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Cloning, Expression, and Sequence Conservation of Pathogenesis-Related Gene Transcripts of Potato

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Treatment of potato tuber disks with arachidonic acid elicits the accumulation of several mRNAs. cDNA clones corresponding to two of these mRNAs were isolated and characterized. Nucleotide sequence analysis reveals that both clones (pSTH-2 and pSTH-21) contain an open-reading frame coding for a 155-amino acid polypeptide. The polypeptides encoded by the two clones differ by only six amino acids and show a high degree of similarity with PR protein sequences from pea (~42%) and parsley (~37%). mRNAs corresponding to the two potato cDNA clones also accumulate in *Solanum chacoense* and in tomato following elicitor treatment. Maximum accumulation of the mRNAs corresponding to the two cDNA clones is reached 24 hr after elicitor treatment of the tuber disks. pSTH-2-related mRNAs also accumulate in tubers after wounding or treatment with eicosapentaenoic acid and are detected in potato and tomato leaves treated with a *Phytophthora infestans* mycelium homogenate. The presence of these conserved genes in species from three plant families and the similarity of their induction pattern suggest an important function during the plant defense response.

Additional keywords: hypersensitive response, gene expression.

Plants respond to environmental stresses by rapid and specific changes in metabolism. The metabolic response of the plant to pathogen stress includes *de novo* synthesis of proteins and enzymes (Dixon *et al.* 1986; Collinge and Slusarenko 1987), reinforcement of the plant cell wall through synthesis of ligninlike material (Kolattukudy and Soliday 1985), and production of antimicrobial phytoalexins (Darvill and Albersheim 1984). These events are often part of a set of defense mechanisms referred to as the "hypersensitive response" and are generally accompanied by changes in specific mRNA accumulation and in gene transcription rates (Bell *et al.* 1986; Lawton and Lamb 1987).

Typical hypersensitive response symptoms appear after inoculation of potato leaves or tubers with an incompatible (avirulent) race of the late blight fungus *Phytophthora infestans* (Mont.) de Bary. While the inoculated tubers show rapid cell death at the site of infection, localized browning, increased lignification (Friend 1976), decreased steroid glycoalkaloid accumulation (Tjamos and Kuć 1982), and *de novo* synthesis of sesquiterpenoid phytoalexins (Kuć and Rush 1985), the inoculated leaves produce small necrotic lesions but do not accumulate phytoalexins (Rohwer *et al.* 1987). Among the various metabolic activities stimulated in potato during the defense response are that of enzymes required for phytoalexin synthesis (Coolbear and Threlfall 1985), chitinases and 1,3-β-glucanases (Kombrink *et al.* 1988), as well as phenylalanine ammonia-lyase and 4-coumarate:CoA ligase (Cuypers *et al.* 1988; Fritzemeier *et al.* 1987). The presence of an intact fungal system is not essential to the appearance of these defense reactions because the hypersensitive response can also be induced in potato tuber disks by treatment with cellfree homogenates of mycelium from *P. infestans*. Arachidonic and eicosapentaenoic acids, two 20-carbon polyunsaturated fatty acids present in these homogenates, were shown to act as elicitors (Bostock *et al.* 1981).

We have previously shown that differential accumulation of several mRNAs occurs during the hypersensitivelike response induced by arachidonic acid in potato tubers (Marineau *et al.* 1987). In this paper, we report the nucleotide sequence of a cDNA clone (pSTH-2) previously shown to correspond to mRNA sequences accumulating specifically in potato tubers following elicitation (Marineau *et al.* 1987). We also report the isolation and sequence of pSTH-21, a closely related cDNA clone. These two sequences show significant similarity with recently sequenced elicitor- and pathogen-induced PR (pathogenesis-related) protein cDNA clones from pea (Fristensky *et al.* 1988) and parsley (Somssich *et al.* 1988). We also describe the accumulation of mRNAs corresponding to pSTH-2 and pSTH-21 in different potato tissues and in tomato leaves following various treatments.

MATERIALS AND METHODS

Materials. Potato tubers (*Solanum tuberosum* L. cv. Kennebec) were obtained from the Quebec Ministry of Agriculture "Les Buissons" Research Station. Tubers were stored in the dark at 4° C and brought to room temperature 24 hr before use. Arachidonic acid ($20{:}4^{\Delta 5,8,11,14}$) and eicosapentaenoic acid ($20{:}5^{\Delta 5,8,11,14,17}$) were purchased from Sigma, St. Louis, MO. Restriction enzymes, linoleic acid ($18{:}2^{\Delta 9,12}$), and oligo(dT)-cellulose were from Pharmacia PL Biochemicals, Baie d'Urfée, Quebec, Canada. Nitrocellulose paper (BA85) was purchased from Schleicher and Schuell,

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Sequence data will appear in GenBank as accession numbers M25155 (pSTH-2) and M25156 (pSTH-21).

Keene, NH, and the Sequenase sequencing kit was purchased from U.S. Biochemical Corp., Cleveland, OH.

