Invited Trends Article:

Fungal Peptide Elicitors: Signals Mediating Pathogen Recognition in Plants

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Highly sensitive and specific recognition systems for microbial pathogens are essential for disease resistance in plants. Proteinaceous elicitors activating plant pathogen defense have been identified in numerous antagonistic plant/fungus interactions. Precisely defined signal structures required for elicitor-mediated activation of plant defense are indicative of the involvement of receptors in elicitor perception and subsequent signal generation. Use of pure elicitor preparations has helped to establish a functional link between binding of elicitors to high-affinity binding sites in plant plasma membranes and activation of plant defense. Thus elicitor binding sites appear to function as physiological receptors. Currently, isolation and molecular characterization of elicitor receptors is under way. Transfer of new recognition specificities into plants is supposed to be a key strategy for engineering pathogen resistance in economically important crops.

Introduction

Plants as sessile organisms have evolved sophisticated and unique defense mechanisms to cope with disadvantageous situations, such as pathogen attack. To trigger appropriate protective measures plants need to distinguish between "self" and "non-self". In contrast to antigen recognition and defense activation by the immune system of vertebrates, which is essentially based on the circulation and interaction of highly specialized cells throughout the whole organism, each plant cell is autonomously capable of sensing the presence of potential phytopathogens as well as mounting defense. Conceptual similarities between the immune response of vertebrates and activation of plant pathogen defense have repeatedly been proposed (Baker *et al.*, 1997; Dangl, 1994). The structural basis of defense systems in organisms from either kingdom may, however, be quite different.

Plant pathogen resistance occurs at the cultivar (host resistance, race/cultivar-specific resistance) or species level (non-host resistance, general resistance) mediated by recognition of either race-specific or general elicitors, respectively (Boller, 1995; De Wit, 1995; Ebel and Scheel, 1997; Keen, 1990). The term "elicitor", originally coined for compounds that induce accumulation of antimicrobial phytoalexins in plants, is now commonly applied to agents stimulating any type of defense response (Ebel and Scheel, 1997; Keen and Bruegger, 1977). General elicitors stimulate defense responses in all cultivars of at least one plant species. Infrequent changes in the host range of phytopathogens over recorded history (Heath, 1997) indicate relative stability of non-host resistance, of which genetic determination is poorly understood. Race-specific pathogen recognition is determined by the action of complementary pairs of (semi)dominant resistance (*R*) genes in the host plant and (semi)dominant avirulence (*avr*) genes in the pathogen (De Wit, 1995). Lack or nonfunctional products of either gene would result in colonization of the plant. A biochemical interpretation of this gene-for-gene concept implies a receptor/ligand-like interaction between plant *R* gene products and the corresponding *avr* gene products from the pathogen (De Wit, 1995; Keen, 1990). Race-specific elicitors are indeed very often direct products of *avr* genes which trigger resistance reactions only in host plant cultivars carrying the matching *R* gene (Alfano and Collmer, 1996; De Wit, 1995; Knogge, 1996).

The spectrum of reactions elicited in plants undergoing either type of resistance includes processes that result from transcriptional activation of

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defense-related genes, such as production of lytic enzymes, phytoalexin biosynthesis, and systemic acquired resistance (Ebel and Scheel, 1997; Hammond-Kosack and Jones, 1996). Other plant responses associated with pathogen defense result from allosteric enzyme activation initiating cell wall reinforcement by oxidative cross-linking of cell wall components, apposition of callose and lignins, and production of reactive oxygen intermediates (Dangl *et al.*, 1996; Ebel and Scheel, 1997; Hammond-Kosack and Jones, 1996; Lamb and Dixon, 1997). The molecular basis for the frequently observed highly localized response, hypersensitive cell death, is still uncertain. There is yet no evidence for a causal link between HR and initiation of other defense responses, such as activation of defense-related genes (Dangl *et al.*, 1996).

Our knowledge on the precise molecular mechanisms underlying non-self recognition and intracellular signal generation in plant-pathogen interactions is rapidly expanding. This is particularly true for the interaction between plants and phytopathogenic fungi. Numerous (poly)peptide elicitors have recently been identified, which activate plant responses associated with pathogen resistance. We therefore confine our review to antagonistic plant-fungus interactions mediated by this particular class of elicitors. Instead of comprehensive coverage of the whole field we exemplarily refer to new discoveries in selected well-studied experimental systems. Related aspects on fungal carbohydrate elicitors as well as on elicitors derived from phytopathogenic bacteria are excellently reviewed elsewhere (Baker *et al.*, 1997; Bonas and Van den Ackerveken, 1997; Ebel and Scheel, 1997; Hahn, 1996).

Elicitors of Plant Defense Responses

Elicitors of diverse chemical nature and from a variety of different plant pathogenic fungi have been isolated and shown to trigger defense responses in intact plants or cultured plant cells. These elicitors include (poly)peptides, glycoproteins, lipids, and oligosaccharides. While the first elicitors characterized were predominantly oligosaccharides (Darvill and Albersheim, 1984), research over recent years has revealed a multitude of (poly)peptides mediating initiation of plant pathogen defense (Ebel and Scheel, 1997). Peptide elicitors from phytopathogenic bacteria and viruses have also been identified (Alfano and Collmer, 1996; Bonas and Van den Ackerveken, 1997; Culver and Dawson, 1989). The tremendous structural diversity of purified elicitors rules out that a universal elicitor as general signal for initiation of plant pathogen defense may exist (Boller, 1995; Ebel and Scheel, 1997). The intrinsic function of elicitors in the life cycle of phytopathogenic fungi remains often elusive. A role as pathogenicity factor has been ascribed to race-specific elicitors, but unequivocal evidence has rarely been presented (Knogge, 1996). Race-specific elicitors are often synthesized and secreted only upon infection of the host plant. In contrast, protein elicitors of the general type appear to be permanently present in the fungal cell wall, e.g. as structural components.

