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Colloquium Paper

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Oligopeptide elicitor-mediated defense gene activation in cultured parsley cells

KLAUS HAHLBROCK, DIERK SCHEEL, ELKE LOGEMANN, THORSTEN NÜRNBERGER, MARTIN PARNISKE, SUSANNE REINOLD, WENDY R. SACKS, AND ELMON SCHMELZER

Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany

ABSTRACT **We have used suspension-cultured parsley cells (*Petroselinum crispum*) and an oligopeptide elicitor derived from a surface glycoprotein of the phytopathogenic fungus *Phytophthora megasperma* f.sp. *glycinea* to study the signaling pathway from elicitor recognition to defense gene activation. Immediately after specific binding of the elicitor by a receptor in the plasma membrane, large and transient increases in several inorganic ion fluxes (Ca^{2+}, H^+, K^+, Cl^-) and H_2O_2 formation are the first detectable plant cell responses. These are rapidly followed by transient changes in the phosphorylation status of various proteins and by the activation of numerous defense-related genes, concomitant with the inactivation of several other, non-defense-related genes. A great diversity of cis-acting elements and trans-acting factors appears to be involved in elicitor-mediated gene regulation, similar to the apparently complex nature of the signal transduced intracellularly. With few exceptions, all individual defense responses analyzed in fungus-infected parsley leaves have been found to be closely mimicked in elicitor-treated, cultured parsley cells, thus validating the use of the elicitor/cell culture system as a valuable model system for these types of study.**

A crucial and rapidly expanding area of research concerns the chemical communication within and among organisms. This basic level of intercellular and interorganismic communication plays a critical role in determining the composition and dynamic behavior of ecosystems through such processes as the discrimination between self and nonself and the determination of symbiotic and pathogenic relationships. In functional terms, the chain of molecular events comprising these pathways can be divided into three parts: (*i*) generation and recognition of extracellular signals, (*ii*) intracellular signal conversion and/or transduction, and (*iii*) signal-specific responses of target cells.

We are studying elements of all three functionally interconnected parts of such a signal–response chain, exploiting the fact that many details of the non-host-resistance response of parsley leaves (*Petroselinum crispum*) to infection with the soybean pathogen *Phytophthora megasperma* f.sp. *glycinea* (*Pmg*) can be mimicked by treatment of suspension-cultured parsley cells with an elicitor preparation from this fungus. We have focused our interest on the following elements:

- the nature and mechanism of action of a *Pmg*-derived molecule with high elicitor activity on parsley cells;
- rapid cell membrane- and cell wall-associated changes, as well as intracellular changes, in metabolic activity; and
- the elicitor-mediated activation and inactivation of numerous defense-related and non-defense-related genes.

Prior to a discussion of some mechanistic details of the responses of cultured parsley cells to treatment with *Pmg* elicitor, the present state of knowledge of the whole-plant interaction of parsley leaves with the intact fungus will be briefly summarized.

Responses of Parsley Leaves to *Pmg* Infection

Young, primary parsley leaves were used for most of our studies (1–4), since a higher rate of infection with *Pmg* was obtained than with old leaves under the conditions used (1). Combining all of the results obtained so far with *Pmg*-infected parsley leaves, three major steps in the overall defense response can be distinguished (Fig. 1).

Following leaf inoculation with fungal zoospores, cyst formation, germination, and formation of appressoria and infection vesicles (≈4 hr postinoculation), the first microscopically visible sign of a plant defense response is hypersensitive (very rapid and highly localized) cell death. This hypersensitive response is associated with reinforcement of the affected cell wall—for example, by apposition of callose and incorporation of phenolics. The newly incorporated phenolics are readily detectable without histochemical staining by their autofluorescence under blue/UV light (1–4). Results obtained recently with a similar system, potato (*Solanum tuberosum* L.) leaves infected with *Phytophthora infestans*, indicate that the penetrating fungal infection vesicle is killed concomitantly with hypersensitive plant cell death (5). In both the parsley and potato systems, as well as in many others, hypersensitive cell death appears to be a particular early-plant-defense response initiated by those cells that are invaded by the fungus or in direct contact with fungal structures. Available evidence suggests a close correlation between the frequency of hypersensitive cell death (provided it occurs at all) and the degree of resistance (5).

The second line of defense consists of numerous rapidly accumulated enzymes, structural proteins, and metabolites, at least some of which possess antifungal activity. Among these antifungal compounds are the so-called phytoalexins, plant species- or family-specific classes of broad-range antibiotics. In parsley, the phytoalexins are a mixture of linear furanocoumarins (6–8), which are easily detectable by their blue autofluorescence under UV light. Many of these second-line defense reactions are activated transcriptionally, as demon-

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Abbreviations: *Pmg, Phytophthora megasperma*; PAL, phenylalanine ammonia-lyase; 4CL, 4-coumarate:CoA ligase; C4H, cinnamate 4-hydroxylase; PR proteins, pathogenesis-related proteins; PRH, homeodomain-containing PR protein; BPF, box P-binding factor; BIF, box I-binding factor; CPRF, common plant regulatory factors.

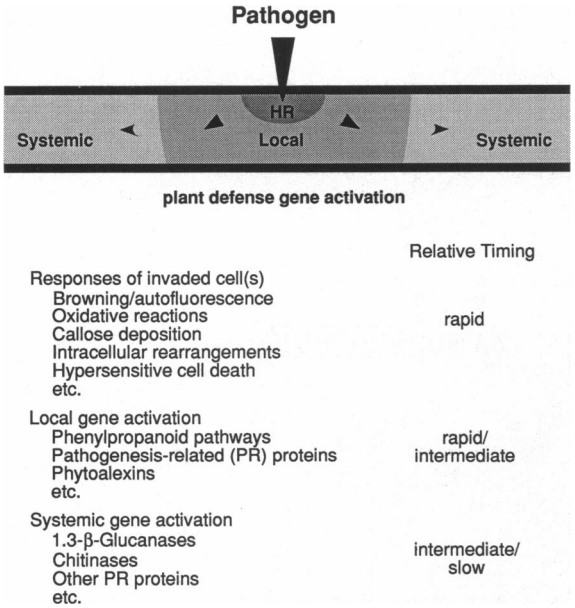

FIG. 1. Schematic representation of three major types of plant defense response to fungal infection. HR, hypersensitive response.

strated by *in situ* mRNA hybridization in *Pmg*-infected parsley leaves (3, 4, 9). The affected tissue is depicted in Fig. 1 as the area undergoing "local gene activation" and is restricted to a defined area surrounding the fungal penetration site. Relative to the small number of cells displaying the hypersensitive response, however, the number of cells exhibiting local gene activation is large.

