Eight apparently unrelated cDNA clones, not including PAL and 4CL, but complementary to other mRNA induced in leaves of the potato cultivar Datura upon infection with *P. infestans* race 4 (incompatible interaction), were obtained after differential screening of 20,000 recombinants. All of these cDNAs were used as probes in RNA blot hybridizations that compared the levels of their corresponding mRNAs in infected leaves. The cDNA, 347 bp, that gave the strongest signal was designated as PR1 and used in the following studies.

**Timing of mRNA induction.** The time course of transcriptional activation of the *prp1* gene(s) in elicitor-treated leaves (Fig. 1A) was similar to that reported previously for 4CL (Fritzemeier *et al.* 1987). The difference in the amounts of PR1 mRNA in uninfected and infected

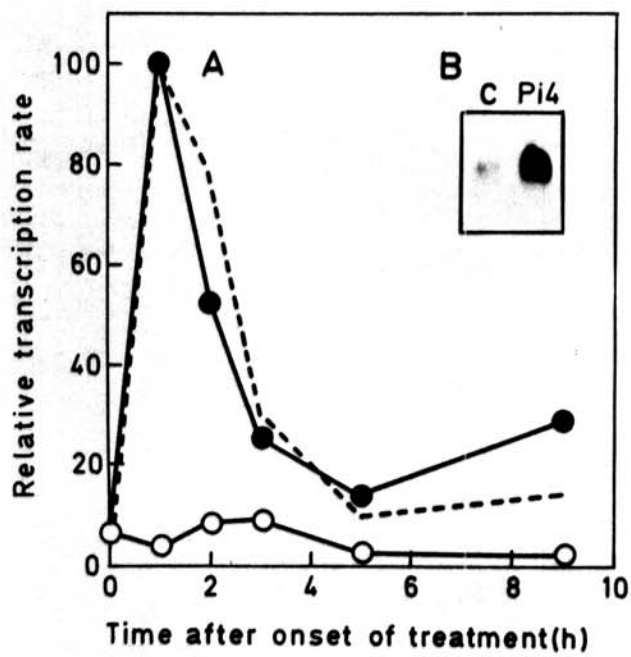


**Fig. 1.** Transcriptional activation of *prp1*. (**A**) Results from densitometrical scans of slot-blot hybridizations of PR1 (closed symbols) or 4CL (broken line) cDNA with $^{32}$P-labeled runoff transcripts. Nuclei were isolated at the indicated times following treatment of potato leaves with culture-filtrate elicitor. Open symbols indicate hybridization of PR1 cDNA with $^{32}$P-labeled RNA isolated from nuclei of water-treated control leaves. (**B**) PR1 mRNA levels in leaves 4 hr after spraying with either water (C = control) or a spore suspension of *Phytophthora infestans* race 4 (*Pi4*); each lane contains 20 μg of total RNA hybridized with $^{32}$P-labeled PR1 cDNA.

leaves 4 hr after inoculation, the time point used for construction of the cDNA library, is shown in Figure 1B.

The extent of induction at this early time point varied considerably among several independent experiments, from very large (Fig. 1B; or Fritzemeier *et al.* 1987) to hardly detectable (Fig. 2). By contrast, a subsequent, large, and concomitant increase in the steady-state PR1 and 4CL mRNA levels about 5–10 hr after inoculation was always observed. As shown in Figure 2, both mRNA types showed, at later time points, the same differential accumulation between compatible and incompatible interactions of cultivar Datura leaves with *P. infestans* races 1 and 4, respectively.

*In situ* hybridization with labeled PR1 antisense RNA showed the accumulation of the PR1 transcript around the site of fungal penetration. Figure 3 shows, for example, the spatial distribution of the accumulated transcript 24 hr after the inoculation of a cultivar Datura leaf with *P. infestans* race 4 (incompatible interaction). A less localized response was observed in the compatible interaction with race 1.

**Translation product.** The encoded PR1 protein was identified by *in vitro* translation of hybrid-selected poly(A)$^+$ mRNA followed by separation on a two-dimensional gel. The translation product migrated as a single spot with an apparent molecular mass of about 25 kDa and a pI of 5.5 (Fig. 4).