Since the first barrier invading fungi have to overcome is the plant cell wall, fungal endohydrolytic enzymes have been suggested to act as elicitors (Cervone *et al.*, 1997). However, elicitor activity of a *Trichoderma viride* endoxylanase stimulating HR, ethylene and phytoalexin production in tobacco and tomato, was found to be independent of enzyme activity (Sharon *et al.*, 1993). Research on endopolygalacturonases has revealed that these enzymes release elicitor-active oligogalacturonide fragments from the plant cell wall rather than being elicitors of defense themselves. This intriguing concept of plant-derived (endogenous) elicitors for activating pathogen defense is very likely to function in many plant-pathogen interactions. Most plants possess a cell wall polygalacturonase-inhibiting protein (PGIP) that can physically interact with fungal cell wall endopolygalacturonases. This interaction may favor release of elicitor-active oligogalacturonides from the plant cell wall over complete depolymerization of cell wall polygalacturonides (Cervone *et al.*, 1997).

In the few cases investigated elicitor activity was found to be determined by small fragments of the intact elicitor molecule, suggesting recognition of "epitope"-like structures by receptors at the plant cell surface. The cultivar or species specificity of elicitors, their ability to induce plant defense responses at trace amounts as well as data from structure-activity relationship studies performed with race-specific and general elicitors also sup-

port the idea that highly specific plant receptors mediate non-self recognition in plant-fungus interactions (Boller, 1995; Ebel and Scheel, 1997). The following examples may illustrate this notion.

Avirulence gene products

Certain races of the barley pathogen *Rhynchosporium secalis* secrete a small protein, NIP1, that acts as a race-specific elicitor of defense gene activation in barley cultivars carrying the resistance gene *Rrs1* (Hahn *et al.*, 1993; Rohe *et al.*, 1995). Proof of avirulence gene function of the *nip1* gene was provided by gene disruption and gene complementation experiments (Knogge, 1996; Rohe *et al.*, 1995). Replacement of the *nip1* gene by a nonfunctional gene in an avirulent race yielded virulent transformants (W. Knogge, personal communication). Transformation of virulent races of the fungus with the *nip1* gene rendered the transformants avirulent only on barley cultivars carrying the *Rrs1* gene. This was further substantiated by experiments in which purified NIP1 protected a barley cultivar carrying *Rrs1* against infection by a virulent fungal race lacking a functional *nip1* allele (Rohe *et al.*, 1995). Avirulence of fungal races on *Rrs1* plants consistently correlated with the production of elicitor-active NIP1. In contrast, virulent races either lack the *nip1* gene or possess a *nip1* allele in which single nucleotide exchanges rendered the corresponding gene product elicitor-inactive (Rohe *et al.*, 1995).

On susceptible barley cultivars, fungal *nip1* disruption transformants exhibited reduced levels of virulence compared with NIP1-expressing wild-type races, suggesting a role of NIP1 as virulence factor (Knogge, 1996). This is corroborated by the non-host specific necrosis-inducing activity of this peptide on all barley cultivars as well as on various mono- and dicotyledonous plants (Knogge, 1996; Wevelsiep *et al.*, 1991). NIP1 exerts its toxic activity partially through indirect activation of the plasma membrane H^+-ATPase (Wevelsiep *et al.*, 1991). Thus, NIP1 may simultaneously act as a general virulence factor and, additionally, as an avirulence factor in resistant barley cultivars. The quantities of NIP1 required to trigger necrosis in barley leaves appear to be substantially higher than those required for defense gene activation in resistant barley cultivars (W. Knogge, personal communication). Intriguingly, resistant host plant cultivars may have acquired the ability to recognize a pathogen through tolerable non-toxic amounts of a fungal virulence factor. At the molecular level, this would be consistent with the existence of two distinct NIP1 receptors differing largely in their ligand affinities. The 82-amino acid product of the *nip1* gene is processed to yield a 60-amino acid mature protein (Rohe *et al.*, 1995). NIP1 contains 10 cysteine residues whose distribution within the complete amino acid sequence is reminiscent of fungal hydrophobins (Wessels, 1996). These cysteines form disulfide bridges which are required for both, the elicitor and toxin activity of this peptide (V. Li and W. Knogge, personal communication).

The products of two *avr* genes (AVR4 and AVR9) from the tomato pathogen *Cladosporium fulvum* act as elicitors of hypersensitive cell death only on tomato cultivars carrying the matching resistance genes *Cf-4* and *Cf-9*, respectively (Joosten *et al.*, 1994; Van den Ackerveken *et al.*, 1992). Gene complementation and disruption experiments similar to those described above for the *nip1* gene have unequivocally proven the role of the *avr4* and *avr9* gene products as determinants of race/cultivar-specific resistance in this interaction (Joosten *et al.*, 1994; Marmeisse *et al.*, 1993; Van den Ackerveken *et al.*, 1992). Expression of the *avr4* and *avr9* genes is specifically induced during pathogenesis (Joosten *et al.*, 1994; Van den Ackerveken *et al.*, 1992). However, *avr4* and *avr9* gene products appear to be dispensible for pathogenicity of the fungus (Joosten *et al.*, 1997). Fungal races virulent on tomato *Cf-9* cultivars completely lack the *avr9* gene (Van den Ackerveken *et al.*, 1992), while virulence on *Cf-4* cultivars results from single point mutations in the coding region of the *avr4* gene (Joosten *et al.*, 1997). Transcripts of the *avr4* gene were found in all virulent fungal races tested upon infection of *Cf-4* tomato cultivars, but elicitor protein was immunologically undetectable. However, infection of *Cf-4* plants with potato virus X expressing *avr4* alleles from virulent races resulted in HR induction. Thus, these gene products appear to be potentially active elicitors. Joosten *et al.* (1997b) conclude that instability of AVR4 isoforms produced by virulent strains are crucial factors in circumvention of *Cf-4*-mediated resistance.