In contrast, the third line of defense is induced systemically throughout the entire infected leaf, and often the entire organism as well, and comprises yet another set of antifungal activities. This systemic response, which in potato (10), tobacco (11), and various other plants has been shown to include the systemic activation of genes encoding several chitinase and 1,3-β-glucanase isoforms, is most likely to occur also in *Pmg*-infected parsley leaves.

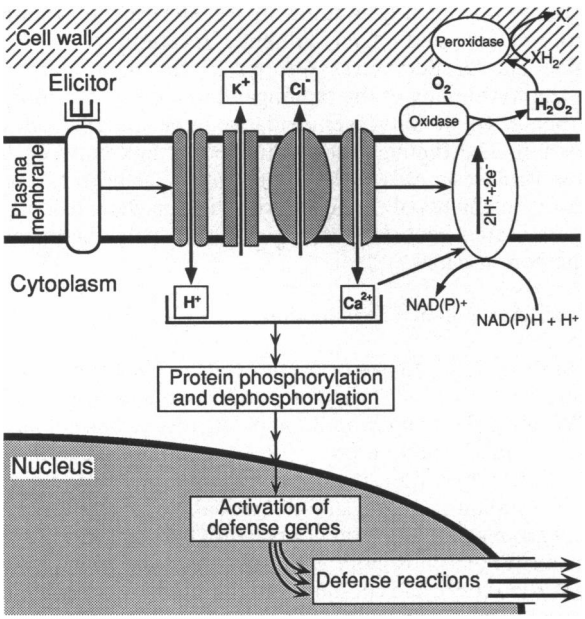

FIG. 2. Schematic illustration of major, partly hypothetical components of the signaling cascade from elicitor recognition to defense gene activation.

Systemic gene activation appears to be slow relative to hypersensitive cell death and local gene activation but gradually leads to the accumulation of large amounts of protein and enzyme activity (12). A particularly interesting aspect of this third-line systemic response is the phenomenon of "systemic acquired resistance," which renders a previously infected plant more resistant to a second infection, even by a different type of pathogen (13).

Thus, the complex, multicomponent defense response of parsley leaves to *Pmg* infection can be subdivided into three distinct stages: (*i*) hypersensitive cell death; (*ii*) concurrent but physically distinct local-defense gene activation in the surrounding tissue; and (*iii*) subsequent systemic activation of additional defense genes. It remains open at the present time whether the two successive steps of local and systemic gene activation are causally linked by sequential signaling events. In contrast, we have recently obtained evidence that cell death is not a prerequisite for concomitant local gene activation to occur (14), suggesting that a fungus-derived signal triggers local gene activation. The following results support this notion.

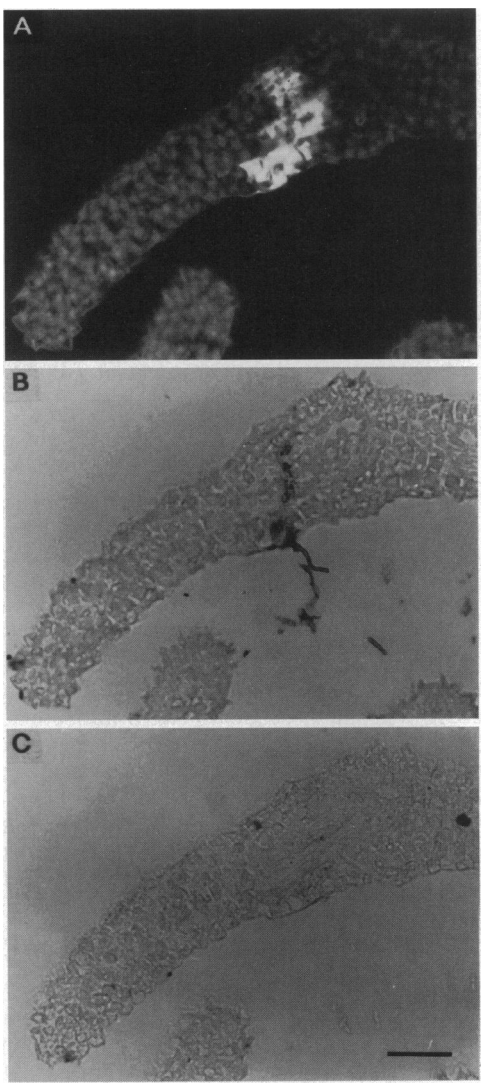

FIG. 3. Immunohistochemical localization of elicitor in a cross section of a *Pmg*-infected parsley leaf bud. Methods were the same as described (1), except that a polyclonal antiserum raised against the purified, deglycosylated elicitor protein (21) was used. (*A*) Autofluorescence under UV-epifluorescent light, indicating hypersensitive cell death. (*B*) Same section under visible light, showing indirect immunoperoxidase staining of elicitor. (*C*) Adjacent section used for the control reaction with preimmune serum. (Bar = 40 μm.)

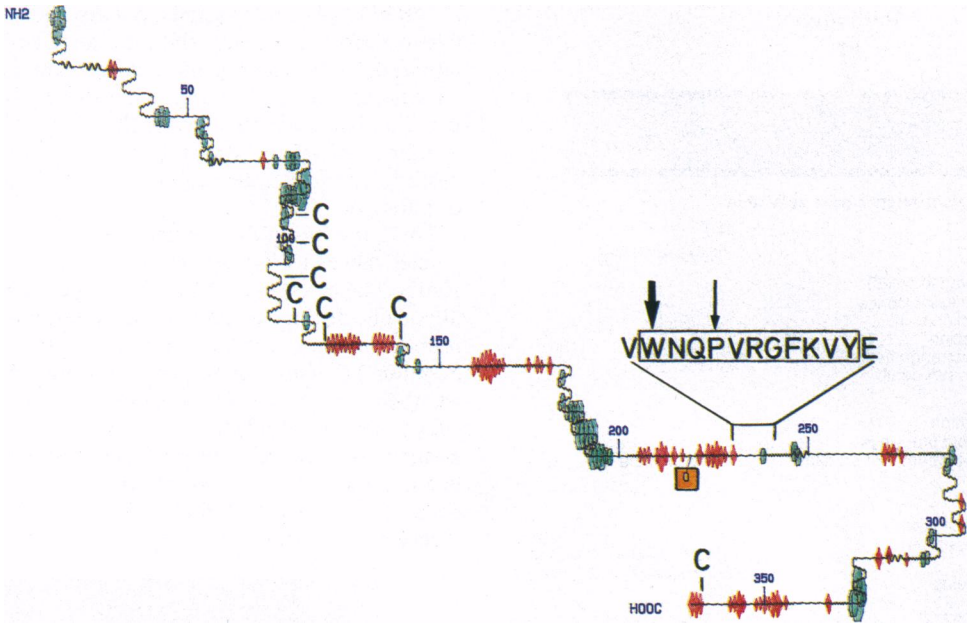

FIG. 4. Chou–Fasman prediction of the structure of the 42-kDa elicitor protein as determined by the PLOTSTRUCTURE program of the GCG (Genetics Computer Group) package (24). The amino acid sequences of the 13-meric oligopeptide elicitor and the smallest elicitor-active derivative (box) as well as their positions within the full-length protein predicted from cDNA sequence information (23) are indicated. Arrows indicate positions critical for activity as determined by alanine substitution mutation analysis (18).