**Genomic organization.** Three clones were isolated from a genomic library of potato cultivar Datura DNA, using the PR1 cDNA as a probe. Two of them were apparently identical. The restriction maps of two partially overlapping

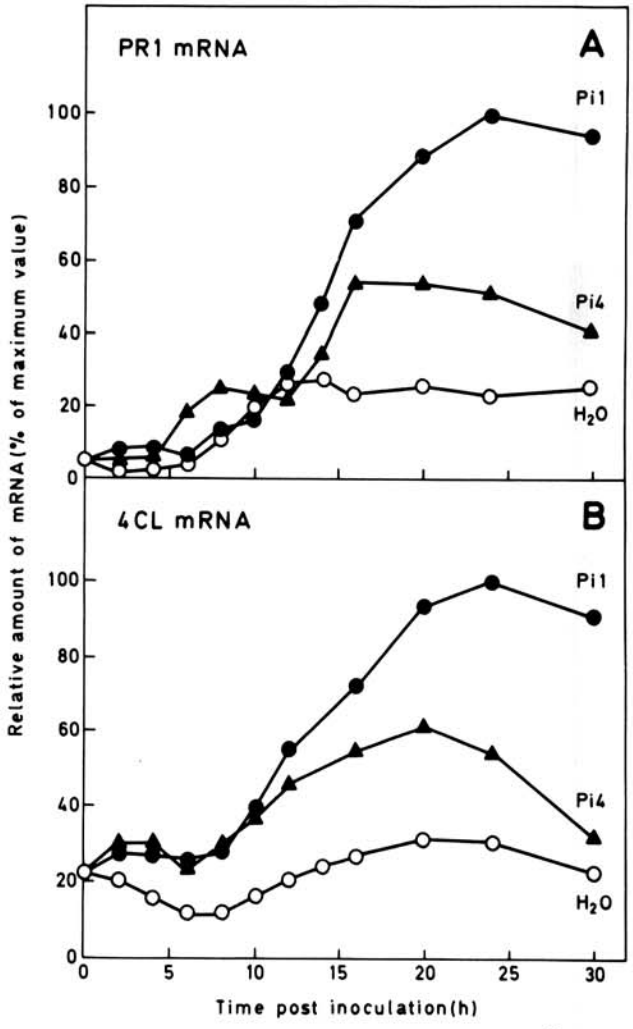


**Fig. 2.** Time course of mRNA accumulation in infected leaves. $^{32}$P-labeled PR1 (**A**) or 4CL (**B**) cDNA was hybridized on slot blots to 5 μg of total RNA from potato leaves at the indicated times after inoculation with *Phytophthora infestans* race 1 (*Pi*1) or race 4 (*Pi*4), or after mock-inoculation with water ($H_2O$). The intensity of the hybridization signals was scanned densitometrically.

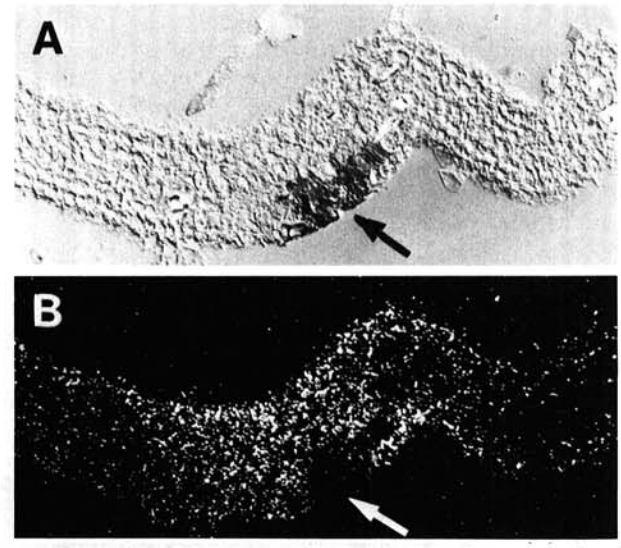


**Fig. 3.** Localization of induced *prp1* transcript at a fungal infection site. Cross sections of a potato cultivar Datura leaf were fixed and embedded in paraffin 24 hr after inoculation with *Phytophthora infestans* race 4 ($10^6$ zoospores per milliliter). (**A**) Bright-field microscopy showing a necrotic spot at the center of an infection site (arrow). (**B**) Autoradiography of an adjacent section after hybridization with $^{35}$S-labeled PR1 antisense RNA. The arrow indicates the site identified in panel **A**.