Elicitor treatments. Tubers were washed with distilled water and surface-sterilized in 70% ethanol for 5 min. Disks (2-mm thick and 25 mm in diameter) were cut from the medullary tissue using a sterile cork borer, washed several times with sterile water, and aged for 6 hr at 19° C in the dark. This aging period allows maximum formation of polysomes in the tuber cells (Sato *et al.* 1980). Treatments were performed by applying 75 μg of fatty acid (arachidonic, eicosapentaenoic, or linoleic acid) (1 μg/μl of emulsion in water) or 75 μl of sterile distilled water (control) to the upper surface of the disks. The treated disks were then incubated in the dark at 19° C for various periods of time.

Stem segments were prepared from 8-week-old plants in the following manner. Segments were cut (4 mm thick and 1 cm in diameter) from internodes and washed with sterile distilled water. The segments were then treated on their cut surfaces with 10 μg of arachidonic acid or with 10 μl of sterile water (control) and incubated in the dark at 19° C for 24 hr.

Homogenates from heat-killed *P. infestans* compatible race 1,2,3,4 were prepared as previously described (Lisker and Kuć 1977). Detached leaves from potato or tomato (*Lycopersicon esculentum* Mill.) plants were cut and floated

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                                                                                                  met
  1  ACACACCTCACAACATTCTCACACATAACATTTTGTATATCCTTTTTGTGTGTTCAATATCTCTAATAACATC                    ATG
                                                  CTT          C

     gly val thr ser tyr thr his glu thr thr thr pro ile ala pro thr arg leu phe lys
 77  GGT GTC ACT AGC TAT ACA CAT GAG ACC ACA ACA CCA ATT GCC CCT ACT AGG TTG TTC AAA
                               T                    G
                              leu                  val

     ala leu val val asp ser asp asn leu ile pro lys leu met pro gln val lys asn ile
137  GCT TTG GTT GTT GAT TCT GAC AAT CTT ATT CCT AAG TTG ATG CCA CAA GTT AAA AAT ATT
         C                                                                   G   C

     glu ala glu gly asp gly ser ile lys lys met asn phe val glu gly ser pro ile lys
197  GAG GCT GAG GGA GAT GGA AGC ATC AAA AAG ATG AAC TTT GTT GAA GGT TCA CCA ATC AAG
                                        G          C
                                                  thr

     tyr leu lys his lys ile his val val asp asp lys asn leu val thr lys tyr ser met
257  TAC TTG AAG CAC AAG ATT CAT GTT GTT GAT GAC AAG AAT TTG GTG ACC AAA TAT TCA ATG
                                                                 T

     ile glu gly asp val leu gly asp lys leu glu ser ile ser tyr asp leu lys phe glu
317  ATT GAA GGA GAT GTT CTT GGA GAC AAA CTT GAA TCC ATT TCC TAT GAT CTC AAA TTT GAA
                                              G

     ala his gly asn gly gly cys val cys lys ser ile thr glu tyr his thr lys gly asp
377  GCT CAT GGA AAT GGA GGA TGT GTT TGC AAG TCT ATA ACT GAG TAC CAC ACA AAA GGT GAT
                                                    G          T
                                                   ala

     tyr val leu lys asp glu glu his asn glu gly gln lys gln gly met glu leu phe lys
437  TAT GTG TTG AAG GAT GAA GAA CAC AAT GAA GGC CAA AAA CAA GGC ATG GAA CTT TTC AAG
                               T                    A
                              asp                  lys

     ile val glu ala tyr leu leu ala asn pro ser val tyr ala ter
497  ATT GTT GAA GCA TAC CTC CTC GCC AAT CCT TCT GTC TAC GCT TAA GTGATGAAAAAAGAATCAGG

562  CCCACACTTGAAATATAACGTGTTGTGACATTATATAATAA----------AGTATACTCTCATTAAAAAGTTTAATC
     T T    G               T              TTAAGAAAATAA    GG    A           -

642  TTTTAAATGAGAT--------GATGGTTTGAGTTTCCATTAATGTTATAGGTCTTTTTGTTGAGTTGTGTGTTCTTTTTC
               ATCATTCT TA           T    ---   T   TGT    C       -

722  CAAGTTTATCATGGGAAGAACTCTTAATAATGTAAGCAACCTTAAATTTTGCTTATCAAATACTTATATTAAAAA-ATGT
                                     -        T                                A     T

802  ATTACTTTTCG-poly(A)
     T
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Fig. 1. Nucleotide sequences of the pSTH-2 and pSTH-21 cDNA clones and the deduced amino acid sequences. Sequences of pSTH-21 that are identical to pSTH-2 are not shown. The dot indicates the first nucleotide in the sequence of pSTH-21.

for 72 hr either on a 10-fold dilution of homogenate solution or on water (control).

