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Update section

Short communication

Isolation of putative defense-related genes from *Arabidopsis thaliana* and expression in fungal elicitor-treated cells

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Abstract

Numerous *Arabidopsis* genes have been cloned that correspond to putative pathogen defense-related genes identified in parsley (*Petroselinum crispum*). Treatment of *Arabidopsis* cells with fungal elicitor leads to rapid accumulation of the respective mRNAs with time courses comparable to those observed for their counterparts in parsley. Evolutionary sequence conservation of many of these genes in several plant species suggests they code for important plant functions.

Plants respond to pathogen attack with rapid activation of a variety of defense reactions. Many of these responses, such as the accumulation of phytoalexins and pathogenesis-related proteins, also the deposition of phenolics and specific proteins in the cell wall are initiated by gene activation [1, 2]. Model systems employing cultured plant cells and elicitor preparations from pathogens have proved extremely useful in the identification and isolation of putative plant defense-related genes [3].

We have previously described numerous parsley cDNAs, designated PR (pathogenesis-related) and ELI (elicitor-activated) representing genes whose transcriptional activation is triggered rapidly by treatment of cultured parsley cells with a fungal elicitor derived from the soybean pathogen *Phytophthora megasperma* f. sp. *glycinea* (*Pmg* [4]). Expression of a few selected representatives of these genes was also found to occur in fungus-infected parsley leaves [5].

Identification of some of the parsley ELI products was achieved by sequencing of the cDNAs and comparison of the deduced proteins with sequences in the database, by hybridization of the cDNAs to heterologous probes encoding products of known functions, and in some instances by expression in *Escherichia coli* (see Table 1). However, many of the functions encoded by the parsley *eli* genes remain to be elucidated.

Assuming that the products of at least some of these genes fulfil important functions in plant defense we checked for evolutionary conservation in other plant species. Nuclear DNA was isolated from alfalfa (*Medicago sativa*), potato (*Solanum tuberosum*) and *Arabidopsis thaliana* [6], digested with various restriction endonucleases, electrophoresed on horizontal agarose gels and trans-

Table 1. Summary of the parsley cDNAs for which corresponding genes in *Arabidopsis thaliana* were detected and subsequently cloned. References refer to the cloning or to the functional identification of the parsley cDNAs.

Parsley cDNA	Function	Genomic cross-hybridization to *Arabidopsis*	*Arabidopsis* gene(s) isolated	Ref.
PR1		–	no	8
PR2		–	no	8
ELI 3		+	yes	4
ELI 4	phenylalanine ammonia-lyase (PAL)	+	yes	7
ELI 5	tyrosine decarboxylase (TyrDC)	+	yes	4
ELI 6		+	yes	4
ELI 7		+	yes	4
ELI 8		+	yes	4
ELI 9	hydroxyproline-rich glycoprotein (HRGP)	+	yes	9
ELI 10		+	yes	4
ELI 11	anionic peroxidase	+	yes	9
ELI 12		+	yes	4
ELI 13		+	yes	4
ELI 14	S-adenosyl-L-homocysteine hydrolase (SHH)	+	yes	10
ELI 15		nt	–	4
ELI 16		+	no	4
ELI 17		–	no	4
ELI 18	*S*-adenosyl-*L*-methionine synthetase 1 (SMS1)	+	yes	10
ELI 19	*S*-adenosyl-*L*-methionine synthetase 2 (SMS2)	nt	–	10
4CL	4-coumarate:CoA ligase (4CL)	+	yes	11
CHS	chalcone synthase (CHS)	+	yes	12

nt = not tested.

ferred to nylon membranes. These genomic DNA blot filters were hybridized with the radiolabelled parsley cDNA probes in 1 M NaCl, 10% dextran sulfate, 1% SDS solution at 50 °C and then washed under low (6 × SSC, 0.5% SDS, 60 °C) moderate (2 × SSC, 0.5% SDS, 60 °C) and high (0.5 × SSC, 0.5% SDS, 65 °C) stringency conditions. Sixteen out of 19 tested probes gave positive autoradiographic signals of diverse complexity at moderate stringency washing conditions indicating the presence of related sequences in all three plants tested. Table 1 shows the results obtained only with *Arabidopsis* DNA; essentially the same results were obtained with alfalfa and potato.