The Oligopeptide Elicitor from *Pmg*: Molecular Characterization and Mode of Perception

Treatment of suspension-cultured parsley cells with a crude *Pmg* mycelial wall preparation (crude *Pmg* elicitor) induces most or all of the defense responses associated with local gene activation in *Pmg*-infected leaf tissue (7–9, 15–17). Numerous rapid and transient metabolic changes have been shown to occur in parsley cells treated with the crude *Pmg* elicitor, including inorganic ion fluxes across the plasma membrane, a release of active oxygen species referred to as the oxidative burst (18, 19), alterations in the phosphorylation status of soluble and membrane-associated proteins (20), activation of defense-related genes and inactivation of other genes (16, 21, 22), and accumulation of furanocoumarin phytoalexins (7). These responses and the more recently identified elicitor receptor (see below) are summarized schematically in Fig. 2 together with their possible causal interrelationships.

To study in detail the molecular mechanisms underlying elicitor recognition and subsequent signal transduction, efforts were undertaken to isolate homogeneous elicitor-active compounds from various fungal sources. A 42-kDa glycoprotein elicitor was purified from fungal culture filtrate whose elicitor activity was found to reside solely in the protein moiety (21). A monospecific polyclonal antiserum raised against the deglycosylated elicitor protein identified the antigen in the cell walls of a few selected *Phytophthora* species but not in several other phytopathogenic fungi (21). Moreover, use of the same antiserum has localized the elicitor *in situ* to hyphal cell walls of *Pmg* growing in or on parsley leaves (Fig. 3).

A peptide consisting of 13 amino acids was identified within the intact glycoprotein to be sufficient to stimulate the same responses as the crude *Pmg* elicitor and the glycoprotein elicitor (18). The isolation of a cDNA encoding the glycoprotein (23) allowed the localization of this oligopeptide to a hydrophilic region within the C-terminal portion of this protein as shown in Fig. 4. A comparison of the peptide sequence with sequences contained in several data bases did not reveal significant similarity to known proteins, as was also found for the glycoprotein elicitor at the amino acid and the nucleotide levels (23). Deletion of one N- and C-terminal amino acid yielded the minimum peptide with full elicitor activity (18) (boxed sequence in Fig. 4). Substitution analysis, in which each individual amino acid of this 11-meric peptide was replaced by alanine, identified two residues critical for activity (arrows in Fig. 4). All other exchanges exerted little or no effect on the elicitor activity of the oligopeptide (18).

With use of the radioiodinated oligopeptide as a ligand in binding assays, a single-class binding site with high affinity (K_d = 2.4 nM) could be detected in parsley microsomal membranes and protoplasts (18). The number of binding sites per protoplast was estimated to be ≈2900. Binding of the peptide elicitor was competable, reversible, saturable, and highly specific with respect to both structural properties of the signal required for binding and plant species that recognized the ligand. The oligopeptide binding site therefore meets a number of criteria expected of an authentic receptor. A series of peptide elicitor derivatives was tested for their ability to compete for binding of the radioligand to parsley microsomes and their effect on ion fluxes, oxidative burst, and phytoalexin formation. The findings show a functional link between the various plant responses to elicitor treatment, which in part may constitute elements of the signal transduction chain leading to defense-related gene activation and subsequent phytoalexin production in parsley.

Intracellular Signal Transduction

The most rapid responses of parsley cells to elicitor treatment detected so far are ion fluxes across the plasma membrane (18, 19). Within 2–5 min upon addition of elicitor, a transient influx of Ca^{2+} and H^+ and an efflux of K^+ and Cl^- are initiated. Immediately thereafter, the concentration of H_2O_2 in the cell culture medium increases greatly (oxidative burst), returning to background levels several hours later (18). Within 5–30 min after elicitation, phosphorylation of several proteins was observed *in vivo* (20), and run-on transcription of the most rapidly activated defense-related genes was detectable (16). Accumulation of the respective mRNAs and increases in activity of the corresponding enzymes were found to occur later but before the formation of phytoalexins (7, 16).