Anaerobic treatments were performed on stem segments or tuber disks that were placed in containers flushed with a constant flow of nitrogen, or with tissues that were submerged in 20 mM sodium phosphate, pH 7.0, containing 50 μg/ml of chloramphenicol (Logemann *et al.* 1988).

cDNA cloning and RNA blot hybridization. Polyribosomal and total RNAs were obtained from potato tissues as described previously (Marineau *et al.* 1987; Jones *et al.* 1985). Poly(A)$^+$ mRNAs from 72-hr arachidonic acid-treated tuber tissue were isolated by chromatography on oligo(dT)-cellulose (Aviv and Leder 1972), and double-stranded cDNAs were prepared according to the procedure of Gubler and Hoffman (1983). Following ligation with *Eco*RI linkers, the cDNA was inserted into the *Eco*RI site of the expression vector lambda ZAP (Stratagene, San Diego, CA) (Short *et al.* 1988). A library containing 8×10^5 recombinant phages was obtained and amplified, after which 1×10^5 plaques were screened using the pSTH-2 cDNA insert as a probe. The M13K07 f1 helper phage was used to rescue plasmids (pBluescript) from phages giving a positive signal after hybridization (Short *et al.* 1988).

RNA blot hybridization was performed using nitrocellulose membranes as described previously using a [^{32}P]-labeled pSTH-2 cDNA insert (specific activity $\approx 5 \times 10^8$ cpm/μg) (Marineau *et al.* 1987). Final washes were performed at 50° C for 20 min in 0.1× SSC (15 mM NaCl, 1.5 mM sodium citrate, pH 7.0). Autoradiograms were optically scanned using an LKB 2002 Ultrascan laser densitometer.

DNA sequencing. The 801-bp cDNA insert from pSTH-2 was subcloned in pUC18, and the nucleotide sequence was determined on both strands using the chemical cleavage procedure of Maxam and Gilbert (1980). The 773-bp cDNA insert from pSTH-21 was sequenced on both strands by the dideoxy chain termination method (Sanger *et al.* 1977).

RESULTS

Sequence analysis. The lambda ZAP library was screened using the cDNA insert of pSTH-2 (Marineau *et al.* 1987) as a probe. Approximately 1% of all clones present in the library contained sequences homologous to the pSTH-2 cDNA insert. Seven of the positive clones were selected and sequenced. They were of two types: four had sequences identical to pSTH-2; the sequences of the others differed slightly. The nucleotide and predicted amino acid sequences of pSTH-2, together with the sequences of one of the latter three clones (pSTH-21), are shown in Figure 1. The cDNA inserts contain 801 and 773 bp, respectively, and their nucleotide sequences are 88% identical. The sequence environment near the methionine initiation codon is the same for both clones (CATCAUGG) and closely matches the consensus sequence for the eucaryotic gene initiation codon (CACCAUGG) (Kozak 1984). The 3′-noncoding region has 250 nucleotides in pSTH-2 and 265 nucleotides in pSTH-21. As is the case for many plant genes (Hunt *et al.* 1987), no AAUAAA consensus polyadenylation signal sequence can be found in the vicinity of the poly(A) tail of either clone.

Both pSTH-2 and pSTH-21 cDNA inserts contain an open-reading frame coding for a polypeptide of 155 amino acids. The amino acid sequences of these polypeptides are very similar (96% amino acid identity), differing in only six amino acids. The calculated molecular masses of the polypeptides (17,275 Da for pSTH-2 and 17,180 Da for pSTH-21) are consistent with the size estimated for the pSTH-2-selected mRNA *in vitro* translation products (molecular mass $\approx$ 17,000 Da) (Marineau *et al.* 1987). The predicted positive charge is the same (pI 6.1) for the polypeptides from pSTH-2 and pSTH-21.

The upper section of Figure 2 shows the hydropathic profile of the polypeptides encoded by pSTH-2 and pSTH-