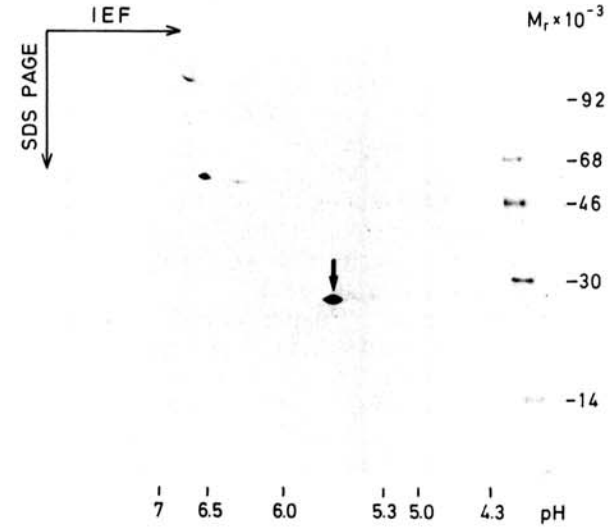


**Fig. 4.** Two-dimensional gel analysis of the *in vitro* translation product (arrow) obtained from mRNA hybrid selected with PR1 cDNA. Proteins not marked by an arrow originate from endogenous mRNA activity in the reticulocyte lysate.

clones, λ-St51 and λ-St128, are shown in Figure 5. Regions hybridizing with PR1 cDNA or end-labeled mRNA, which detected 5′ portions not present on the cDNA, were identified (Fig. 5, regions A–D) and the nucleotide sequences determined (see below).

The simple genomic organization of the *prp1* gene(s) is shown in Figure 6. After digestion of genomic DNA with *Eco*RI, only a single fragment hybridized to the 1.6-kilobase (kb) *Eco*RI fragment of λ-St128 under stringent conditions. This band corresponds to a defined restriction fragment in region A of Figure 5 on λ-St128. Copy number reconstruction based on a 1C value of 2.1 pg (Bennet and Smith 1976) indicated that this fragment exists in approximately 10–15 copies per haploid genome. Essentially the same result was obtained with *Hin*dIII, and only a very limited restriction fragment length polymorphism (with 3 of 12 tested restriction enzymes) was observed within the apparent multiple copies of the 1.6-kb *Eco*RI fragment from λ-St128.

**Nucleotide and amino acid sequences.** The nucleotide sequence of the *prp1* gene encompassed in region A of Figure 5 and the deduced amino acid sequence are presented in Figure 7. The major transcription start site, determined by primer extension, was located 54 bp upstream of the translation start site. A minor site was found 6 bp farther downstream; its relation to this or other copies of the *prp1* gene was, however, not further investigated. Promoter features that are indicated in Figure 7 are a sequence similar (8 of 10 nucleotides) to the heat shock element consensus (Pelham 1985) and a putative "TATA" box.

The 5′ border of the single intron (408 bp) was deduced by matching the sequence to known splice-junction sites (Brown 1986). The 3′ border was determined by S1 mapping. The resulting calculated sizes of the PR1 mRNA (870 nucleotides) and protein (217 amino acids, $M_r$ = 25,054) concur with the values estimated above (Figs. 1B and 4, respectively). The cDNA ranged from the *Eco*RI site at position +947 to position +1274 (Fig. 7) and was completely identical in nucleotide sequence with the respective portion of region A of Figure 5.

The Gap program from the University of Wisconsin Genetics Computer Group (Devereux *et al.* 1984) was used to compare the *prp1* gene (region A in Fig. 5) with regions B, C, and D. On λ-St51, areas of sequence similarity are confined to regions B and C. Region B consists of 325 bp with 83% identity to the first exon of *prp1*, immediately followed by 344 bp whose identity to the second exon also averages 83%. No sequence similarity to the promoter, intron, or 3′ untranslated region of *prp1* was found in this area. Region C is comprised of 186 bp with 85% identity to the first exon of *prp1*, beginning 10 bp upstream of the translation start site. Region D, which is on λ-St128, encompasses 433 bp that are 91% identical to a portion of the second exon and the 3′ untranslated region of the *prp1* gene. No sequence similarity to the first exon of *prp1* was detected in this area.