Only the parsley ELI 4 and CHS probes gave autoradiographic signals upon washing of the filters at high-stringency conditions. Therefore, assuming that the probes had an average G + C nucleotide content of 45–50%, the sequences detected by most of them should share ca. 70–80% identity over long stretches of the sequence and even more for ELI 4 and CHS. For CHS this was expected, as the *Arabidopsis chs* gene has previously been shown to be more than 85% homologous to that of parsley [13].

Arabidopsis combines numerous properties that have made it an excellent organism for various molecular genetic studies. Several groups have recently shown that *Arabidopsis* may also be well suited as a model system to study plant-pathogen interactions [14, 15, 16]. Therefore, we cloned the counterpart genes from *Arabidopsis*, as elucidation of the functions encoded by these genes should be facilitated using this plant species.

All parsley cDNAs that gave positive signals on DNA blots were used to screen an EMBL4 genomic library of *Arabidopsis thaliana* ecotype Columbia (constructed and kindly provided by Dr Csaba Koncz, MPI, Köln, FRG), using the moderate-stringency hybridization conditions described above. Table 1 summarizes the probes for

which we were able to identify and plaque-purify phage containing genomic *Arabidopsis* DNA counterparts. One set of additional genomic *Arabidopsis* clones (At-*ap3*) was isolated using a potato cDNA encoding a highly anionic peroxidase ([17]; kindly provided by Dr P.E. Kolattukudy, Ohio State University). These phage clones showed no cross-hybridization to those isolated with the parsley ELI 11 cDNA, despite the fact that this cDNA also encodes an anionic peroxidase (Table 1). The sizes of the genomic DNA fragments contained in the EMBL4 phages were 12–18 kb.

Identification and subcloning of the regions corresponding to the parsley cDNAs were achieved using combinations of restriction enzymes for digestion followed by separation of the restriction fragments by electrophoresis, transfer to nylon membranes and hybridization with the parsley probes. To verify that the correct fragments were subsequently subcloned into the vector (pUC19), the subcloned *Arabidopsis* DNAs were radiolabelled and used as probes in rehybridization experiments to their respective filter-bound parsley cDNAs. In addition, these *Arabidopsis* fragments were used as probes for hybridizations to restriction-fragmented genomic *Arabidopsis* DNA under high-stringency conditions to estimate the genome complexity (Fig. 1). For the gene families At-*eli* 5, 14, 18 and *4cl* simple patterns were observed whereas At-*eli* 11 detected rather complex patterns. The degree of complexity for the other gene families varied greatly and ranged from low (At-*eli* 3, 4, 12, 16 and *chs*) to very high (At-*eli* 6, 8, 9, 10, 12) (results not shown).