Both, AVR4 and AVR9, are synthesized as larger precursors which upon secretion are proteolytically processed into 105-mer and 28-mer polypeptides, respectively, by either fungal or plant proteases (Joosten *et al.*, 1994; Van den Ackerveken *et al.*, 1993). Elicitor activity of both peptides was shown to depend on disulfide bridge formation between cysteine residues. 2D-^{1}H-NMR studies on the secondary structure and global fold of AVR9 revealed a rigid, barrel-like structure containing three antiparallel β-sheets connected by two loops and three disulfide bridges linking all six cysteine residues in a cystine knot (Vervoort *et al.*, 1997). This structural motif is also found in proteinase inhibitors or animal growth factors which are known to interact with specific target proteins, such as enzymes or receptors. Substitution of single amino acids within AVR9 revealed residues that are essential for elicitor activity. Particularly, the hydrophobic β-loop of AVR9 appears to be crucial for necrosis-inducing activity in *Cf-9* tomato cultivars (Kooman-Gersmann *et al.*, 1997).

Two elicitor peptides from the rust fungus, *Uromyces vignae*, that induce hypersensitive cell death in resistant cowpea cultivars have been purified to homogeneity (D'Silva and Health, 1997). These peptides are the first race-specific elicitors to be isolated from an obligate biotrophic fungus. The heat-stable, acidic, and hydrophobic peptides did not show significant sequence similarity to any known protein. Unlike other fungal race-specific elicitors, these peptides lack cysteine residues. A striking feature of these elicitors is the presence of proline-rich regions, which may define a rapid and strong protein-binding capacity.

General elicitors

Elicitins constitute a family of highly conserved, non-glycosylated 10-kDa proteins that are present in the entire *Phytophthora* genus (except in some highly virulent isolates of the tobacco pathogen *P. parasitica var. nicotianae, Ppn*) as well as some *Pythium* species (Huet *et al.*, 1995; Ricci *et al.*, 1989; Yu, 1995). Elicitins stimulate HR-like leaf necrosis in tobacco, other *Nicotiana* spp. and apparently in a cultivar-specific manner in some radish and turnip cultivars (Bonnet *et al.*, 1996; Kamoun *et al.*, 1994; Kamoun *et al.*, 1993; Ricci *et al.*, 1989). HR-like necrosis is accompanied by systemic protection of the plant against subsequent infection with virulent *Ppn* isolates or the unrelated pathogen, *Sclerotinia sclerotiorum* (systemic acquired resistance, SAR) (Bonnet *et al.*, 1996; Keller *et al.*, 1996). Induction of SAR, however, is not dependent on the presence of elicitins in leaves remote from the site of elicitor application (Keller *et al.*, 1996). Recently, a low-molecular weight diffusible signal was found to be released from cultured tobacco cells treated with the *P. cryptogea* elicitin, cryptogein (Chappell *et al.*, 1997). This compound is capable of triggering activation of the same defense genes as cryptogein, but in cells which are not in immediate contact with this elicitor. Thus, cells directly stimulated by fungal elicitors appear to secrete secondary signal molecules that activate defense responses in neighboring cells thereby amplifying the overall response of challenged plants.

The virulence of *Ppn* on tobacco is inversely correlated with elicitin secretion, implying that elicitins are avirulence factors acting as genus-specific determinants in this plant (Bonnet *et al.*, 1994; Kamoun *et al.*, 1994). This has recently been demonstrated by an elegant approach to inhibit elicitin production in *P. infestans* by gene silencing. Fungal mutants incapable of producing elicitin became highly virulent on *N. benthamiana*, a nonhost plant to wild type *P. infestans* (S. Kamoun and F. Govers, personal communication).

Elicitins fall into two classes according to their leaf necrosis-inducing activity. Acidic α-elicitins, such as capsicein (from *P. capsici*), are 100-fold less toxic than basic β-elicitins, such as cryptogein. Similarly, basic elicitins are 10–50-fold more active than acidic elicitins in inducing SAR in tobacco (Bonnet *et al.*, 1996). Use of recombinant structural derivatives of cryptogein revealed that point mutations consistently affected both, HR- and SAR-inducing activity, in the same way (I. Penot and P. Ricci, personal communication). Elicitation of necrosis and SAR appears therefore to be mediated by a single elicitin receptor. To identify domains within elicitins that are sufficient for elicitor activity, Perez *et al.* (1997) used synthetic 10- to 18-mer peptides covering different parts of capsicein and cryptogein, respectively, as elicitors of HR and PR gene expression. This study, however, concludes that two different defense pathways are independently induced by different domains of elicitins.

The 10 elicitins sequenced so far share more than 60% sequence homology at the amino acid level (Perez *et al.*, 1997). Only very few residues were identified as key determinants accounting for much of the observed difference in necrotic activity of the two elicitin types (O'Donohue *et al.*, 1995). Six conserved cysteine residues form three disulfide bridges crucial for necrotic activity (Kamoun *et al.*, 1997; Ricci *et al.*, 1989). X-ray crystallography of cryptogein revealed a complex structure of six a-helices, an antiparallel two-stranded β-sheet, and an Ω-loop. This motif is assumed to be a recognition site for a putative receptor (Boissy *et al.*, 1996).

A 42-kDa cell wall glycoprotein of *Phytophthora sojae* induces transcriptional activation of defense genes and accumulation of furanocoumarin phytoalexins in parsley cell cultures and protoplasts (Nürnberger *et al.*, 1994; Parker *et al.*, 1991). This response can be also observed in parsley seedlings upon infection with zoospores of the fungus (Hahlbrock *et al.*, 1995). Characterization of corresponding cDNA clones revealed that the gene encodes a 57-kDa precursor protein (Sacks *et al.*, 1995). This suggests proteolytic processing of the gene product into the mature 42-kDa protein. An internal peptide of 13 amino acids (Pep-13) was found to be necessary and sufficient for elicitor activity of the intact glycoprotein (Hahlbrock *et al.*, 1995; Nürnberger *et al.*, 1994; Sacks *et al.*, 1995). The amino acid sequences of the oligopeptide and the intact protein elicitor did not show any significant homology to known sequences (Sacks *et al.*, 1995). Substitution analysis, in which individual amino acids of Pep-13 were progressively replaced by alanine, identified only two residues critical for activity. ^{1}H-NMR studies revealed a random-coil-like structure of the oligopeptide in aqueous solution (J. Vervoort, personal communication).