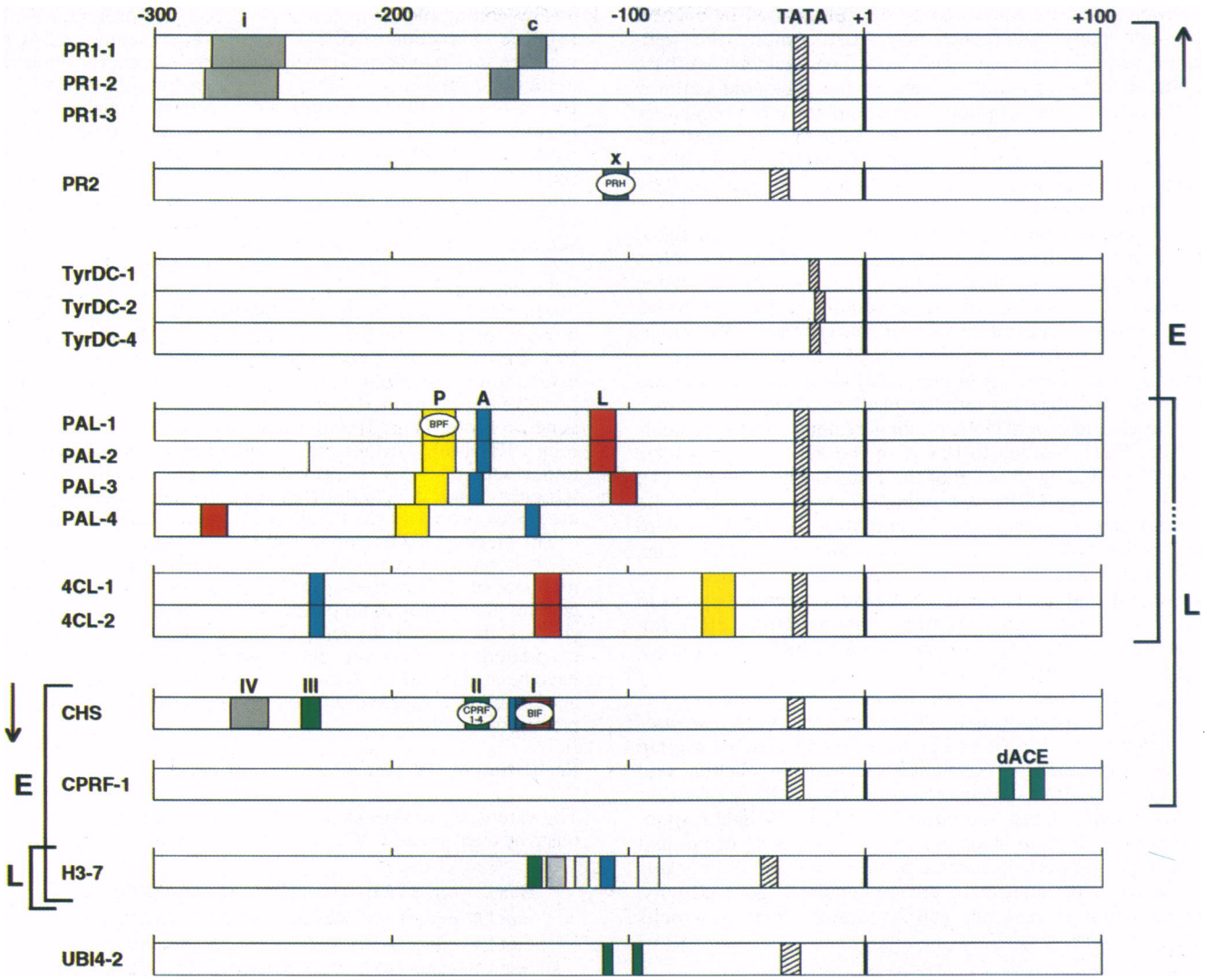

FIG. 5. Schematic representation of TATA-proximal regions of genes shown to be elicitor-responsive (E brackets) in cultured parsley cells. Positions −300 to +100 are represented, except for the PAL-2 gene, for which sequence is available only to position −242. Elicitor-activated genes are indicated by an arrow directed upwards; elicitor-repressed genes, by an arrow directed downwards. For comparison, genes responding to UV light (L brackets) and one control gene responding neither to elicitor nor to light (UBI4-2; ref. 39) are also shown. Boxes represent cis-acting elements, with sequence similarity indicated by color or shading. Letters or numbers indicate elements identified functionally. DNA-binding proteins are depicted as ovals located on the fragment used for their identification. See text for descriptions of these proteins. CHS, chalcone synthase; TyrDC, tyrosine decarboxylase; H3-7, histone H3 subclass; PRH, homeodomain-containing PR protein; BPF, box P-binding factor; BIF, box I-binding factor; dACE, duplicated ACGT-containing element.

Loss- and gain-of-function experiments demonstrated that all of these elicitor responses were strictly dependent on the presence of Ca^{2+} in the cell culture medium (19, 25, 26), were prevented by ion-channel inhibitors that suppress elicitor-mediated fluxes of one or more ions (26), and were initiated by compounds that stimulate all elicitor-induced ion fluxes in the absence of elicitor (25, 26). Therefore, transient activation of plasma membrane-located ion channels appears to be an early component of elicitor signal transduction.

While both the oxidative burst and protein phosphorylation/dephosphorylation could be located downstream of ion channel activation, their possible causal interconnections with other signaling components remain to be elucidated. Circumstantial evidence suggests that the intracellular signal transduction chain involves inositol phosphates but not GTP-binding proteins or cAMP (27).

The scheme shown in Fig. 2 includes the most clearly demonstrated components of the signal transduction chain together with their potential causal relationships. A major target of the signaling cascade is the up- or down-regulation of numerous elicitor-responsive genes.

Regulation of Elicitor-Responsive Genes

The list of genes responding to elicitor treatment of cultured parsley cells with rapid, transient activation is large. It includes the genes or gene families encoding phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL), the first and last steps of general phenylpropanoid metabolism (28, 29); tyrosine decarboxylase (TyrDC) (30); *S*-adenosyl-L-methionine synthetase (SMS) and *S*-adenosyl-L-homocysteine hydrolase (SHH), two enzymes of the activated methyl-group cycle (31); one particular isoform of chitinase (32); anionic peroxidase (POX) and a hydroxyproline-rich glycoprotein (HRGP), two cell wall-associated proteins (33); and two intracellular "pathogenesis-related" (PR) proteins, PR1 and PR2 (9, 34). In addition, several functionally unidentified, elicitor-responsive genes have been shown to behave similarly (16). The genes encoding *S*-adenosyl-L-methionine:bergaptol *O*-methyltransferase [(BMT); a specific enzyme of furanocoumarin biosynthesis (7, 35)] and another chitinase isoform (32) were activated more slowly than the other genes mentioned.

Fewer genes are known to be down-regulated by elicitor, although many such genes may exist. Among the well-established cases are the genes encoding chalcone synthase (CHS), a light-regulated enzyme of the flavonoid pathway (22); a putative transcription factor, common plant regulatory factor 1 (CPRF-1), possibly involved in light-dependent chalcone synthase gene activation (refs. 36 and 37; and A. Block, personal communication); and the histone H3 subclass H3-7 (unpublished data).

It is generally believed that the stimulus-dependent signal transduction pathway modulating gene expression involves transcription factors binding to stimulus-specific cis-acting elements. A comparison of elicitor-responsive genes for which TATA-proximal sequences are available is presented schematically in Fig. 5. Cis-acting elements identified either experimentally or by homology to previously identified elements are also presented, together with the locations of sequences shown to bind characterized DNA-binding proteins. These proteins include: PRH, binding to box X of the PR2 gene promoter (40); BPF, binding to box P of the PAL-1 gene promoter (4); CPRF-1 through CPRF-4, binding to box II of the chalcone synthase gene promoter (36, 37); and Myb-like BIF, binding to box I of the chalcone synthase gene promoter (M. Feldbrügge, M.P., K.H., and B. Weisshaar, unpublished results). It is noteworthy that each type of DNA-binding protein belongs to a different class of putative transcription factors (PRH, homeodomain-containing; BPF, novel class; CPRF, bZIP (basic/leucine zipper); BIF, Myb-like).

In parsley, there is an intimate metabolic connection between elicitor- (pathogen-) and UV light-triggered responses (see below). This is reflected in particular by a partial overlap in the involvement of cis-acting elements in elicitor- and light-mediated PAL-1 gene activation (boxes P, A, and L in elicitor responsiveness and boxes P and L in UV light responsiveness; ref. 28) and in general by the complexity of up- and down-regulatory effects exerted by these two stimuli (brackets in Fig. 5). With respect to elicitor-regulated gene activity, several major conclusions can be drawn from the results obtained so far.