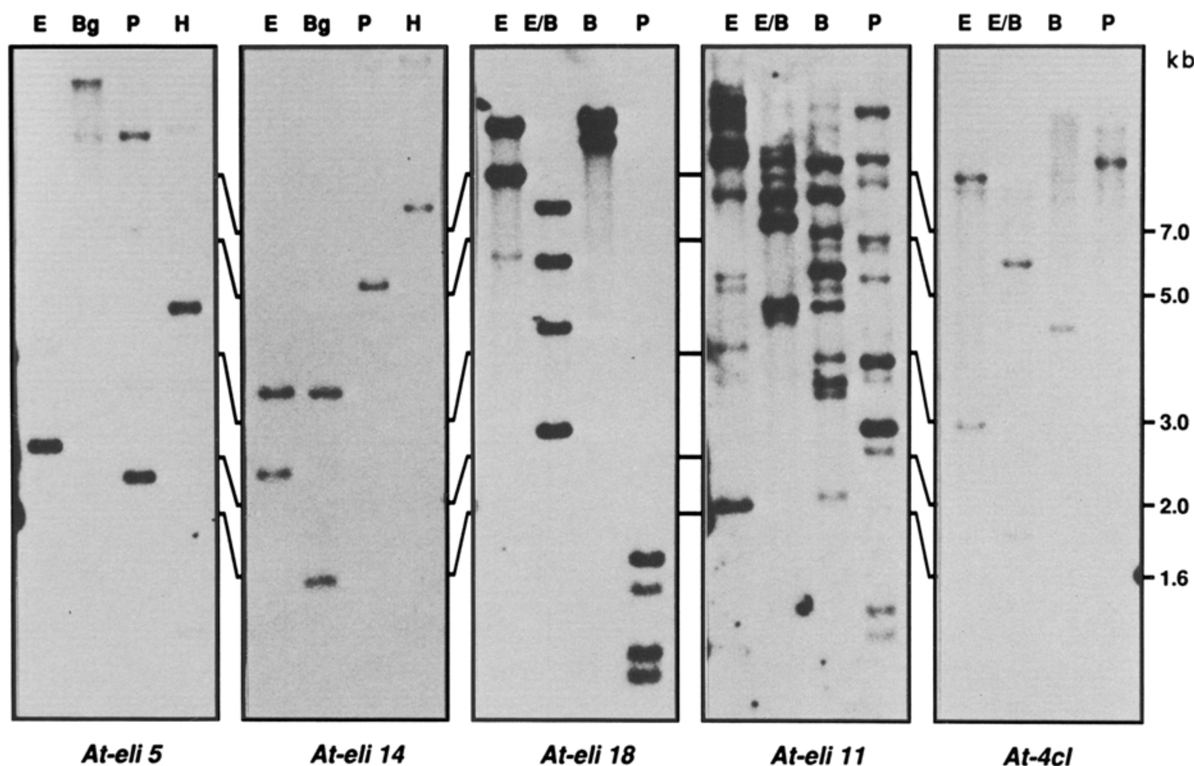

Fig. 1. Examples of varying complexities observed for the isolated *Arabidopsis* genes within the *Arabidopsis* genome detected by DNA blot hybridization. Genomic DNA was isolated from *Arabidopsis* leaves, digested with the indicated restriction enzymes and electrophoresed (3 μg per lane) on 0.8% horizontal native agarose gels. The DNA was transferred to nylon membranes, hybridized to the indicated radiolabelled *Arabidopsis* subcloned genomic fragments and the filter washed under high-stringency conditions as described in the text prior to autoradiography. B, *Bam* HI; Bg, *Bgl* II; E, *Eco* RI; H, *Hin*d III; P, *Pst* I.

To compare the expression behavior of the *Arabidopsis* genes and their parsley counterparts, an *Arabidopsis* cell suspension culture (initiated by Dr D. Scheel, MPI, Köln, FRG, starting from surface-sterilized seeds of *A. thaliana* ecotype Columbia) was treated with 50 μg/ml of *Pmg* elicitor for various periods of time. Total RNA was then extracted and enriched for poly(A)$^+$ RNA. The RNA was run on denaturing agarose gels, transferred to nylon membranes and hybridized with the respective radiolabelled *Arabidopsis* subclones under high-stringency conditions (Fig. 2). Elicitation of the cells resulted in a strong and rapid increase in the mRNA levels detected by the probes. In most cases, the timing of accumulation was very similar to that found in *Pmg* elicitor-treated parsley cells [4]. However, we also noted some differences. No expression was detected with the At-*eli* 6 probe (results not shown). Furthermore, greatly elevated or constant mRNA levels were observed for At-*eli* 7, 8 and 11 in the *Arabidopsis* cells but not for their