Elicitor Perception

Highly sensitive perception systems for either pathogen-derived (exogenous) or plant-derived (endogenous) elicitors are the key to successful plant pathogen defense. Plant receptors are instrumental for the recognition of these signals as well as for the initiation of an intracellular signal transduction cascade leading to activation of multifacetted defense reactions. A contact-dependent transfer mechanism similar to the type III secretory pathway of mammalian bacterial pathogens appears to mediate delivery of *avr* gene products from phytopathogenic bacteria into host plant cells. In the host cell cytosol bacterial *avr* gene products are assumed to interact directly with their corresponding plant *R* gene products (Bonas and Van den Ackerveken, 1997). The molecular architecture of such perception systems for fungal *avr* gene products, however, appears to be different (Joosten *et al.*, 1997; Kooman-Gersmann *et al.*, 1996).

While fungal *avr* genes often encode race-specific elicitors (Knogge, 1996), the role of plant *R* gene products in elicitor perception remains rather unclear. A saturable high-affinity binding site for AVR9 from *C. fulvum* (K_d=0.07 nM) was indistinguishably detectable in all tomato cultivars including those lacking the *Cf-9* resistance gene (Kooman-Gersmann *et al.*, 1996). Moreover, all solanaceous plant species tested possessed this binding site while non-solanaceous plants did not. The presence of the AVR9 binding site did correlate with the presence of members of the *Cf-9* gene family, but apparently not with the presence of a functional allele of this *R* gene (Kooman-Gersmann *et al.*, 1996). Synthetic mutant AVR9 peptides as well as AVR9 mutant peptides purified from PVX::AVR9-infected tobacco plants were used as competitors in binding assays and as elicitors of HR in resistant tomato cultivars (P. De Wit, personal communication). Since binding activity of these peptides always correlated with their HR-inducing activity, receptor function of the AVR9 binding site seems plausible. Taken together, these data appear to argue against the *Cf-9* gene product being the primary target site for the elicitor. It may, however, be possible that nonfunctional *Cf-9* homologs are still capable of binding AVR9 but lack the ability to initiate an intracellular signaling cascade. It is also conceivable that the *Cf-9* gene product is recruited by the AVR9 elicitor/receptor complex to form a heteromeric complex.

Isolated plant *R* genes conferring resistance to phytopathogenic fungi share common sequence motifs such as transmembrane domains, nucleotide binding sites and multiple leucine-rich repeats (Baker *et al.*, 1997; Hammond-Kosack and Jones, 1997). This latter domain in particular is thought

to mediate protein-protein interactions and ligand binding in eukaryotic signal-transducing proteins (Kobe and Deisenhofer, 1994). Leucine-rich repeats are found in various proteins that differ in their function and cellular location. Determination of the cellular location of *R* gene products will therefore be important to answering the question whether they are implicated in *avr* gene product recognition or downstream signaling at early rate-limiting steps in the signal transduction cascade (Hammond-Kosack and Jones, 1997). However, differences in the molecular function of *R* gene products from different plants may also be anticipated.

High-affinity receptors for fungal elicitors of the general type have been reported to reside in the plasma membrane of plant cells (Boller, 1995; Ebel and Scheel, 1997). A single class, high-affinity receptor for the *P. sojae*-derived oligopeptide Pep-13 has recently been identified in parsley membrane preparations and protoplasts (K_d=2.4 nM) (Nürnberger *et al.*, 1994). Binding sites with very similar affinity constants in tobacco membrane preparations have been reported for the *P. cryptogea* β-elicitin cryptogein (Wendehenne *et al.*, 1995) and for the *T. viride* endoxylanase (Hanania and Avni, 1997) as well as for a yeast invertase glycopeptide fragment (gp8c) in tomato (Basse *et al.*, 1993). While significantly different from the K_d of the AVR9 receptor, these affinity constants appear to be characteristic for receptors of proteinaceous as well as non-proteinaceous general elicitors (Ebel and Scheel, 1997). Another common feature of all elicitor binding sites is their low abundance, e. g. parsley cells harbor approximately 1600 Pep-13 binding sites per cell (Nürnberger *et al.*, 1994). In general, kinetic properties of these elicitor-binding proteins, such as high affinity, saturability, and reversibility of ligand binding together with a direct correlation between the binding affinities and the elicitor activities of the respective ligands, indicate that such elicitor binding sites function as physiological receptors. Use of structural derivatives of Pep-13 or gp8c, respectively, allowed the demonstration of a functional link between signal perception and elicitation of a physiological response in the plant cell (Basse *et al.*, 1993; Nürnberger *et al.*, 1994). The dual function of receptors, i.e., perception of an extracellular signal on the one hand and initiation of an intracellular signal transduction cascade on the other hand, is nicely exemplified by the tomato gp8c-glycopeptide receptor. While the carbohydrate moiety of gp8c was found to compete binding of intact gp8c to its receptor, ethylene production-inducing activity of gp8c was dependent on the intact ligand (Basse *et al.*, 1993). Thus, ligand domains responsible for signal recognition and intracellular signal generation can be structurally separated from each other in this molecule.