- No common stimulus-specific cis-acting elements have been detected among the various elicitor- and UV light-responsive gene promoters.
- The combinations of those cis-acting elements that have been shown functionally to be involved in elicitor-mediated gene activation (boxes i, X, P, A, and L in Fig. 5) may be similar within gene families or between particularly closely interconnected gene families, such as PAL and 4CL, but generally differ greatly among gene families.
- Notable exceptions appear to be ACGT-containing elements (ACE; green boxes in Fig. 5), which occur frequently in plant gene promoters (37). These elements are present in all promoters (or functional units thereof; refs. 37 and 41) tested that are either down-regulated (CHS, CPRF-1, and H3-7 gene promoters among others) or unaffected (UBI4-2) by elicitor (ref. 22 and unpublished data).
- These ACEs and dACE versions thereof (see Fig. 5) are likely to be involved in both gene activation and gene repression, depending on the sequence context (36) and metabolic conditions (unpublished data).
- The three boxes (P, A, and L) originally identified in the PAL-1 promoter by *in vivo* DNA footprinting (28) are present in similar form in all known PAL and 4CL gene promoters from numerous plant species, strongly indicating their functional importance.

Further evidence for the functional relevance of box P was provided by the formation of specific DNA–protein complexes in mobility-shift assays with nuclear extracts from parsley cells and by cloning and characterization of one such protein, BPF-1 (4). The interaction of BPF-1 with its target sequence box P could be shown to occur *in vivo*: in cotransfection experiments, a chimeric protein containing the DNA-binding domain of BPF-1 fused to the transcriptional activation domain of viral protein VP16 (42) could specifically activate transcription from an artificial promoter containing a tetramer of box P (M.P. and K.H., unpublished results). Box L has also been found to interact with nuclear proteins. The formation of one of the DNA–protein complexes exhibits the property of being stimulus (elicitor)-inducible (Fig. 6). The same nucleotide sequence that was previously shown to display an elicitor-inducible footprint was now found to form elicitor-inducible interactions *in vitro*. Such inducibility by elicitor was observed neither for the complexes formed with box P, although the BPF-1 mRNA level in elicitor-treated parsley cells is to a large extent transcriptionally regulated (4), nor for a recently reported complex formed specifically between box X of the PR2 gene promoter (Fig. 5) and a homeodomain-containing protein, PRH (40). Further strong evidence for the functional importance of box L is provided by the observation that a tetramer of box L activates transcription when fused to a heterologous promoter (M.P. and K.H., unpublished results).

The biochemical functions of PR1, PR2, and some products of elicitor-responsive genes and the overall physiological significance of elicitor-mediated accumulation of enzymes of the general phenylpropanoid pathway [PAL, cinnamate 4-hydroxylase (C4H), and 4CL; refs. 43 and 44] have yet to be fully elucidated. Fig. 7 outlines those functional relationships that have been established to date for elicitor-stimulated genes or gene families associated directly or indirectly with phenylpropanoid metabolism.

Regulation of UV Light-Responsive Genes

The extensive overlap in the regulation of secondary metabolism by elicitor and UV light is manifest in the responsiveness

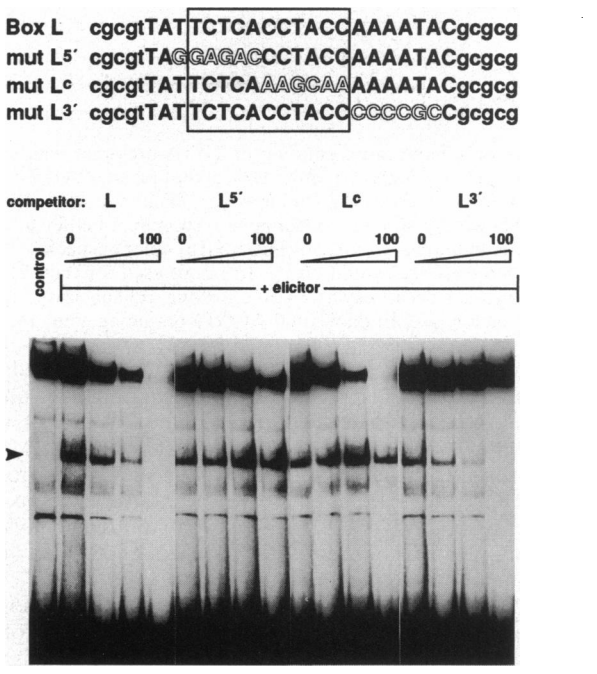

FIG. 6. Gel mobility-shift assays performed with nuclear extracts from untreated (control) or 3-hr elicitor-treated parsley cells and a box L-containing labeled probe (*Upper*, top line; box L enframed). Unlabeled competitors were either the same box L-containing probe or mutated versions (*Upper*) as indicated, used at 0-, 25-, 50- and 100-fold molar excess (*Lower*). By this criterion, the elicitor-inducible shift (arrowhead in *Lower*) is specific for intact box L. Methods were essentially the same as those described previously for box P-binding proteins (4).

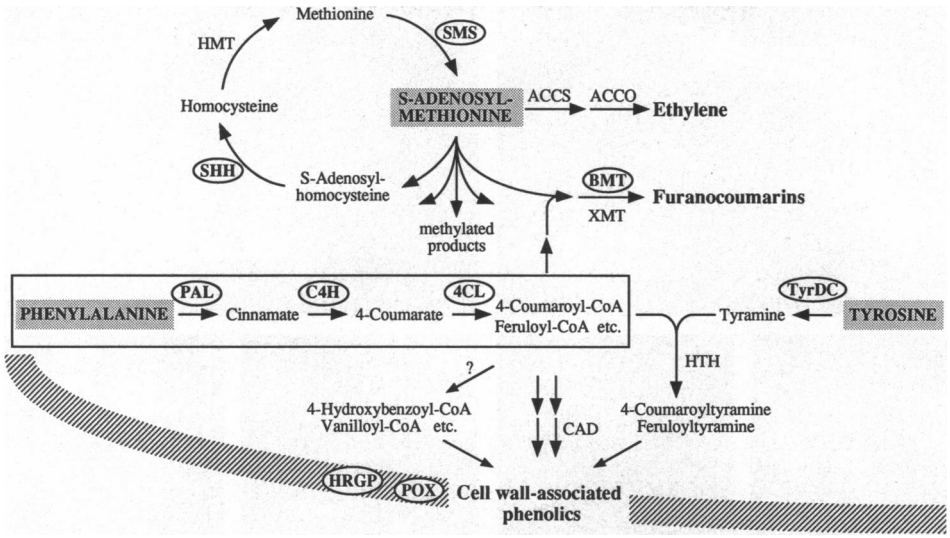

FIG. 7. Metabolic interconnections among various selected elicitor-inducible reactions in cultured parsley cells. Ovals indicate enzymes for which elicitor-mediated gene activation has been demonstrated with the corresponding cDNAs. CAD, cinnamyl alcohol dehydrogenase; HTH, hydroxycinnamoyl-CoA:tyramine hydroxycinnamoyltransferase; HMT, homocysteine *S*-methyltransferase; ACCS and ACCO, 1-aminocyclopropane-1-carboxylic acid synthase and oxidase; XMT, *S*-adenosyl-L-methionine:xanthotoxol *O*-methyltransferase; SHH, *S*-adenosyl-L-homocysteine hydrolase; SMS, *S*-adenosyl-L-methionine synthetase; BMT, *S*-adenosyl-L-methionine:bergaptol *O*-methyltransferase; TyrDC, tyrosine decarboxylase; HRGP, hydroxyproline-rich glycoprotein; POX, anionic peroxidase.