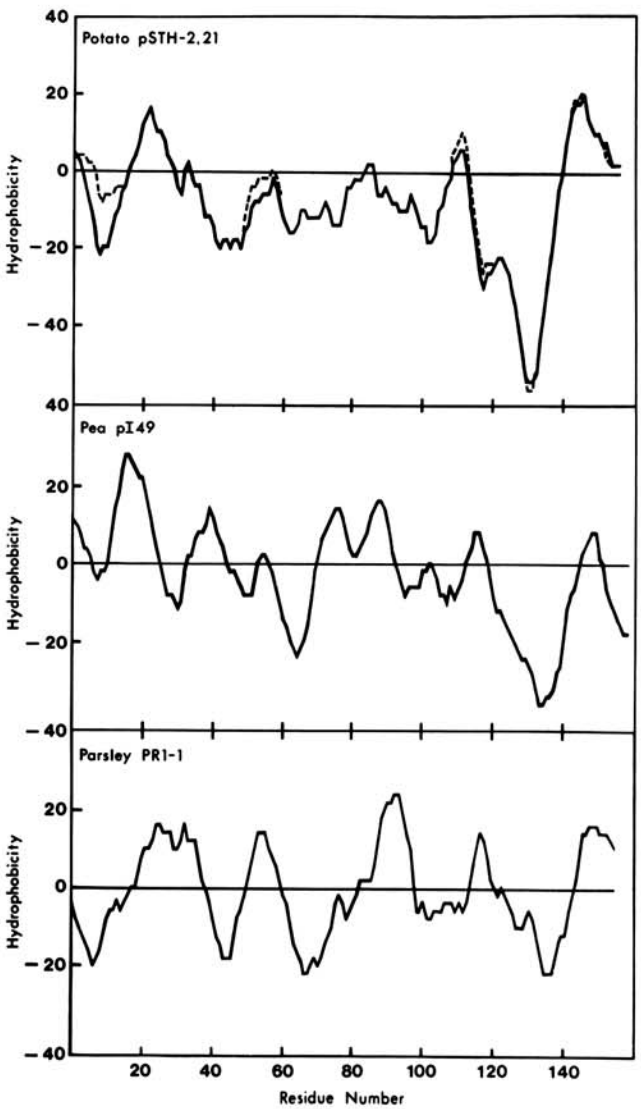

Fig. 2. Hydropathic profiles. Hydropathic profiles of the encoded polypeptides for pSTH-2 (upper section, uninterrupted line), pSTH-21 (upper section, dashed line), pea cDNA clone pI49 (middle section), and parsley PR1-1 cDNA clone (lower section). Each value was calculated as the average hydropathic index of a sequence of nine amino acids and plotted to the middle residue of each sequence (Kyte and Doolittle 1982). Positive and negative values indicate hydrophobic and hydrophilic regions of the protein, respectively.

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PR1-1   MGVQKSEVETTSSVSAEKLFKGLCLDIDTLLPQVLPGAIK  40
pSTH-21 MGVTSYTLETTTPVAPTRLFKALVVDSDNLIPKLMP-QVK  39
pI49    MGVFNVEDEITSVVAPAILYKALVTDADNLTPKVI D-AIK  39

PR1-1   SSE----TLEGDGGVGTVKLVHLGDASPFKTMKQKVDAID  76
pSTH-21 NIE----A-EGD---GSIKKMTFVEGSPIKYLKHKIHVVD  71
pI49    SIEIVEGN-GGA---GTIKKLTFVEDGETKHVLHKVELVD  75

PR1-1   KATFTYSYSIIDGDILLGFIESINNHFTAVPNADGGCTVK 116
pSTH-21 DKNLVTKYSMIEGDVLGDKLESISYDLKFEAHGNGGGCVCK 111
pI49    VANLAYNYSIVGGVGFPDTVEKISFEAKLSAGPNGGSIAK 115

PR1-1   STIIFNTKGDAVVPEENIKFANDQNLTIFKAVEAYLIAN* 155
pSTH-21 SIAEYHTKGDYVLKDEDHNEGKKQGMELFKIVEAYLLANP 151
pI49    LSVKYFTKGDAAPSEEQLKTDKAKGDGLFKALEGYCLAHP 155

pSTH-21 SVYA* 155
pI49    D-YN* 158
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Fig. 3. Amino acid sequence alignments. Protein sequences corresponding to the parsley PR1-1 cDNA clone (Somssich *et al.* 1988), potato pSTH-21 cDNA clone, and pea pI49 cDNA clone (Fristensky *et al.* 1988) are represented. Boxes show residues common to at least two sequences.

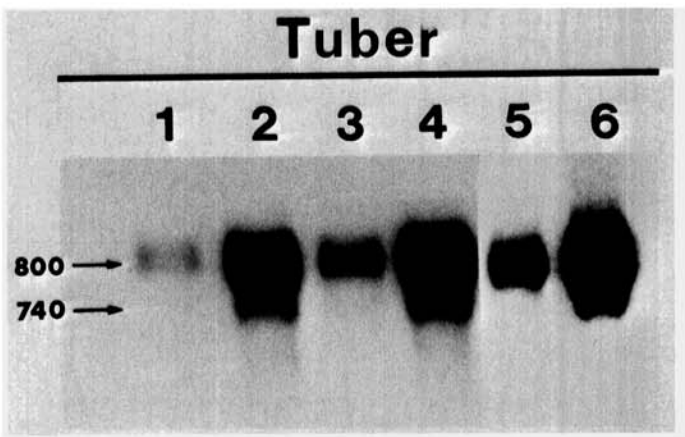

Fig. 4. Accumulation of pSTH-2 transcripts in potato tubers following elicitor treatment. Poly(A)$^+$ mRNAs were extracted from *Solanum tuberosum* tubers after a 72-hr treatment with water (lane 1), arachidonic acid (lane 2), linoleic acid (lane 3), or eicosapentaenoic acid (lane 4). RNAs were also extracted from *S. chacoense* tubers treated for 24 hr with water (lane 5) or arachidonic acid (lane 6). Each lane contained 1 μg of poly(A)$^+$ mRNAs. Arrows indicate mRNA species of different sizes.

21. Except for the N-terminal region, the profiles are nearly identical. No structural features suggest the presence of a signal peptide or membrane-anchoring domain in these proteins. Because there is no evidence for precursor processing in *in vitro* translations of pSTH-2-selected mRNAs by canine pancreatic microsomes (not shown), a cytoplasmic localization is likely for both of these polypeptides.