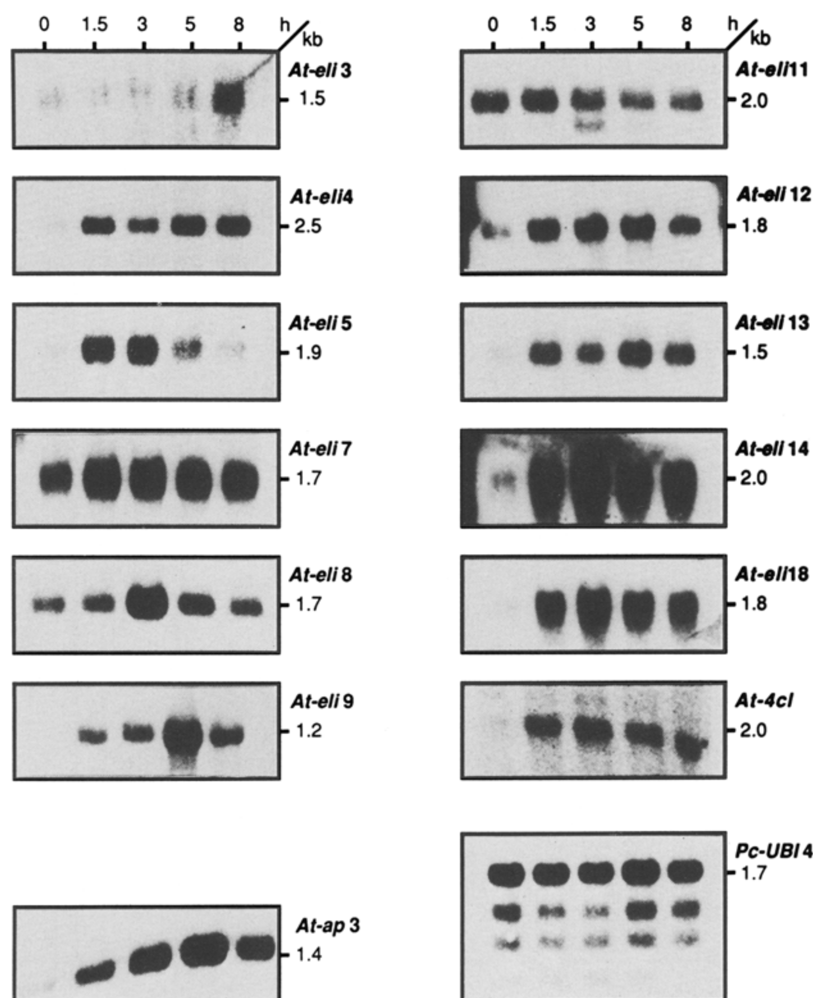

Fig. 2. Expression of the isolated *Arabidopsis* genes upon treatment of *Arabidopsis* cells with fungal elicitor. 4 μg of poly(A)$^+$-enriched RNA, isolated from cells treated with *Pmg* elicitor for various periods of time, was loaded per lane and electrophoresed on 1% denaturing gels, transferred to nylon membranes and hybridized to the indicated radiolabelled *Arabidopsis* genomic subcloned DNA fragments. Filters were then washed under high-stringency conditions and autoradiographed. The probe Pc-UBI (CON2) is a parsley polyubiquitin cDNA [4] used as control to check for RNA loading differences.

counterparts in parsley. Interestingly, the expression patterns of the two types of *Arabidopsis* peroxidase genes detected by At-*eli* 11 and At-*ap3* differed markedly.

Nonetheless, our data clearly demonstrate in both plant species the presence of corresponding elicitor-responsive genes.

Assigning functions to these genes and particularly identifying the roles they may play in pathogen defense are the ultimate goals. In this respect it is noteworthy that recent experiments have shown At-*eli* 3 to be strongly activated upon infiltration of *Arabidopsis* leaves with avirulent *Pseudomonas* strains [15]. In addition, sequence comparison of the translated region of the At-*eli* 3 gene with that of the parsley ELI 3 cDNA showed high sequence similarity throughout (S. Kiedrowski *et al.*, manuscript in preparation), demonstrating that our cloning strategy had indeed resulted in the isolation of corresponding genes very likely encoding similar or identical functions.