of the three steps of general phenylpropanoid metabolism to both stimuli (Fig. 8). While the synthesis of the three enzymes PAL, C4H, and 4CL is strongly and coordinately induced by both fungal elicitor and UV-containing white light, the subsequent phenylpropanoid branch pathways are regulated in a more narrow, stimulus-specific manner (44). Thus, induction of the flavonoid branch pathway is strongly dependent on specific light conditions (45, 46) and is repressed by elicitor (22). On the other hand, formation of wall-bound phenolics (1), furanocoumarin phytoalexins (6, 7), and phenolic esters (47) is induced by elicitor, whereas light exerts little, if any, effect (22).

This differential response of parsley cells to light and elicitor raised the question of whether the individual members of the PAL and 4CL gene families respond differentially to the two stimuli as well. Although certain methodological difficulties precluded a definite answer for 4CL (48), all available evidence suggests that the two 4CL genes respond similarly to light and elicitor. In contrast, recent studies using gene-specific probes for the four PAL genes (ref. 48; E.L. and K.H., unpublished results) demonstrated clear-cut differential behavior of PAL-1, PAL-2, and PAL-3 on the one hand and PAL-4 on the other. In cultured parsley cells, all four PAL genes responded to elicitor, whereas only the first three, but not PAL-4, were responsive to light (see also Fig. 5). Surprisingly, the enzyme kinetic properties of all four PAL proteins (49), as well as those of the two 4CL proteins (50), are almost indistinguishable, making the need for differential induction of isoenzymes difficult to explain.

PAL Gene Expression Patterns in Parsley Plants

These unexplained findings obtained in cultured parsley cells prompted us to investigate the mode of PAL gene expression in intact plants as well. Through use of RNA probes for mRNA localization *in situ*, total PAL gene expression was detected predominantly in three cell types of every organ tested, including stem and flower (Fig. 9 *A* and *B*): epidermal cells, where PAL is probably involved in flavonoid biosynthesis (51); oil-duct epithelial cells, the site of furanocoumarin production (8); and collenchyma and lignifying cells of the vascular bundles (38). At certain stages of flower development, anthers (not shown) and ovaries (Fig. 9*B*) are additional sites of abundant PAL mRNA accumulation.

Examination of the expression of individual PAL gene family members with gene-specific probes for *in situ* hybrid-

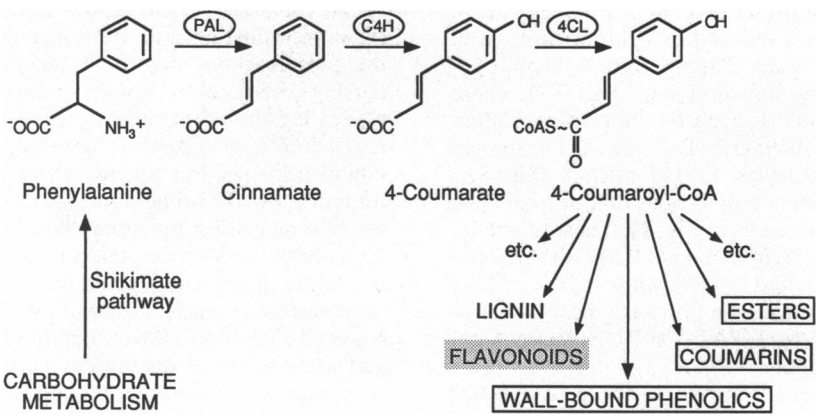

FIG. 8. Biosynthetic relationship of general phenylpropanoid metabolism with various branch pathways, one of which (stippled) is light-induced and at least three of which (boxed) are elicitor-induced in cultured parsley cells.