Sequence comparison. Comparison of the pSTH-2 and pSTH-21 cDNA nucleotide and deduced amino acid sequences with those of other PR proteins revealed a strong similarity to the parsley PR1-1 and PR1-3 sequences (Somssich *et al.* 1988), as well as to pathogen- and elicitor-induced sequences (pI49 and pI176) from pea (Fristensky *et al.* 1988). Figure 3 represents an amino acid sequence comparison of pSTH-21, PR1-1, and pI49. Only one clone from each species is presented because of the very high similarity between clones obtained from any one of the species. Areas of amino acid identity extend throughout the proteins and are not restricted to any particular region. Percent identities in these sequences range from 41 to 43% between potato and pea PR polypeptides, 36 to 38% between potato and parsley PR polypeptides, and 37 to 38% between pea and parsley. No other sequence similarity was found with other PR proteins and mRNAs, including tobacco PR-1a, 1b, and 1c (Cornelissen *et al.* 1986) and potato wound-induced genes *win1* and *win2* (Stanford *et al.* 1989).

Another wound-inducible gene from potato (*wun1*, Logemann *et al.* 1988) has been characterized recently. This gene has a transcript size and wound-inducible kinetics similar to those of pSTH-2. However, it is unlikely that *wun1* and pSTH-2 represent the same gene because the coding region of *wun1* (318 bp, Logemann *et al.* 1989) is smaller than the coding region of pSTH-2 (455 bp).

Structural analogy of the potato, pea, and parsley PR polypeptides is also evident when comparing their hydropathic profiles (Fig. 2). Alignment of the hydrophobic and hydrophilic regions shows a higher similarity in the N- and C-termini of the proteins than is observed between residues 25 and 80.

Accumulation of mRNAs corresponding to pSTH-2 in tubers. The accumulation of pSTH-2-related mRNAs in *S. tuberosum* tuber tissue was monitored after wounding and treatment with several elicitors (Fig. 4). At least two RNA species hybridizing to pSTH-2 (Fig. 4, arrows, 800 and 740 nucleotides) accumulate to high levels in disks after a 72-hr treatment with arachidonic or eicosapentaenoic acid (Fig. 4, lanes 2 and 4, respectively). The control disks also show a small accumulation of transcripts in response to slicing (Fig. 4, lane 1; see also Fig. 5); linoleic acid (Fig. 4, lane 3) has only a slightly larger effect on the accumulation of the mRNAs. Wounding and treatment with arachidonic acid also induce transcript accumulation

in tuber disks of *S. chacoense* Bitt., a tuber-bearing diploid potato (Fig. 4, lanes 5 and 6, respectively).

A time-course comparison of the levels of pSTH-2-related poly(A)$^+$ mRNA accumulation in control tissues and tissues treated with arachidonic acid was conducted (Fig. 5, upper section). No pSTH-2 transcripts can be detected in freshly cut tubers (Fig. 5, upper section, UN) or in untreated disks 6 hr after cutting (Fig. 5, upper section, time 0). Disks were treated with elicitor after this 6-hr aging period. The mRNAs become detectable 2 hr after water (control) or arachidonic acid treatment. The accumulation of pSTH-2-related mRNAs is identical in control tissues and in tissues treated with arachidonic acid for up to 8 hr after treatment, after which time it declines in control tissues (Fig. 5, upper and lower sections) and continues to increase in elicitor-treated material until 24 hr after treatment. mRNAs remained at this level after 48 (not shown) and 72 hr.

Accumulation of pSTH-2-related mRNAs in stems and leaves. No pSTH-2-related mRNAs can be detected in freshly cut stems (Fig. 6, lane 1) and leaves (not shown). Figure 6, lane 2, shows the level of pSTH-2-related mRNA in stems 24 hr after wounding. In leaves, no mRNA can be detected after the same period (not shown) or 72 hr after wounding (Fig. 6, lane 4). Arachidonic acid has no detectable effect in either tissue (not shown). However, transcripts hybridizing to pSTH-2 accumulate in potato and tomato leaves following treatment with a homogenate from *P. infestans* mycelium (Fig. 6, lanes 5 and 6, respectively).