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References

1. Dixon RA, Harrison MJ: Activation, structure, and organization of genes involved in microbial defense in plants. Adv Genet 28: 165–234 (1990).
2. Bowles DJ: Defense-related proteins in higher plants. Annu Rev Biochem 59: 873–907 (1990).
3. Hahlbrock K, Scheel D: Physiology and molecular biology of phenylpropanoid metabolism. Annu Rev Plant Mol Biol 40: 347–369 (1989).
4. Somssich IE, Bollmann J, Hahlbrock K, Kombrink E, and Schulz W: Differential early activation of defense-related genes in elicitor-treated parsley cells. Plant Mol Biol 12: 227–234 (1989).
5. Schmelzer E, Krüger-Lebus S, Hahlbrock K: Temporal and spatial patterns of gene expression around sites of attempted fungal infection in parsley leaves. Plant Cell 1: 993–1001 (1989).
6. Murray MG, Thompson WF: Rapid isolation of high molecular weight plant DNA. Nucl Acids Res 8: 6323–6327 (1980).
7. Lois R, Dietrich A, Hahlbrock K, Schulz W: A phenylalanine ammonia-lyase gene from parsley: structure, regulation and identification of elicitor and light responsive *cis*-acting elements. EMBO J 8: 1641–1648 (1989).
8. Somssich IE, Schmelzer E, Bollmann J, Hahlbrock K: Rapid activation by fungal elicitor of genes encoding 'pathogenesis-related' proteins in cultured parsley cells. Proc Natl Acad Sci USA 83: 2427–2430 (1986).
9. Kawalleck P: Pathogenresistenz in Planzen: Identifizierung von Genen, deren Produkte an induzierten Abwehrreaktionen in Petersilie beteiligt sind. Dissertation, University of Cologne, FRG (1991).
10. Kawalleck P, Plesch G, Hahlbrock K, Somssich IE: Induction by fungal elicitor of S-adenosyl-L-methionine synthetase and *S*-adenosyl-*L*-homocysteine hydrolase mRNAs in cultured cells and leaves of *Petroselinum crispum*. Proc Natl Acad Sci USA 89: 4713–4717 (1992).
11. Douglas C, Hoffmann H, Schulz W, Hahlbrock K: Structure and elicitor or u.v.-light-stimulated expression of two 4-coumarate: CoA ligase genes in parsley. EMBO J 6: 1189–1195 (1987).
12. Reimold U, Kroger M, Kreuzaler F, Hahlbrock K: Coding and 3′ non-coding nucleotide sequence of chalcone synthase mRNA and assignment of amino acid sequence of the enzyme. EMBO J 2: 1801–1805 (1983).
13. Feinbaum RL, Ausubel FM: Transcriptional regulation of the *Arabidopsis thaliana* chalcone synthase gene. Mol Cell Biol 8: 1985–1992 (1988).
14. Davis KR, Schott E, Dong X, Ausubel FM: *Arabidopsis thaliana* as a model system for studying plant-pathogen interactions. In: Lugtenberg BJJ (ed) Signal Molecules in Plants and Plant-Microbe Interactions, pp. 99–106. Springer-Verlag, Berlin (1989).
15. Dangl JL, Lehnacker H, Kiedrowski S, Debener T, Rupprecht C, Arnold M, Somssich IE: Interactions between *Arabidopsis thaliana* and phytopathogenic *Pseudomonas* pathovars: a model for the genetics of disease resistance. In: Hauke H, Verma DPS (eds) Current Plant Science and Biotechnology in Agriculture, Vol. 1: Advances in Molecular Genetics of Plant-Microbe Interactions, pp. 84–89. Kluwer Academic Publishers, Dordrecht (1991).
16. Koch E, Slusarenko A: *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell 2: 437–445 (1990).
17. Roberts E, Kutchan T, Kolattukudy PE: Cloning and sequencing of cDNA for a highly anionic peroxidase from potato and the induction of its mRNA in suberizing potato tubers and tomato fruits. Plant Mol Biol 11: 15–26 (1988).