Current efforts are directed towards the isolation of elicitor receptors and the cloning of cDNAs encoding these molecules. Unfortunately, the apparent lack of specific plant target cells or tissues, in which the respective elicitor receptor is predominantly expressed, has significantly hampered isolation of elicitor receptors. In a first step, chemical cross-linking experiments with homobifunctional or photoaffinity reagents, respectively, as well as labeled ligands have been performed to elucidate the subunit structure of elicitor receptors. For example, a 91-kDa monomeric parsley plasma membrane protein was identified that most likely represents the Pep-13 receptor (Nürnberger *et al.*, 1995). Very recently, purification of a 75-kDa soybean plasma membrane protein was reported. This protein interacts with elicitors of phytoalexin production in soybean seedlings, such as a *P. sojae*-derived mixture of structural isomers of β-glucans or a synthetic hepta-β-glucoside (Mithöfer *et al.*, 1996; Umemoto *et al.*, 1997). A cDNA encoding this protein was isolated and used for production of recombinant receptor in *E. coli*, cultured tobacco cells (Umemoto *et al.*, 1997) or baculovirus-infected insect cells (A. Mithöfer and J. Ebel, personal communication). Evidence for the recombinant protein representing the hepta-β-glucan elicitor, however, is lacking.

At present no elicitor receptor has been isolated nor has receptor function unequivocally been ascribed to any of the cloned plant resistance genes conferring resistance to fungal pathogens. In addition, molecular genetic analysis of various plant mutants impaired in pathogen defense has not revealed genes whose products may function as elicitor receptors (Hammond-Kosack and Jones, 1997).

Intracellular Signal Generation

Ligand binding to cell-surface receptors is assumed to impose a conformational change on the

receptor, resulting in initiation of an intracellular signal transduction cascade and finally in activation of a specific cellular response. Defined by the transduction mechanism involved, most cell-surface receptors of eukaryotic cells belong to either the class of ion-channel-linked receptors, G-protein-linked receptors or enzyme-linked receptors (e.g. receptor protein kinases). In contrast to the multitude of cell surface receptors characterized in animal cells, the molecular structure of plant receptors in general is barely understood. Use of specific antibodies and various pharmacological effectors has provided evidence for the involvement of heterotrimeric as well as small GTP-binding proteins, a number of serine/threonine protein kinases, elements of the MAP-kinase pathway, and protein phosphatases in elicitor-mediated plant defense responses (Ebel and Scheel, 1997; Felix *et al.*, 1994; Hammond-Kosack and Jones, 1996; Kieffer *et al.*, 1997; Ligterink *et al.*, 1997; Viard *et al.*, 1994). These elements are constituents of numerous well-defined signal transduction cascades in animal cells and are activated upon ligand binding to either G-protein-linked receptors or receptor protein kinases. Plant signal transduction chains may therefore be connected to similar receptor types mediating extracellular signal perception and generation of an intracellular signal.

Plant cells carry cell surface proteins with intrinsic protein kinase activity. These plant receptor-like kinases (RLK) structurally resemble receptor protein kinases of animal cells containing large extracytoplasmic domains, single transmembrane spanning segments and cytoplasmic kinase domains (Braun and Walker, 1996). While animal receptor kinases are predominantly tyrosine kinases recognizing exclusively peptide ligands, plant receptor-like kinases are serine/threonine kinases whose cognate ligands are very often unknown. Plant RLK's are involved in many physiological processes, such as sporophytic self-incompatibility, plant growth and organogenesis, and cell differentiation (Braun and Walker, 1996). Interestingly, the rice gene *Xa21* conferring resistance to the bacterial pathogen, *Xanthomonas oryzae*, encodes a receptor-like protein kinase (Song *et al.*, 1995). It remains to be seen whether plant cells employ RLK's to recognize fungus-derived peptide ligands and trigger pathogen defense. However, a particular mode of action will only be ascribed to plant elicitor receptors upon isolation of these proteins and the encoding genes.

Depolarization of the plant plasma membrane is among the earliest responses of plant cells to elicitor treatment, which likely results from changes in the ion permeability of the plasma membrane (Ebel and Scheel, 1997; Pugin *et al.*, 1997). Ion fluxes observed in Pep-13-treated parsley cells comprise influxes of H^+ and Ca^{2+} as well as effluxes of K^+ and Cl^- (Jabs *et al.*, 1997; Nürnberger *et al.*, 1994). Similarly, tobacco cells respond to treatment with cryptogein or xylanase with H^+/Ca^{2+} influx and Cl^- or K^+efflux, respectively (Bailey *et al.*, 1992; Pugin *et al.*, 1997; Tavernier *et al.*, 1995). In addition, xylanase caused extracellular alkalinization in cultured tomato cells (Felix *et al.*, 1994).

Extracellular Ca^{2+} appears to be crucial to induction of plant pathogen defense since absence of extracellular Ca^{2+}, or inhibition of Ca^{2+} influx by Ca^{2+} channel inhibitors blocked not only elicitor-induced Ca^{2+} influx but also plant defense reactions in the tobacco and parsley system (Jabs *et al.*, 1997; Nürnberger *et al.*, 1994; Pugin *et al.*, 1997; Tavernier *et al.*, 1995). Recently, patch-clamp analyses on parsley protoplasts led to the identification of a Pep-13-activated Ca^{2+}-inward ion channel (Zimmermann *et al.*, 1997) that may contribute to elevated levels of cytosolic Ca^{2+} concentrations in elicitor-treated parsley cells (Nürnberger *et al.*, 1994). Activation of defense responses in both tobacco and parsley cells required sustained Ca^{2+} influx (Jabs *et al.*, 1997; Tavernier *et al.*, 1995). Artificial stimulation by certain ionophores of transient ion fluxes in parsley cells resulted in activation of only a subset of elicitor-induced defense genes, while other ionophores induced the same set of defense genes and phytoalexin production as did the elicitor (Jabs *et al.*, 1997). This is reminiscent of a recent report on differential activation of transcription factors induced by Ca^{2+} response amplitude and duration in animal cells, which suggests that specificity in signaling cascades can be determined by signal quality as well as signal quantity (Dolmetsch *et al.*, 1997).