A computer search of GenBank using the Gap program revealed a sequence with great similarity to *prp1*. This sequence represents a gene, designated Gmhsp26-A, encoding a member of the group of low molecular mass heat shock proteins in soybean (Czarnecka *et al.* 1988). The similarity of *prp1* to Gmhsp26-A, as assigned by the program, was 61% at the nucleotide level and 51% at the amino acid level (Fig. 8).

## DISCUSSION

Several extracellular PR proteins from potato have recently been reported (Kombrink *et al.* 1988; Parent and Asselin 1987). The PR protein described here was not found in the intercellular washing fluid and is probably located intracellularly, similar to the PR1 and PR2 proteins in parsley (Somssich *et al.* 1986). PR1 from potato is a typical PR protein with the characteristic properties of accumulation at infection sites, low molecular mass, and an acidic isoelectric point.

So far, the biochemical function of PR1 from potato, like most other PR proteins except for the recently identified chitinases and 1,3-β-glucanases (Kombrink *et al.* 1988), is not known. It was therefore particularly interesting to note a striking similarity between the nucleotide and deduced amino acid sequences of the potato PR1 and the heat shock protein HSP26 from soybean. The gene encoding the soybean HSP26, Gmhsp26-A (Czarnecka *et al.* 1988), is identical in nucleotide sequence with a soybean gene designated as G2-4, which is transcriptionally activated by auxin and certain heavy metal ions (Hagen *et al.* 1988).

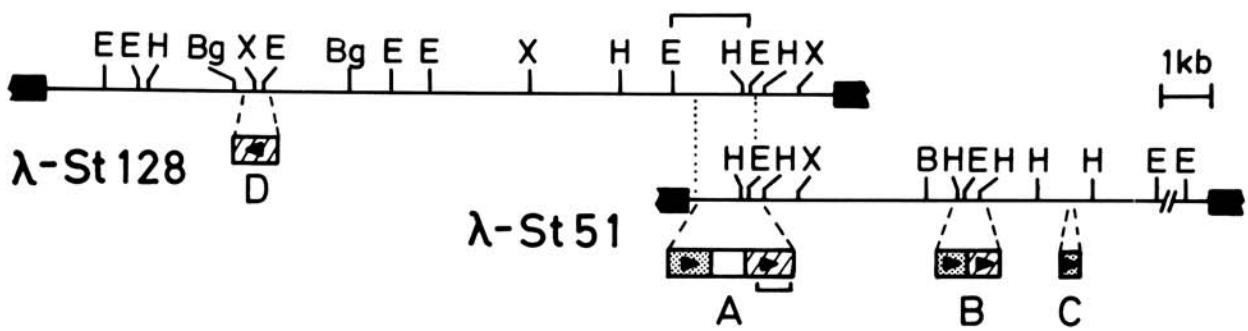


**Fig. 5.** Comparison of restriction maps for two partially overlapping genomic *prp1* clones. The restriction enzymes used were *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E), *Hin*dIII (H), and *Xho*I (X). Regions hybridizing to PR1 cDNA or end-labeled RNA are marked A–D. Region A represents the complete *prp1* gene; regions B, C, and D are fragments with partial sequence similarity to region A (see text). Exons 1 and 2, as well as corresponding, similar nucleotide sequences, are marked as stippled and hatched areas, respectively. The single intron is depicted as an open box in A. The lower bracket in A indicates identity with the cDNA. The upper bracket marks the *Eco*RI fragment used in Figure 6.

Although the promoter of the *prp1* gene described here contains one heat shock elementlike sequence at position −188 (Fig. 7) and three additional ones with slightly less similarity (7 of 10 nucleotides) between −600 and −700 (data not shown), their location is well beyond the distance from the TATA box found to be optimal for heat shock response (Pelham 1982; Pelham and Bienz 1982; Schöffl *et al.* 1986). Moreover, the promoter region of the *prp1* gene lacks a typical CCAAT box that was found to be crucial for the function of more distal heat shock elements (Bienz and Pelham 1986). In a preliminary experiment, no PR1 mRNA induction was detected in heat-shocked potato leaves. This is in accord with other systems, where the occurrence of such elements in the promoter region of several, but not all, genes encoding PR proteins has been noted (Somssich *et al.* 1988).