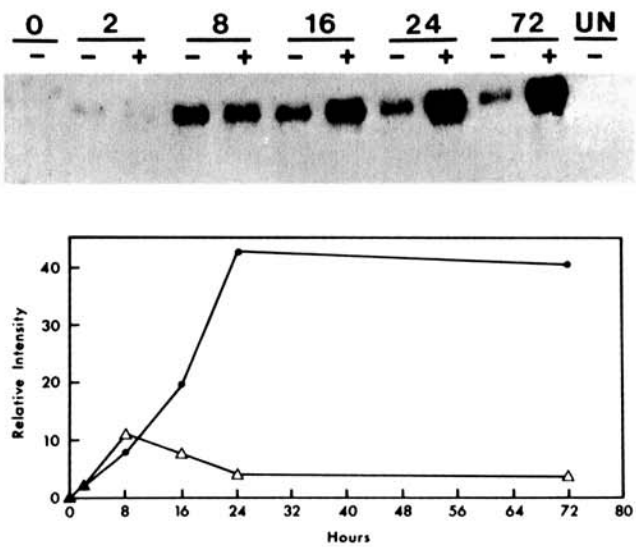

Fig. 5. Time course of pSTH-2-related mRNA accumulation. Upper section: elicitor- and water-treated potato tuber disks. Poly(A)$^+$ mRNAs were extracted from tuber disks treated with water (−) or arachidonic acid (+) for various periods of time. Numbers represent hours after the beginning of treatment. All tissues were aged for 6 hr in the dark before treatment, except for freshly cut tubers (UN), which were frozen immediately after cutting. Each lane contained 2 μg of poly(A)$^+$ mRNAs. Lower section: time course of pSTH-2-related mRNA accumulation expressed as the relative intensity of the hybridization signals shown in the upper section of this figure. Full circles: elicitor-treated samples. Open triangles: control samples. Arrows indicate mRNA species of different size.

Effect of anaerobiosis on the accumulation of pSTH-2-related mRNAs. Anaerobiosis is known to have a dramatic effect on gene activity and protein synthesis in plants (Sachs *et al.* 1980). pSTH-2 transcript accumulation was inhibited in both control tuber disks and disks treated with arachidonic acid under anaerobic conditions (Fig. 7, lanes 2 and 3, respectively). However, the mRNA steady-state level remained approximately the same if the tuber disks were treated for 24 hr with the elicitor before anaerobic treatment (Fig. 7, lane 4). Inhibition of transcript accumulation was also observed in stem segments during anaerobiosis (Fig. 5, lane 3).

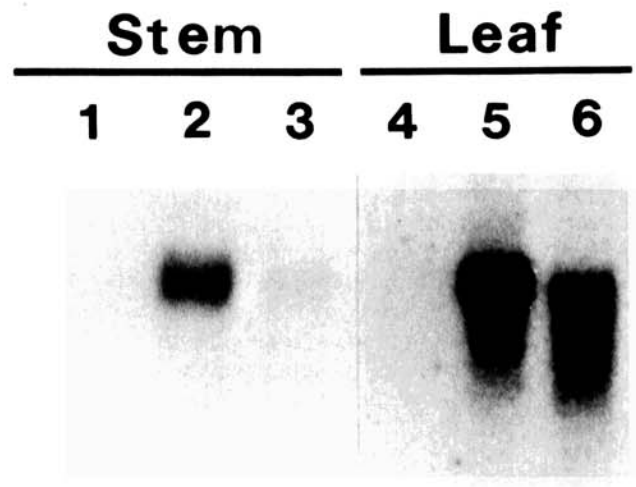

Fig. 6. Accumulation of pSTH-2 transcripts in stems and leaves. mRNAs were extracted from fresh potato stems (lane 1), from stem segments 24 hr after slicing (lane 2), stem segments incubated for 24 hr under anaerobic conditions (lane 3), potato leaves treated for 72 hr with water (lane 4), and potato and tomato leaves treated for 72 hr with a fungal homogenate of *Phytophthora infestans* (lanes 5 and 6, respectively). Each lane contained 1 μg of poly(A)$^+$ mRNAs (stems) and 5 μg of poly(A)$^+$ mRNAs (leaves).

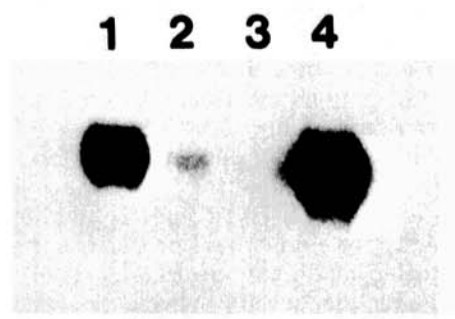

Fig. 7. Effect of anaerobiosis on the fate of pSTH-2 transcripts. Poly(A)$^+$ mRNAs were extracted from tuber disks treated for 24 hr with arachidonic acid (lane 1), placed under anaerobic conditions (N_2) for 24 hr with (lane 3) or without (lane 2) arachidonic acid treatment, or incubated under anaerobic conditions (24 hr) after a previous 24-hr aerobic treatment with arachidonic acid (lane 4).

DISCUSSION

The high sequence similarity that exists between the two potato PR cDNA clones (88% nucleotide identities) and the fact that the deduced amino acid sequences are not identical suggest that the corresponding mRNAs are transcribed from two nonallelic members of a gene family. This is consistent with our previous results indicating that several distinct polypeptides are obtained when mRNAs selected from elicitor-treated tissues by hybridization with pSTH-2 are translated *in vitro* (Marineau *et al.* 1987).

The high proportion (~1%) of pSTH-2-hybridizing cDNA clones present in the library is probably representative of the abundance of pSTH-2-related mRNAs in elicitor-treated tuber cells. These transcripts accumulate rapidly following wounding or treatment with the elicitor (Fig. 5). Rapid transcript accumulation appears to be important in the defense response of plants and has been reported in other systems following wounding, elicitor treatment, or attempted infection by a pathogen (Bell *et al.* 1986; Lawton and Lamb 1987).