Protein phosphorylation appears to be required for activation of elicitor-induced ion fluxes. Inhibitors of serine/threonine kinases such as staurosporine were found to inhibit Ca^{2+} influx in cryptogein-treated tobacco cells, which in itself is

necessary for induction of the other ion fluxes observed in this system as well as phytoalexin production (Tavernier *et al.*, 1995; Viard *et al.*, 1994). Similarly, staurosporine inhibited extracellular alkalinization and ethylene biosynthesis in xylanase-treated tomato cells, while a phosphatase inhibitor, calyculin A, activated both responses in absence of elicitor (Felix *et al.*, 1994). Direct regulation of elicitor receptor function by phosphorylation/dephosphorylation, however, has yet to be demonstrated.

Conclusion

Striking similarities in the pattern of plant defense responses upon fungal infection as well as among elements implicated in elicitor-activated signal transduction cascades have been observed in plants establishing either cultivar-specific or species-specific resistance (Ebel and Scheel, 1997; Hammond-Kosack and Jones, 1996). This is in clear contrast to the bewildering diversity of race-specific and general elicitors identified, which may reflect a complementary diversity of elicitor receptors with distinct ligand specificities in plants. Thus, specificity in the interaction between plants and phytopathogenic fungi appears to be mediated largely at the level of signal perception. Molecular analysis of elicitor receptors, studies on the subcellular localization of plant *R* gene products and on their possible physical interaction with fungal *avr* gene products will ultimately answer the question whether molecular mechanisms of signal perception and intracellular signal generation are conserved regardless of the type of resistance involved. Molecular characterization of elicitor receptors will also shed light on how receptor/ligand interactions trigger intracellular signal transduction cascades mediating activation of plant defense.

A novel strategy in molecular plant breeding aims at conferring recognition mechanisms for surface components of phytopathogenic fungi from resistant plants (of either type of resistance) to economically important, but susceptible host plants. Plant resistance genes as well as genes encoding elicitor receptors mediating pathogen recognition in non-host plants appear to be suitable for this purpose. In addition, genes encoding non-host plant elicitor receptors will prove valuable not only for engineering this rather stable type of resistance in crop plants, but also for uncovering the genetic basis of non-host resistance in plants.

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Alfano J. R., Collmer A. (1996), Bacterial pathogens in plants: Life up against the wall. Plant Cell **8**, 1683–1698.

Bailey B. A., Korcak R. F., Anderson J. D. (1992), Alterations in *Nicotiana tabacum* L. cv *Xanthi* cell membrane function following treatment with an ethylene biosynthesis-inducing endoxylanase. Plant Physiol. **100**, 749–755.

Baker B., Zambryski P., Staskawicz B., Dinesh-Kumar S. P. (1997), Signaling in plant-microbe interactions. Science **276**, 726–733.

Basse C. W., Fath A., Boller T. (1993), High affinity binding of a glycopeptide elicitor to tomato cells and microsomal membranes and displacement by specific glycan suppressors. J. Biol. Chem. **268**, 14724–14731.

Boissy G., Delafortelle E., Kahn R., Huet J. C., Bricogne G., Pernollet J. C., Brunie S. (1996), Crystal structure of a fungal elicitor secreted by *Phytophthora cryptogea*, a member of a novel class of plant necrotic proteins. Structure **4**, 1429–1439.

Boller T. (1995), Chemoperception of microbial signals in plant cells. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46**, 189–214.

Bonas U., Van den Ackerveken G. (1997), Recognition of bacterial avirulence proteins occurs inside the plant cell: a general phenomenon in resistance to bacterial diseases? Plant J. **12**, 1–7.

Bonnet P., Bourdon E., Ponchet M., Blein J. P., Ricci P. (1996), Acquired resistance triggered by elicitins in tobacco and other plants. Eur. J. Plant Pathol. **102**, 181–192.

Bonnet P., Lacourt I., Venard P., Ricci P. (1994), Diversity in pathogenicity of tobacco and in elicitin production among isolates of *Phytophthora parasitica*. J. Phytopathol. **141**, 25–37.

Braun D. M., Walker J. C. (1996), Plant transmembrane receptors: new pieces in the signaling puzzle. Trends. Biochem. Sci. **21**, 70–74.

Cervone F., Castoria R., Leckie F. D., De Lorenzo G. (1997), Perception of fungal elicitors and signal trans-