In light of these results, the presence of apparently nonfunctional heat shock elements in the *prp1* gene takes on greater significance. Further study may indicate the evolutionary relationship between the different proteins that are produced in response to stress. It should be noted, however, that no sequence similarity could be found between PR1 and several known PR proteins from two other Solanaceous species, tobacco (Cornelissen *et al.* 1987) and tomato (Lucas *et al.* 1985).

Within experimental error, the timing of transcriptional activation and mRNA accumulation in elicitor-treated or fungus-infected potato leaves is the same for PR1 and 4CL, as well as for PAL (Fritzemeier *et al.* 1987), including the differential behavior at late time points in compatible and incompatible interactions with *P. infestans*. This suggests that all three proteins/enzymes are related to the process in which the plant attempts to limit fungal penetration.

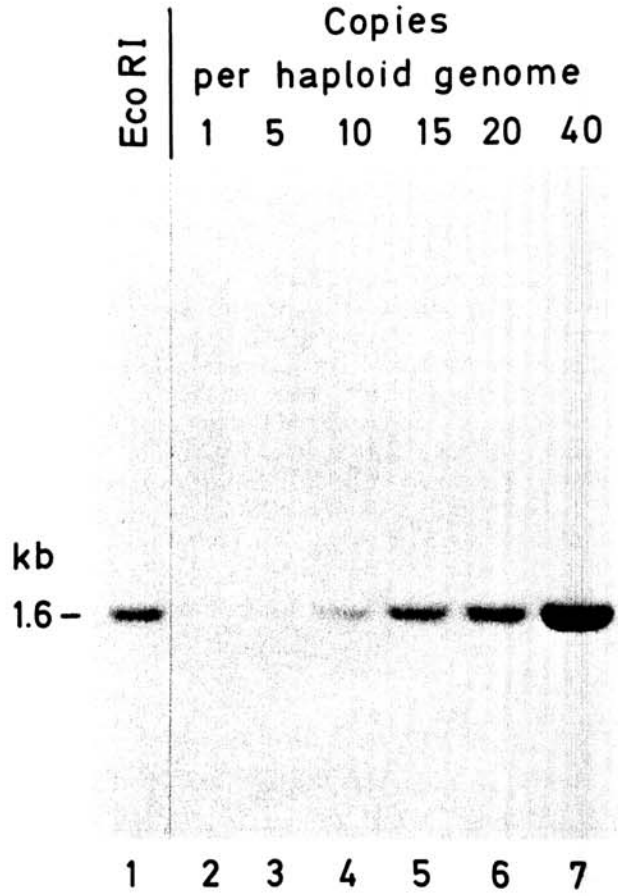


**Fig. 6.** Genomic complexity of the *prp1* gene. Twenty micrograms of DNA from leaves of the tetraploid potato cultivar Datura was digested with *Eco*RI (lane 1); lanes 2–7 contain 1, 5, 10, 15, 20, and 40 haploid genome equivalents, respectively, of the subcloned 1.6-kb *Eco*RI fragment from λ-St128 (Fig. 5) comprising most of the *prp1* gene. After blotting to a nylon membrane, the DNA was hybridized to the same $^{32}$P-labeled fragment.

```
-240 TCTTTAACAAATTAAAATTGAAAATATGATAAATAAATTAAACTATTCTATCATTGATTT

-180 TTCTAGCCACCAGATTTGACCAAACAGTGGGTGACATGAGCACATAAGTCATCTTTATTG

-120 TATTTTATTACTCACTCCAAAAATATAGGGAATATGTTTACTACTTAATTTAGTCAAATA

 -60 TAATTTTATATTAGAATAATTGAATAGTCAAACAAGAAACTTTAATGCATCCTTATTTTT

   1 TCCTCTATAAAAAAAAGACTAGACACCAAGGGAGACCAACCACACATAATTAAGATGGCA
                                                              MetAla

  61 GAAGTGAAGTTGCTTGGTCTAAGGTATAGTCCTTTTAGCCATAGAGTTGAATGGGCTCTA
     GluValLysLeuLeuGlyLeuArgTyrSerProPheSerHisArgValGluTrpAlaLeu

 121 AAAATTAAGGGAGTGAAATATGAATTTATAGAGGAAGATTTACAAAATAAGAGCCCTTTA
     LysIleLysGlyValLysTyrGluPheIleGluGluAspLeuGlnAsnLysSerProLeu

 181 CTTCTTCAATCTAATCCAATTCACAAGAAAATTCCAGTGTTAATTCACAATGGCAAGTGC