Induction of pSTH-2 transcript accumulation in tuber disks after treatment with arachidonic or eicosapentaenoic acid (Fig. 4) is consistent with the elicitor activity reported for these molecules in the mycelium of *P. infestans* (Bostock *et al.* 1981). Linoleic acid, an 18-carbon unsaturated fatty acid which does not induce phytoalexin synthesis in potato, did not cause a significantly higher transcript accumulation than that caused by slicing after 72 hr. It is possible, however, that the kinetics of mRNA accumulation following treatment with linoleic and eicosapentaenoic acids differ from that shown for arachidonic acid treatment (Fig. 5). While arachidonic acid had little effect on the expression of the pSTH-2-related genes in stems and leaves of potato, a homogenate from *P. infestans* mycelium had significant eliciting activity in leaves. This result can be explained by assuming an inefficient penetration of the fatty acid into the tissue or a differential sensitivity to the elicitor by various potato tissues, or even by assuming the presence of additional factors in the homogenate that enhance the activity of the fatty acid. It is indeed known that some compounds present in mycelium homogenates (among which are various glucans) are inactive themselves as elicitors of phytoalexin accumulation in potato tuber disks but can increase the eliciting activity of arachidonic acid, or even that of other unsaturated fatty acids with little elicitor activity of their own (Preisig and Kuć 1985).

Anaerobiosis inhibits the accumulation of pSTH-2 and pSTH-21 transcripts induced by wounding or treatment with arachidonic acid. A similar result has also been reported during wounding for the potato *wun1* gene (Logemann *et al.* 1988). This could result from a general inhibition of the protein synthesis processes normally maintained in aerobic conditions (Sachs *et al.* 1980). However, it is also possible that specific enzyme-mediated oxidative metabolic processes (Preisig and Kuć 1987) are involved in the induction of pSTH-2-related mRNA accumulation. Finally, the observation that the steady-state level of pSTH-2-related mRNAs remains unchanged if the elicitor is applied 24 hr before placing the disks under anaerobic conditions (Fig. 6) indicates that the effect of anaerobiosis on pSTH-2-related mRNAs is not mediated by a change in the stability of these mRNAs.

The amino acid sequences deduced from pSTH-2 and pSTH-21 show a strong similarity to PR protein sequences from parsley and pea. Furthermore, the corresponding PR genes are all activated by pathogen or elicitor stress. Parsley PR1-1 mRNAs are synthesized rapidly in elicitor-treated cell suspension cultures (Somssich *et al.* 1986) and also accumulate at sites of fungal infection in parsley leaves (Somssich *et al.* 1988). Similarly, mRNAs corresponding to the pea pI49 and pI176 cDNA clones accumulate rapidly following elicitor treatment or inoculation of pea pods with a pathogen (Fristensky *et al.* 1985; Riggleman *et al.* 1985). The occurrence of homologous genes expressed under pathogen or elicitor stress in three different plant families suggests that these genes are evolutionarily related and that they probably share a common ancestor. It also suggests an important role in the plant defense response against pathogens. Currently, however, the function of these genes is obscure because comparison of their sequences with data banks indicates that they do not belong to any previously known class of proteins. It is also very interesting to note that the defense-related proteins described in this paper and those recently characterized in parsley and pea apparently belong to a new class of PR proteins because they do not show sequence similarities to any of the previously characterized PR proteins (Van Loon 1985). Isolation of the proteins encoded by these genes and characterization of their structure and function will be conducted to clarify their role during the defense response in plants.

NOTE ADDED IN PROOF

The pSTH-2 and pSTH-21 cDNA deduced amino acid sequences were found to have a strong similarity (46% amino acid identity) with the recently published sequence of the major birch pollen allergen *Betv1* (Breiteneder *et al.* 1989).

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LITERATURE CITED

Aviv, H., and Leder, P. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA 69:1408-1412.

Bell, J. N., Ryder, T. B., Wingate, V. P. M., Bailey, J. A., and Lamb, C. J. 1986. Differential accumulation of plant defense gene transcripts in a compatible and an incompatible plant-pathogen interaction. Mol. Cell. Biol. 6:1615-1623.

Bostock, R. M., Kuć, J., and Laine, R. A. 1981. Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in the potato. Science 212:67-69.

Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., and Breitenbach, M. 1989. The gene coding for the major birch pollen allergen *Betv1* is highly homologous to a pea disease resistance gene. EMBO J. 8:1935-1938.

Collinge, D. B., and Slusarenko, A. J. 1987. Plant gene expression in response to pathogens. Plant Mol. Biol. 9:389-410.