duction. In: Signal Transduction in Plants (P. Aducci, ed.), Birkhäuser Verlag, Basel, 153–177.
Chappell J., Levine A., Tenhaken R., Lusso M., Lamb C. (1997), Characterization of a diffusible signal capable of inducing defense gene expression in tobacco. Plant Physiol. **113**, 621–629.
Culver J. N., Dawson W. O. (1989), Tobacco mosaic virus elicitor coat protein: An elicitor of the hypersensitive response but not required for the development of mosaic symptoms in *Nicotiana sylvestris*. Virology **173**, 755–758.
Dangl J. L. (1994), The major histocompatibility complex à la carte: are there analogies to plant disease resistance genes on the menu? Plant J. **2**, 3–11.
Dangl J. L., Dietrich R. A., Richberg M. H. (1996), Death dont have no mercy: cell death programs in plant-microbe interactions. Plant Cell **8**, 1793–1807.
Darvill A. G., Albersheim P. (1984), Phytoalexins and their elicitors – a defense against microbial infection in plants. Annu. Rev. Plant. Physiol. **35**, 243–275.
De Wit P. J. G. M. (1995), Fungal avirulence genes and plant resistance genes: Unravelling the molecular basis of gene-for-gene interactions. In: Advances in Botanical Research (J. H. Andrews and I. C. Tommerup, eds.), Academic Press, London, **Vol. 21**, 147–185.
Dolmetsch R. E., Lewis R. S., Goodnow C. C., Healy J. I. (1997), Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. Nature **386**, 855–858.
D'Silva I., Heath M. C. (1997), Purification and characterization of two novel hypersensitive response-inducing specific elicitors produced by the cowpea rust fungus. J. Biol. Chem. **272**, 3924–3927.
Ebel J., Scheel D. (1997), Signals in host-parasite interactions. In: The Mycota. Plant Relationships, Part A (G. C. Carroll and P. Tudzynski, eds.), Springer-Verlag, Berlin Heidelberg, **Vol. V**, 85–105.
Felix G., Regenass M., Spanu P., Boller T. (1994), The protein phosphatase inhibitor calyculin A mimics elicitor action in plant cells and induces rapid hyperphosphorylation of specific proteins as revealed by pulse labeling with [^{33}P]phosphate. Proc. Natl. Acad. Sci. USA **91**, 952–956.
Hahlbrock K., Scheel D., Logemann E., Nürnberger T., Parniske M., Reinhold S., Sacks W. R., Schmelzer E. (1995), Oligopeptide elicitor-mediated defense gene activation in cultured parsley cells. Proc. Natl. Acad. Sci. USA **92**, 4150–4157.
Hahn M., Jüngling S., Knogge W. (1993), Cultivar-specific elicitation of barley defense reactions by the phytotoxic peptide NIP1 from *Rhynchosporium secalis*. Mol. Plant-Microbe Interact. **6**, 745–754.
Hahn M. G. (1996), Microbial elicitors and their receptors in plants. Annu. Rev. Phytopathol. **34**, 387–412.
Hammond-Kosack K. E., Jones J. D. G. (1996), Resistance gene-dependent plant defense responses. Plant Cell **8**, 1773–1791.
Hammond-Kosack K. E., Jones J. D. G. (1997), Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**, 575–607.
Hanania U., Avni A. (1997), High-affinity binding site for ethylene-inducing xylanase elicitor on *Nicotiana tabacum* membranes. Plant J. **12**, 113–120.
Heath M. C. (1997), Evolution of plant resistance and susceptibility to fungal parasites. In: Plant Relationships, Part B (G. C. Carroll and P. Tudzynski, eds.), Springer-Verlag, Berlin Heidelberg, **Vol. V**, 257–276.
Huet J. C., Le C. J. P., Nespoulous C., Pernollet J. C. (1995), The relationships between the toxicity and the primary and secondary structures of elicitinlike protein elicitors secreted by the phytopathogenic fungus *Pythium vexans*. Mol. Plant Microbe Interact. **8**, 302–310.
Jabs T., Tschöpe M., Colling C., Hahlbrock K., Scheel D. (1997), Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc. Natl. Acad. Sci. USA **94**, 4800–4805.
Joosten M. H. A. J., Cozijnsen T. J., de Wit P. J. G. M. (1994), Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature **367**, 384–386.
Joosten M. H. A. J., Honee G., Van Kan J. A. L., De Wit P. J. G. M. (1997), The gene-for-gene concept in plant-pathogen interactions: Tomato-*Cladosporium fulvum*. In: The Mycota. Plant Relationships Part B (G. C. Carroll and P. Tudzynski, eds.), Springer-Verlag, Berlin Heidelberg, **Vol. V**, 3–16.
Joosten M. H. A. J., Vogelsang R., Cozijnsen T. J., Verberne M. C., De Wit P. J. G. M. (1997), The biotrophic fungus *Cladosporium fulvum* circumvents Cf-4-mediated resistance by producing unstable AVR4 elicitors. Plant Cell **9**, 367–379.
Kamoun S., van West P., de Jong A. J., de Groot K. E., Vleeshouwers V. G. A. A., Govers F. (1997), A gene encoding a protein elicitor of *Phytophthora infestans* is down-regulated during infection of potato. Mol. Plant-Microbe Interact. **10**, 13–20.
Kamoun S., Young M., Foerster H., Coffey M. D., Tyler B. M. (1994), Potential role of elicitins in the interaction between Phytophthora species and tobacco. Appl. Environ. Microbiol. **60**, 1593–1598.
Kamoun S., Young M., Glascock C. B., Tyler B. M. (1993), Extracellular protein elicitors from Phytophthora: host-specificity and induction of resistance to bacterial and fungal phytopathogens. Mol. Plant Microbe Interact. **6**, 15–25.
Keen N. T. (1990), Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. **24**, 447–463.
Keen N. T., Bruegger B. (1977), In: Host Plant Resistance to Pests (P. A. Hedin, ed.), American Chemical Society, Washington, 1–26.
Keller H., Blein J. P., Bonnet P., Ricci P. (1996), Physiological and molecular characteristics of elicitin induced systemic acquired resistance in tobacco. Plant Physiol. **110**, 365–376.
Kieffer F., Simon-Plas F., Maume B. F., Blein J. P. (1997), Tobacco cells contain a protein, immunologically related to the neutrophil small G protein Rac2 and involved in elicitor-induced oxidative burst. FEBS Lett. **403**, 149–153.
Knogge W. (1996), Fungal infection of plants. Plant Cell **8**, 1711–1722.
Kobe B., Deisenhofer J. (1994), The leucine-rich repeat: A versatile binding motif. Trends Biochem. **19**, 415–421.
Kooman-Gersmann M., Vogelsang R., Hoogendijk E. C. M., De Wit P. J. G. M. (1997), Assignment of

amino acid residues of the AVR9 peptide of *Cladosporium fulvum* that determine elicitor activity. Mol. Plant-Microbe Interact. **10**, 821–829.

Kooman-Gersmann M., Honee G., Bonnema G., De Wit P. J. G. M. (1996), A high-affinity binding site for the AVR9 peptide elicitor of *Cladosporium fulvum* is present on plasma membranes of tomato and other solanaceous plants. Plant Cell **8**, 929–938.

Lamb C., Dixon R. A. (1997), The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**, 251–275.

Ligterink W., Kroj T., zur Nieden U., Hirt H., Scheel D. (1997), Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science **276**, 2054–2057.

Marmeisse R., van den Ackerveken G. F. J. M., Goosen T., de Wit P. J. G. M., van den Broeck H. W. J. (1993), Disruption of the avirulence gene *avr9* in two races of the tomato pathogen *Cladosporium fulvum* causes virulence on tomato genotypes with the complementary resistence gene *Cf9*. Mol. Plant-Microbe Interact. **6**, 412–417.