     LeuLeuGlnSerAsnProIleHisLysLysIleProValLeuIleHisAsnGlyLysCys

 241 ATTTGTGAGTCTATGGTCATTCTTGAATACATTGATGAGGCATTTGAAGGCCCTTCCATT
     IleCysGluSerMetValIleLeuGluTyrIleAspGluAlaPheGluGlyProSerIle

 301 TTGCCTAAAGACCCTTATGATCGCGCTTTAGCACGATTTTGGGCTAAATACGTCGAAGAT
     LeuProLysAspProTyrAspArgAlaLeuAlaArgPheTrpAlaLysTyrValGluAsp

 361 AAGGTATATTGCTTTTAAGTTATTCCAATTGATTGAAAAGTTTGTTTTAGTTACGTTATT
     Lys

 421 ACATATACTTTAGGTCTCATGCTTTTTAATAATCTTTTATAAAATTCGACTAAGACGAAC

 481 TTCTCGTATAGTCAACAATACTAACATATTTGTCTAGTAGTTGGTTAGGAAATAAGTTAT

 541 CCGAATATTAAATTCTGGATAAGTAATGAATACCATATTTGATAGTTGATTTGGAGATAA

 601 ATTATTCGTGTATAAAATTAATATGATATTTGATTTGCAATTTAGAAATACATAACTATT

 661 TATATGCATAGATCCATTATAACTAATTGATATATTATTAATATCTGTATAACTCTAACC

 721 AGCTATCGAAACGAGTCAACGAACCTTATTAAGTTTTGTTTGTTGGGCAGGGGGCAGCAG
                                                            GlyAlaAlaV

 781 TGTGGAAAAGTTTCTTTTCGAAAGGAGAGGAACAAGAGAAAGCTAAAGAGGAAGCTTATG
     alTrpLysSerPhePheSerLysGlyGluGluGlnGluLysAlaLysGluGluAlaTyrG

 841 AGATGTTGAAAATTCTTGATAATGAGTTCAAGGACAAGAAGTGCTTTGTTGGTGACAAAT
     luMetLeuLysIleLeuAspAsnGluPheLysAspLysLysCysPheValGlyAspLysP

 901 TTGGATTTGCTGATATTGTTGCAAATGGTGCAGCACTTTATTTGGGAATTCTTGAAGAAG
     heGlyPheAlaAspIleValAlaAsnGlyAlaAlaLeuTyrLeuGlyIleLeuGluGluV

 961 TATCTGGAATTGTTTTGGCAACAAGTGAAAAATTTCCAAATTTTTGTGCTTGGAGAGATG
     alSerGlyIleValLeuAlaThrSerGluLysPheProAsnPheCysAlaTrpArgAspG

1021 AATATTGCACACAAAACGAGGAATATTTTCCTTCAAGAGATGAATTGCTTATCCGTTACC
     luTyrCysThrGlnAsnGluGluTyrPheProSerArgAspGluLeuLeuIleArgTyrA

1081 GAGCCTACATTCAGCCTGTTGATGCTTCAAAATGAGTATACCTCAAGTGAATTTCAAGAT
     rgAlaTyrIleGlnProValAspAlaSerLysEnd

1141 TTTGTGTGGCAATAAAAATTGAGTTTTTGTAAATTCAATTGAAATATATTAAAGTTGCAT

1201 GTTATAAGATTTATCTTTATTTCACTAGTTAATATAAATTTTGGATTCACGTATAAATAA

1261 AAGTATTGTTAAGAGAAAGAAAGCTT
```

**Fig. 7.** Nucleotide and deduced amino acid sequences of *prp1*. The transcriptional start site corresponds to +1 in the numbering of the nucleotide sequence. The following nucleotide sequences are underlined or boxed: a heat shock consensus element, a putative "TATA" box, and two putative polyadenylation signals. The cDNA described in the text starts with the *Eco*RI site at position +947 and ends at position +1274.