Coolbear, T., and Threlfall, D. R. 1985. A comparison of the sites of

phytoalexin accumulation and of biosynthetic activity in potato tuber tissue inoculated with biotic elicitors. Phytochemistry 24:2219-2224.
Cornelissen, B. J. C., Hooft van Huijsduijnen, R. A. M. H., Van Loon, L. C., and Bol, J. F. 1986. Molecular characterization of messenger RNAs for "pathogenesis-related" proteins 1a, 1b and 1c, induced by TMV infection of tobacco. EMBO J. 5:37-40.
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.
Darvill, A. G., and Albersheim, P. 1984. Phytoalexins and their elicitors - a defense against microbial infection in plants. Annu. Rev. Plant Physiol. 35:243-275.
Dixon, R. A., Bailey, J. A., Bell, J. N., Bolwell, G. P., Cramer, C. L., Edwards, K., Hamdan, M. A. M. S., Lamb, C. J., Robbins, M. P., Ryder, T. B., and Schuch, W. 1986. Rapid changes in gene expression in response to microbial elicitation. Philos. Trans. R. Soc. London B314:411-426.
Friend, J. 1976. Lignification in infected tissue. Pages 291-303 in: Biochemical Aspects of Plant-Parasite Relationships. J. Friend and D. R. Threlfall, eds. Academic Press, London.
Fristensky, B., Horovitz, D., and Hadwiger, L. A. 1988. cDNA sequences for pea disease resistance response genes. Plant Mol. Biol. 11:713-715.
Fristensky, B., Riggleman, R. C., Wagoner, W., and Hadwiger, L. A. 1985. Gene expression in susceptible and disease resistant interactions of peas involved with *Fusarium solani* pathogens and chitosan. Physiol. Plant Pathol. 27:15-28.
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.
Gubler, U., and Hoffman, B. J. 1983. A simple and very efficient method for generating cDNA libraries. Gene 25:263-269.
Hunt, A. G., Chu, N. M., Odell, J. T., Nagy, F., and Chua, N.-H. 1987. Plant cells do not properly recognize animal gene polyadenylation signals. Plant Mol. Biol. 8:23-35.
Jones, J. D. G., Dunsmuir, P., and Bedbrook, J. 1985. High level expression of introduced chimeric genes in regenerated transformed plants. EMBO J. 4:2411-2418.
Kolattukudy, P. E., and Soliday, C. L. 1985. Effects of stress on the defensive barriers of plants. Pages 381-400 in: Cellular and Molecular Biology of Plant Stress. J. L. Key and T. Kosuge, eds. Alan R. Liss, New York.
Kombrink, E., Schroder, M., and Hahlbrock, K. 1988. Several "pathogenesis-related" proteins in potato are 1,3-beta-glucanase and chitinase. Proc. Natl. Acad. Sci. USA 85:782-786.
Kozak, M. 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucleic Acids Res. 12:857-872.
Kuć, J., and Rush, J. S. 1985. Phytoalexins. Arch. Biochem. Biophys. 236:455-472.
Kyte, J., and Doolittle, R. F. 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-132.
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.
Lisker, N., and Kuć, J. 1977. Elicitors of terpenoid accumulation in potato tuber slices. Phytopathology 67:1356-1359.
Logemann, J., Mayer, J. E., Schell, J., and Willmitzer, L. 1988. Differential expression of genes in potato tubers after wounding. Proc. Natl. Acad. Sci. USA 85:1136-1140.
Logemann, J., Lipphardt, S., Lorz, H., Hauser, I., Willmitzer, L., and Schell, J. 1989. 5′-upstream sequences from the *wun1* gene are responsible for gene activation by wounding in transgenic plants. Plant Cell 1:151-158.
Marineau, C., Matton, D. P., and Brisson, N. 1987. Differential accumulation of potato tuber mRNAs during the hypersensitive response induced by arachidonic acid elicitor. Plant Mol. Biol. 9:335-342.
Maxam, A. M., and Gilbert, W. 1980. Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. 65:499-560.
Preisig, C. L., and Kuć, J. A. 1985. Arachidonic acid-related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans* (Mont.) de Bary. Arch. Biochem. Biophys. 236:379-389.
Preisig, C. L., and Kuć, J. A. 1987. Inhibition by salicylhydroxamic acid, BW755C, eicosatetraynoic acid and disulfiram of hypersensitive resistance elicited by arachidonic acid or poly-L-lysine in potato tuber. Plant Physiol. 84:891-894.
Riggleman, R. C., Fristensky, B., and Hadwiger, L. A. 1985. The disease resistance response in pea is associated with increased levels of specific mRNAs. Plant Mol. Biol. 4:81-86.
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.
Sachs, M. M., Freeling, M., and Okimoto, R. 1980. The anaerobic proteins of maize. Cell 20:761-767.
Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.
Sato, T., Ishizuka, M., Watanabe, A., Imaseki, H. 1980. The synthesis and properties of polysomal RNA in potato tuber slices during the early stage of aging. Plant Cell Physiol. 21:137-147.
Short, J. M., Fernandez, J. M., Sorge, J. A., and Musc, D. 1988. ZAP: A bacteriophage expression vector with *in vivo* excision properties. Nucleic Acids Res. 16:7583-7600.
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.
Stanford, A., Bevan, M., and Northcote, D. 1989. Differential expression within a family of novel wound-induced genes in potato. Mol. Gen. Genet. 215:200-208.
Tjamos, E. C., and Kuć, J. 1982. Inhibition of steroid glycoalkaloid accumulation by arachidonic and eicosapentaenoic acids in potato. Science 217:542-544.
Van Loon, L. C. 1985. Pathogenesis-related proteins. Plant Mol. Biol. 4:111-116.