Mithöfer A., Lottspeich F., Ebel J. (1996), One-step purification of the beta-glucan elicitor-binding protein from soybean (*Glycine max* L.) roots and characterization of an anti-peptide antiserum. FEBS Lett. **381**, 203–207.

Nürnberger T., Nennstiel D., Hahlbrock K., Scheel D. (1995), Covalent cross-linking of the *Phytophthora megasperma* oligopeptide elicitor to its receptor in parsley membranes. Proc. Natl. Acad. Sci. USA **92**, 2338–2342.

Nürnberger T., Nennstiel D., Jabs T., Sacks W. R., Hahlbrock K., Scheel D. (1994), High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. Cell **78**, 449–460.

O'Donohue M. J., Gousseau H., Huet J.-C., Tepfer D., Pernollet J.-C. (1995), Chemical synthesis, expression and mutagenesis of a gene encoding β-cryptogein, an elicitin produced by *Phytophthora cryptogea*. Plant Mol. Biol. **27**, 577–586.

Parker J. E., Schulte W., Hahlbrock K., Scheel D. (1991), An extracellular glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* elicits phytoalexin synthesis in cultured parsley cells and protoplasts. Mol. Plant-Microbe Interact. **4**, 19–27.

Perez V., Huet J.-C., Nespoulos C., Pernollet J.-C. (1997), Mapping the elicitor and necrotic sites of Phytophthora elicitins with synthetic peptides and reporter genes controlled by tobacco defense gene promoters. Mol. Plant-Microbe Interact. **10**, 750–760.

Pugin A., Frachisse J.-M., Tavernier E., Bligny R., Gout E., Douce R., Guern J. (1997), Early events induced by the elicitor cryptogein in tobacco cells: Involvement of a plasma membrane NADPH oxidase and activation of glycolysis and pentose phosphate pathway. Plant Cell **9**, 2077–2091.

Ricci P., Bonnet P., Huet J.-C., Sallantin M., Beauvais-Cante F., Bruneteau M., Billard V., Michel G., Pernollet J.-C. (1989), Structure and activity of proteins from pathogenic fungi Phytophthora eliciting necrosis and acquired resistance in tobacco. Eur. J. Biochem. **183**, 555–563.

Rohe M., Gierlich A., Hermann H., Hahn M., Schmidt B., Rosahl S., Knogge W. (1995), The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the *Rrs1* resistance genotype. EMBO J. **14**, 4168–4177.

Sacks W., Nürnberger T., Hahlbrock K., Scheel D. (1995), Molecular characterization of nucleotide sequences encoding the extracellular glycoprotein elicitor from *Phytophthora megasperma*. Mol. Gen. Genet. **246**, 45–55.

Sharon A., Fuchs Y., Anderson J. D. (1993), The elicitation of ethylene biosynthesis by a Trichoderma xylanase is not related to the cell wall degradation activity of the enzyme. Plant Physiol. **102**, 1325–1329.

Song W. Y., Wang G. L., Chen L. L., Kim H. X., Pi L. Y., Holsten T., Gardner J., Wang B., Zhai W. X., Zhu L. H., Fauquet C., Ronald P. (1995), A receptor kinase-like protein encoded by the rice disease resistence gene *Xa21*. Science **270**, 1804–1806.

Tavernier E., Wendehenne D., Blein J. P., Pugin A. (1995), Involvement of free calcium in action of cryptogein, a proteinaceous elicitor of hypersensitive reaction in tobacco cells. Plant Physiol. **109**, 1025–1031.

Umemoto N., Kakitani M., Iwamatsu A., Yoshikawa M., Yamaoka N., Ishida I. (1997), The structure and function of a soybean beta glucan elicitor binding protein. Proc. Natl. Acad. Sci. USA **94**, 1029–1034.

Van den Ackerveken G. F. J. M., Van Kan J. A. L., De Wit P. J. G. M. (1992), Molecular analysis of the avirulence gene *avr9* of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. **2**, 359–366.

Van den Ackerveken G. F. J. M., Vossen P., De Wit P. J. G. M. (1993), The AVR9 race-specific elicitor of *Cladosporium fulvum* is processed by endogenous and plant proteases. Plant J. **103**, 91–96.

Vervoort J. J. M., Berg A., Vossen P., Vogelsang R., Joosten M. H. A. J., De Wit P. J. G. M. (1997), The race-specific elicitor AVR9 of the tomato pathogen *Cladosporium fulvum*: A cystine knot protein. Sequence-specific 1H NMR assignments, secondary structure and global fold of the protein. FEBS Lett. **404**, 153–158.

Viard M.-P., Martin F., Pugin A., Ricci P., Blein J.-P. (1994), Protein phosphorylation is induced in tobacco cells by the elicitor cryptogein. Plant Physiol. **104**, 1245–1249.

Wendehenne D., Binet M.-N., Blein J.-P., Ricci P., Pugin A. (1995), Evidence for specific, high-affinity binding sites for a proteinaceous elicitor in tobacco plasma membranes. FEBS Lett. **374**, 203–207.

Wessels J. G. H. (1996), Fungal hydrophobins: proteins that function at an interface. Trends Plant Sci. **1**, 9–14.

Wevelsiep L., Kogel K.-H., Knogge W. (1991), Purification and characterization of peptides from *Rhynchosporium secalis* inducing necrosis in barley. Physiol. Mol. Plant Pathol. **39**, 471–482.

Yu L. M. (1995), Elicitins from Phytophthora and basic resistance in tobacco. Proc. Natl. Acad. Sci. USA **92**, 4088–4094.

Zimmermann S., Nürnberger T., Frachisse J.-M., Wirtz W., Guern J., Hedrich R., Scheel D. (1997), Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc. Natl. Acad. Sci. USA **94**, 2751–2755.