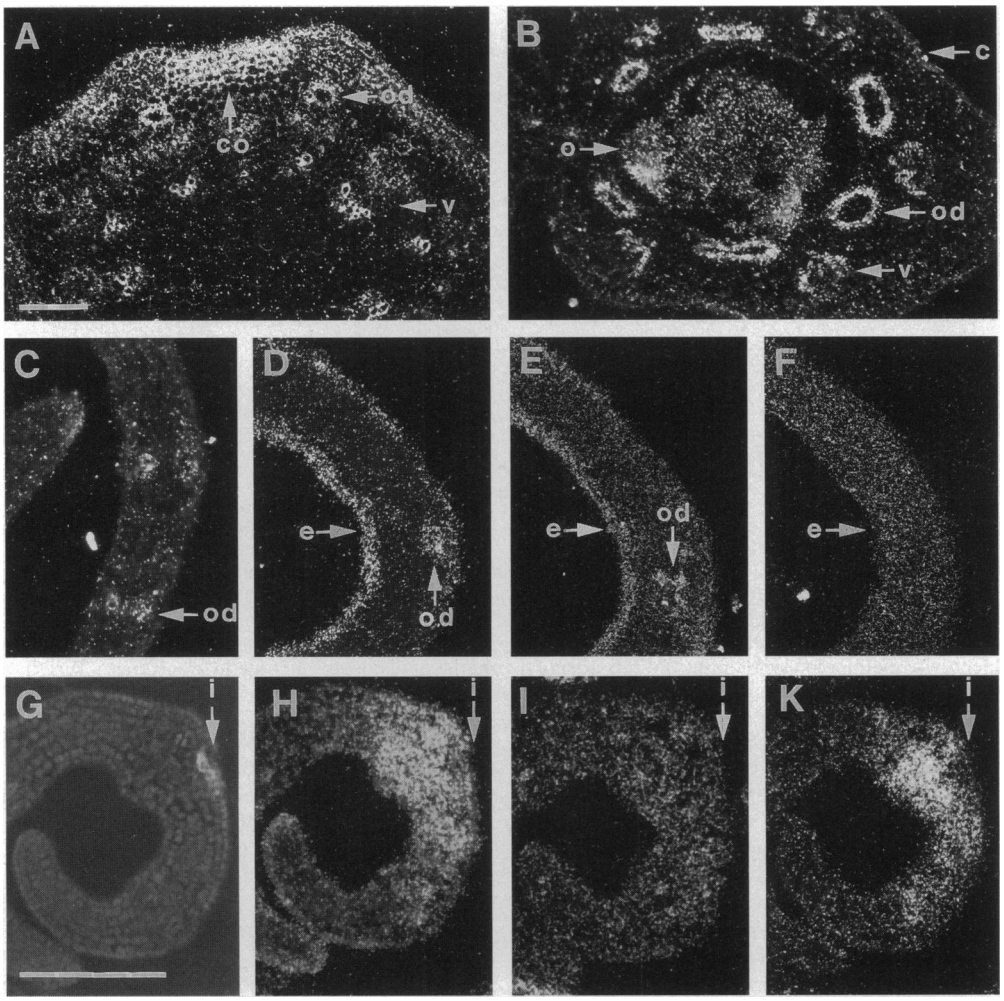

FIG. 9. PAL gene expression patterns in various parsley tissues. Cross sections of stem (*A*), gyneceum (*B*), and primary leaf buds that were 24-hr dark-adapted (*C*), 16-hr reilluminated (*D–F*), or 6-hr *Pmg*-infected (*G–K*) were hybridized *in situ* (3, 4) with PAL-1 antisense RNA (hybridizing with all PAL mRNA species) (*A–D* and *H*) or with PAL-1/PAL-2-specific (*E* and *I*) or PAL-4-specific (*F* and *K*) antisense RNA probes derived from the 5′ untranslated regions of the respective mRNAs. *C–F* and *H–K* are serial sections, respectively. *G* is identical to *H* but is shown under UV-epifluorescent light for identification of the infection site (i) (arrow). c, Carpel; co, collenchyma; e, epidermis; o, ovary; od, oil duct; v, vascular bundle. (Bars in *A* for *A* and *B* and in *G* for *C–K* = 100 μm.)

ization has so far been successful for PAL-4 and for PAL-1 and PAL-2 in combination (PAL-1/PAL-2). The results obtained for PAL induction by light and *Pmg* infection are shown in Fig. 9 *C–K*. Total PAL mRNA was present in oil ducts in both etiolated and illuminated leaf buds (Fig. 9 *C* and *D*) but accumulated preferentially in the upper and lower epidermis only in the latter. The same expression pattern was found with the PAL-1/PAL-2-specific probe (Fig. 9*E*), whereas PAL-4 mRNA (Fig. 9*F*) was neither induced by light nor present in detectable amounts in oil ducts. The opposite induction behavior was observed at *Pmg*-infection sites (Fig. 9*G*), where PAL mRNA had accumulated strongly by 6 hr postinoculation with fungal spores (Fig. 9*H*). While PAL-1 mRNA (Fig. 9*I*) was not induced to detectable levels, PAL-4 mRNA (Fig. 9*K*) exhibited the same accumulation pattern as that of total PAL mRNA (see local gene activation in Fig. 1). Thus, in whole-plant tissue as in cultured cells, induction of PAL-1/PAL-2 but not PAL-4 mRNA was triggered by light, while PAL-4 mRNA accumulated strongly at fungal infection sites. In contrast to cultured cells, however, PAL-1/PAL-2 mRNA was not detected in response to infection.

This discrepancy represents the first example of a major difference in induction behavior of parsley cells in culture and in infected tissue, although a large number of properties have been examined. We tentatively assume that this apparent discrepancy is associated with a particularly strict mode of cell type-specific gene expression at infection sites, an intriguing possibility for further investigation.

Conclusions

Suspension-cultured cells have proven to be an excellent model system for studies of the various aspects of elicitor recognition, signal transduction, and defense gene activation. In particular, the proteinaceous nature of the *Pmg* elicitor that acts on parsley cells enabled us to perform functional analysis by expressing the corresponding gene and mutated versions in *Escherichia coli* (23). Furthermore, the identification of an oligopeptide as the elicitor-active determinant within the protein allowed binding studies to be carried out, leading to the establishment of a functional link between signal perception via a peptide receptor on the cell surface, transient stimulation of plasma membrane-located ion channels, and activation of defense-related nuclear genes (18). Although several components of elicitor signal transduction have now been identified and interconnected, the present picture is still not complete. In particular, a question that remains to be answered is how the high specificity of the incoming elicitor signal is maintained and transduced through the entire chain of events from extracellular recognition to the activation and inactivation of

numerous defense-related and non-defense-related genes. We expect, however, that the combination of defined elicitors and cultured cells will continue to serve as useful model systems for expanding our knowledge about this particular type of signaling between and within organisms.

An additional advantage of this cell culture system is the fact that single cells or cell aggregates can be infected by the intact fungus. This infection system represents an intermediate stage between elicitor-treated cultured cells and fungus-infected, intact plant tissue (14). Since infection stimulates all responses analyzed thus far in both intact tissue and cultured cells (3, 5, 14) while elicitor treatment fails to stimulate hypersensitive cell death and callose accumulation (19), cultured cells can be used for micromanipulation, replacing the invading fungus by local application of the elicitor. In particular, it will be interesting to determine whether the physical force exerted by the fungal penetration peg contributes to the recognition and signaling mechanisms between the two organisms. In summary, we are beginning to appreciate how the great diversity of organismic interactions in ecosystems is reflected by an enormous complexity of integrated molecular networks within individual, affected cells.