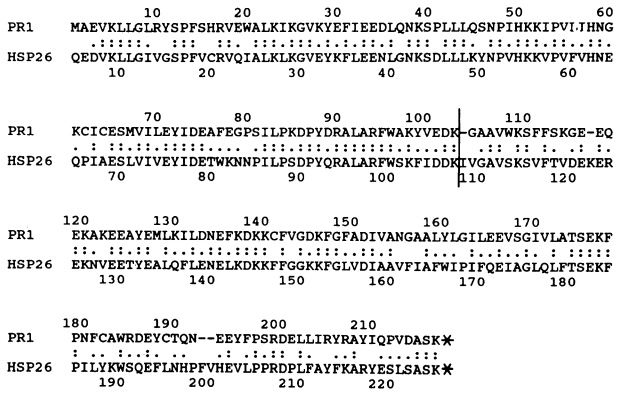


**Fig. 8.** Comparison of deduced amino acid sequences for protein PR1 from potato (Fig. 7) and a 26-kDa heat shock protein, HSP26, from soybean (Czarnecka *et al.* 1988). Identical amino acids (:) and neutral changes (.) are indicated. The position of single introns in the corresponding genes is marked by a vertical line; asterisks denote termination codons in the nucleotide sequences.

In the incompatible interaction, this process is successful and results in the formation of small local lesions, possibly explaining the smaller extent and shorter duration in mRNA accumulation observed for PR1 in this interaction compared to the compatible one.

One aim of our studies is the molecular analysis of the disease resistance response in potato, including the mechanisms of gene activation in infected tissue. In this connection, a gene with the simplest structure and genomic complexity that responds rapidly and strongly to infection is of great interest. The *prp1* gene fits this description better than any other potato gene so far investigated. All available information from restriction mapping indicates that the *prp1* gene exists in the potato cultivar Datura as multiple copies of great similarity. The three closely linked regions B–D (Fig. 5) showing a considerable degree of sequence similarity to *prp1* appear to be pseudogenes. Their similarity to only the coding region of *prp1* may indicate that they originated from a reverse transcription and reintegration type of event. Analysis of the regulatory elements present in the *prp1* gene should prove to be interesting, particularly in view of its similarity to the soybean gene regulated by heat shock, auxin, and heavy metal ions.

## ACKNOWLEDGMENTS

We thank Jennifer Söhne for excellent technical assistance. This work was supported by a postdoctoral fellowship (to J. L. T.) from the Max Planck Society and by the Federal Ministry of Science and Technology of the Federal Republic of Germany (BMFT project 1.02).

## LITERATURE CITED

Bennet, M. D., and Smith, J. B. 1976. Nuclear DNA amounts in angiosperms. Philos. Trans. R. Soc. London B 274:227-274.

Bienz, M., and Pelham, H. R. B. 1986. Heat-shock regulatory elements function as an inducible enhancer in the *Xenopus hsp70* gene and when linked to a heterologous promoter. Cell 45:753-760.

Brown, J. W. S. 1986. A catalogue of splice junction and putative branch point sequences from plant introns. Nucleic Acids Res. 14:9549-9559.

Cornelissen, B. J. C., Horowitz, J., van Kan, J. A. L., Goldberg, R. E., and Bol, J. L. 1987. Structure of tobacco genes encoding pathogenesis-related proteins from the PR-1 group. Nucleic Acids Res. 15:6799-6811.

Cuypers, B., Schmelzer, E., and Hahlbrock, K. 1988. *In situ* localization of rapidly accumulated phenylalanine ammonia-lyase mRNA around penetration sites of *Phytophthora infestans* in potato leaves. Mol. Plant-Microbe Interact. 1:157-160.

Czarnecka, E., Nagao, R. T., Key, J. L., and Gurley, W. B. 1988. Characterization of Gmhsp26-A, a stress gene encoding a divergent heat shock protein of soybean: Heavy-metal-induced inhibition of intron processing. Mol. Cell. Biol. 8:1113-1122.

Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.

Dunsmuir, P., Bond, D., Lee, K., Gidoni, D., and Townsend, J. 1988. Stability of introduced genes and stability in expression. Section C1, pages 1-17 in: Plant Molecular Biology Manual. S. B. Gelvin and R. A. Schilperoort, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Fritzemeier, K.-H., Cretin, C., Kombrink, E., Rohwer, F., Taylor, J., Scheel, D., and Hahlbrock, K. 1987. Transient induction of phenylalanine ammonia-lyase and 4-coumarate:CoA ligase mRNAs in potato leaves infected with virulent or avirulent races of *Phytophthora infestans*. Plant Physiol. 85:34-41.

Hagen, G., Uhrhammer, N., and Guilfoyle, T. J. 1988. Regulation of expression of an auxin-induced soybean sequence by cadmium. J. Biol. Chem. 263:6442-6446.

Kauffmann, S., Legrand, M., Geoffroy, P., and Fritig, B. 1987. Biological function of 'pathogenesis-related' proteins: Four PR proteins of tobacco have 1,3-$\beta$-glucanase activity. EMBO J. 6:3209-3212.

Kombrink, E., Schröder, M., and Hahlbrock, K. 1988. Several "pathogenesis-related" proteins in potato are 1,3-$\beta$-glucanases and chitinases. Proc. Natl. Acad. Sci. USA 85:782-786.

Lapeyre, B., and Amalric, F. 1985. A powerful method for the preparation of cDNA libraries: Isolation of cDNA encoding a 100-k-Dal nucleolar protein. Gene 37:215-220.

Legrand, M., Kauffmann, S., Geoffroy, P., and Fritig, B. 1987. Biological function of pathogenesis-related proteins: Four tobacco pathogenesis-related proteins are chitinases. Proc. Natl. Acad. Sci. USA 84:6750-6754.

Lucas, J., Camacho-Henriquez, A., Lottspeich, F., Henschen, A., and Sanger, H.-L. 1985. Amino acid sequence of the "pathogenesis-related" leaf protein p14 from viroid-infected tomato reveals a new type of structurally unfamiliar proteins. EMBO J. 4:2745-2749.

Maniatis, T. E., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Parent, J.-G., and Asselin, A. 1987. Acidic and basic extracellular pathogenesis-related leaf proteins from fifteen potato cultivars. Phytopathology 77:1122-1125.

Pelham, H. R. B. 1982. A regulatory upstream promoter element in the *Drosophila hsp 70* heat-shock gene. Cell 38:517-528.

Pelham, H. R. B. 1985. Activation of heat-shock genes in eukaryotes. Trends Genet. 1:31-35.

Pelham, H. R. B., and Bienz, M. 1982. A synthetic heat-shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. EMBO J. 1:1473-1477.

Rohwer, F., Fritzemeier, K.-H., Scheel, D., and Hahlbrock, K. 1987. Biochemical reactions of different tissues of potato (*Solanum tuberosum*) to zoospores or elicitors from *Phytophthora infestans*. Planta 170:556-561.

Sanger, F., Nicklen, S., and Coulsen, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.

Schmelzer, E., Jahnen, W., and Hahlbrock, K. 1988. *In situ* localization of light-induced chalcone synthase mRNA, chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. Proc. Natl. Acad. Sci. USA 85:2989-2993.

Schöffl, F., Baumann, G., Raschke, E., and Bevan, M. 1986. The expression of heat-shock genes in higher plants. Philos. Trans. R. Soc. London B 314:453-468.

Somssich, I. E., Schmelzer, E., Bollmann, J., and Hahlbrock, K. 1986. Rapid activation by fungal elicitor of genes encoding "pathogenesis-related" proteins in cultured parsley cells. Proc. Natl. Acad. Sci. USA 83:2427-2430.

Somssich, I. E., Schmelzer, E., Kawalleck, P., and Hahlbrock, K. 1988. Gene structure and *in situ* transcript localization of pathogenesis-related protein 1 in parsley. Mol. Gen. Genet. 213:93-98.

Van Loon, L. C. 1985. Pathogenesis-related proteins. Plant Mol. Biol. 4:111-116.