1. Jahnen, W. & Hahlbrock, K. (1988) *Planta* **173,** 197–204.
2. Scheel, D., Dangl, J., Douglas, C., Hauffe, K. D., Herrmann, A., Hoffmann, H., Lozoya, E., Schulz, W. & Hahlbrock, K. (1987) in *Plant Molecular Biology*, eds. v. Wettstein, D. & Chua, N.-H. (Plenum, New York), pp. 315–326.
3. Schmelzer, E., Krüger-Lebus, S. & Hahlbrock, K. (1989) *Plant Cell* **1,** 993–1001.
4. Da Costa e Silva, O., Klein, L., Schmelzer, E., Trezzini, G. F. & Hahlbrock, K. (1993) *Plant J.* **4,** 125–135.
5. Freytag, S., Arabatzis, N., Hahlbrock, K. & Schmelzer, E. (1994) *Planta* **194,** 123–135.
6. Tietjen, K. G., Hunkler, D. & Matern, U. (1983) *Eur. J. Biochem.* **131,** 401–407.
7. Hauffe, K. D., Hahlbrock, K. & Scheel, D. (1986) *Z. Naturforsch. C* **41,** 228–239.
8. Scheel, D., Hauffe, K. D., Jahnen, W. & Hahlbrock, K. (1986) in *Recognition in Microbe–Plant Symbiotic and Pathogenic Interactions*, ed. Lugtenberg, B. (Springer, Berlin), pp. 325–331.
9. Somssich, I., Schmelzer, E., Kawalleck, P. & Hahlbrock, K. (1988) *Mol. Gen. Genet.* **21,** 93–98.
10. Schröder, M., Hahlbrock, K. & Kombrink, E. (1992) *Plant J.* **2,** 161–172.
11. Ward, E. R., Ukues, S. J., Williams, S. C., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Métraux, J.-P. & Ryals, J. A. (1991) *Plant Cell* **3,** 1085–1094.
12. Kombrink, E., Schröder, M. & Hahlbrock, K. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 782–786.
13. Ryals, J., Ukues, S. & Ward, E. (1994) *Plant Physiol.* **104,** 1109–1112.
14. Gross, P., Julius, C., Schmelzer, E. & Hahlbrock, K. (1993) *EMBO J.* **12,** 1735–1744.
15. Kombrink, E. & Hahlbrock, K. (1986) *Plant Physiol.* **81,** 216–221.
16. Somssich, I. E., Bollmann, J., Hahlbrock, K., Kombrink, E. & Schulz, W. (1989) *Plant Mol. Biol.* **12,** 227–234.
17. Dangl, J. L., Hauffe, K. D., Lipphardt, S., Hahlbrock, K. & Scheel, D. (1987) *EMBO J.* **9,** 2551–2556.
18. Nürnberger, T., Nennstiel, D., Jabs, T., Sacks, W. R., Hahlbrock, K. & Scheel, D. (1994) *Cell* **78,** 449–460.
19. Scheel, D., Colling, C., Hedrich, R., Kawalleck, P., Parker, J. E., Sacks, W. R., Somssich, I. E. & Hahlbrock, K. (1991) in *Advances in Molecular Genetics of Plant–Microbe Interactions*, eds. Hennecke, H. & Verma, D. P. S. (Kluwer, Dordrecht, The Netherlands), Vol. 1, pp. 373–380.
20. Dietrich, A., Mayer, J. E. & Hahlbrock, K. (1990) *J. Biol. Chem.* **265,** 6360–6368.
21. Parker, J. E., Schulte, W., Hahlbrock, K. & Scheel, D. (1991) *Mol. Plant-Microbe Interact.* **4,** 19–27.
22. Lozoya, E., Block, A., Lois, R., Hahlbrock, K. & Scheel, D. (1991) *Plant J.* **1,** 227–234.
23. Sacks, W., Nürnberger, T., Hahlbrock, K. & Scheel, D. (1995) *Mol. Gen. Genet.* **246,** 45–55.
24. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
25. Sacks, W. R., Ferreira, P., Hahlbrock, K., Jabs, T., Nürnberger, T., Renelt, A. & Scheel, D. (1993) in *Advances in Molecular Genetics of Plant–Microbe Interactions*, eds. Nester, E. W. & Verma, D. P. S. (Kluwer, Dordrecht, The Netherlands), Vol. 2, pp. 485–495.
26. Nürnberger, T., Colling, C., Hahlbrock, K., Jabs, T., Renelt, A., Sacks, W. R. & Scheel, D. (1994) *Biochem. Soc. Symp.* **60,** 173–182.
27. Renelt, A., Colling, C., Hahlbrock, K., Nürnberger, T., Parker, J. E., Sacks, W. R. & Scheel, D. (1993) *J. Exp. Bot.* **44,** 257–268.
28. Lois, R., Dietrich, A., Hahlbrock, K. & Schulz, W. (1989) *EMBO J.* **8,** 1641–1648.
29. Douglas, C., Hoffmann, H., Schulz, W. & Hahlbrock, K. (1987) *EMBO J.* **6,** 1189–1195.
30. Kawalleck, P., Keller, H., Hahlbrock, K., Scheel, D. & Somssich, I. E. (1993) *J. Biol. Chem.* **268,** 2189–2194.
31. Kawalleck, P., Plesch, G., Hahlbrock, K. & Somssich, I. E. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4713–4717.
32. Vollberg, H. (1994) Diploma thesis (Univ. of Cologne, Cologne, Germany).
33. Kawalleck, P. (1991) Doctoral thesis (Univ. of Cologne, Cologne, Germany).
34. van de Löcht, U., Meier, I., Hahlbrock, K. & Somssich, I. E. (1990) *EMBO J.* **9,** 2945–2950.
35. Hauffe, K. D. (1988) Doctoral thesis (Univ. of Cologne, Cologne, Germany).
36. Feldbrügge, M., Sprenger, M., Dinkelbach, M., Yasaki, K. & Weisshaar, B. (1994) *Plant Cell* **6,** 1607–1621.
37. Weisshaar, B., Armstrong, G. A., Block, A., da Costa e Silva, O. & Hahlbrock, K. (1991) *EMBO J.* **10,** 1777–1786.
38. Wu, S. C. & Hahlbrock, K. (1992) *Z. Naturforsch. C* **47,** 591–600.
39. Kawalleck, P., Somssich, I. E., Feldbrügge, M., Hahlbrock, K. & Weisshaar, B. (1993) *Plant Mol. Biol.* **21,** 673–684.
40. Korfhage, U., Trezzini, J. F., Meier, I., Hahlbrock, K. & Somssich, I. E. (1994) *Plant Cell* **6,** 695–708.
41. Schulze-Lefert, P., Dangl, J. L., Becker-André, M., Hahlbrock, K. & Schulz, W. (1989) *EMBO J.* **8,** 651–656.
42. Labow, M. A., Baim, S. B., Shenk, T. R. & Levine, A. J. (1990) *Mol. Cell. Biol.* **10,** 3343–3356.
43. Hahlbrock, K., Lamb, C. J., Purwin, C., Ebel, J., Fautz, E. & Schäfer, E. (1981) *Plant Physiol.* **67,** 768–773.
44. Hahlbrock, K. & Scheel, D. (1989) *Annu. Rev. Plant Physiol. Mol. Biol.* **40,** 347–369.
45. Ohl, S., Hahlbrock, K. & Schäfer, E. (1989) *Planta* **177,** 228–236.
46. Frohnmeyer, H., Ehmann, B., Kretsch, T., Rocholl, M., Harter, K., Nagatani, A., Furuya, M., Batschauer, A., Hahlbrock, K. & Schäfer, E. (1992) *Plant J.* **2,** 899–906.
47. Kauss, H., Franke, R., Krause, K., Conrath, U., Jeblick, W., Grimmig, B. & Matern, U. (1993) *Plant Physiol.* **102,** 459–466.
48. Lois, R. & Hahlbrock, K. (1992) *Z. Naturforsch. C* **47,** 90–94.
49. Appert, C., Logemann, E., Hahlbrock, K., Schmid, J. & Amrhein, N. (1994) *Eur. J. Biochem.* **225,** 491–499.
50. Lozoya, E., Hoffmann, H., Douglas, C., Schulz, W., Scheel, D. & Hahlbrock, K. (1988) *Eur. J. Biochem.* **176,** 661–667.
51. Schmelzer, E., Jahnen, W. & Hahlbrock, K. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2989–2993.