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# Signal perception in plant pathogen defense

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**Abstract.** Highly sensitive and specific recognition systems for microbial pathogens are essential for disease resistance in plants. Structurally diverse elicitors from various pathogens have been identified and shown to trigger plant defense mechanisms. Elicitor recognition by the plant is assumed to be mediated by receptors. Plant receptors for fungus-derived elicitors appear to reside preferentially in the plasma membrane, whereas viral and bacterial elicitors may enter the plant cell and are perceived intracellularly. Receptor activation initiates an intracellular signal transduction cascade leading to stimulation of a characteristic set of plant defense responses. Isolation of plant elicitor receptors and their encoding genes is expected to provide significant information on the molecular basis of signal perception and intracellular signal generation in plant-pathogen interactions.

**Key words.** Avirulence gene; elicitor; elicitor receptor; pathogen defense; resistance gene; signal transduction.

## Introduction

Adaptation to environmental conditions is a general feature of all living organisms. In particular, plants as sessile organisms have evolved sophisticated and unique mechanisms to tolerate nonoptimal life situations or to resist microbial pathogen attack. To trigger appropriate protective measures against invading pathogens plants need to distinguish between 'self' and 'nonself'. 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 defenses have repeatedly been proposed [1, 2]. The structural basis of defense systems in organisms from either kingdom may, however, be quite different.

In their natural environment plants encounter a vast array of potential phytopathogens, such as viruses, bacteria, fungi and nematodes. Nevertheless, in the majority of cases plants withstand pathogen attack, and successful colonization of the plant is the exception rather than the rule in plant-pathogen interactions (nonhost resistance, species resistance). The reasons why a plant may be an inappropriate host for most potential phytopathogens are several [3]: (i) the plant does not support the lifestyle of an invading pathogen and thus does not serve as a substrate for microbial growth; (ii) plants possess preformed structural barriers or are equipped with antimicrobial compounds that prevent pathogen ingress and spread; or (iii) the plant may recognize the pathogen and initiate its endogenous multicomponent defense system. Attempted infection of a nonhost plant by a particular pathogen may thus not necessarily be counteracted by an active plant defense response. It is yet unknown to what extent induced defense responses contribute to nonhost resistance in plants. Infrequent changes in the host range of phytopathogens over recorded history [4] indicate relative genetic stability of nonhost resistance. This is likely due to functionally redundant signal perception and plant defense mechanisms, which may constitute the molecular basis of this particular type of resistance. Generally, the genetic determination of nonhost resistance is poorly understood.

Only in the minority of cases have pathogens developed effective mechanisms for circumventing the defense machinery of a particular plant species, allowing them to successfully colonize the species (basic or host susceptibility). Particular cultivars of these species, however, possess the ability to recognize certain strains or races

of the pathogen species and, consequently, mount an efficient resistance response (host resistance, race/cultivar-specific resistance) [4–6]. 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 [5, 6]. Lack or nonfunctional products of either gene would result in colonization of the plant. Genetic evidence for this gene-for-gene relationship was first provided by Flor's [7] pioneering work on the interaction between flax and the causal agent of flax rust, the phytopathogenic fungus *Melampsora lini*. 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 [6, 8].

The spectrum of reactions elicited in plants undergoing either type of resistance is complex, but nevertheless strikingly similar. Plant defense mechanisms include processes that result from transcriptional activation of defense-related genes, such as production of lytic enzymes, phytoalexin biosynthesis and systemic acquired resistance [3, 9]. 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 [3, 9–11]. The molecular basis for a very frequently observed highly localized response, hypersensitive cell death, is still uncertain. A fundamental requirement for the activation of the plant's surveillance system are highly sensitive and specific perception mechanisms for microbial pathogens. A widely accepted hypothesis assumes that activation of plant defense in incompatible plant-microbe interactions results from recognition by the plant of either cell surface constituents of the pathogen or factors that are produced and secreted by the pathogen upon contact with the host plant. Plant-derived elicitors released from the plant cell wall by fungal hydrolytic enzymes are thought to act in a way similar to pathogen-derived elicitors [9, 12]. Accumulating evidence indicates that high-affinity receptors for pathogen-derived signals do function either at the plant cell surface or intracellularly [6, 9, 13], mediating conversion of an extracellular signal into an intracellular signal. Subsequently, an intracellular signal transduction cascade is initiated, triggering activation of the defense arsenal of the challenged host plant cell [3, 9].

Although still fragmentary, our knowledge of the precise molecular mechanisms underlying nonself recognition and intracellular signal transduction in plant-pathogen interactions is rapidly expanding. Instead of attempting comprehensive coverage of the whole field I therefore focus on recent discoveries in selected well-studied experimental systems which are representative of a much larger number of antagonistic plant-microbe interactions. Further information on topics which receive limited attention here can be obtained from excellent reviews published recently [2, 3, 6, 9–11, 13–18].

## Elicitors of plant pathogen defense

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 [9, 19]. Elicitors of diverse chemical nature and from a variety of different plant pathogenic microbes have been characterized and shown to trigger defense responses in intact plants or cultured plant cells. These elicitors include (poly)peptides, glycoproteins, lipids and oligosaccharides (table 1). While the first elicitors characterized were predominantly oligosaccharides [20], research over recent years has revealed a multitude of viral, bacterial or fungal (poly)peptides, respectively, which trigger initiation of plant pathogen defense [9, 21–23]. The tremendous structural diversity of purified elicitors rules out the existence of a universal structural motif as a general signal for initiation of plant pathogen defense [9, 24].

Plant pathogen resistance occurs at the cultivar or species level, and is believed to be mediated by recognition of race-specific or race-nonspecific (general) elicitors, respectively [6, 8, 9]. General elicitors stimulate defense responses in all cultivars of at least one plant species or even at the plant family level. In contrast, according to the gene-for-gene hypothesis, race-specific elicitors are considered to be either direct or indirect products of avirulence genes, conferring resistance only to host plant cultivars carrying the corresponding resistance gene [4–6]. Race-specific elicitors have indeed very often been found to be direct products of *avr* genes [6, 15, 21]. An exception to this rule are syringolides, which are glycolipid elicitors produced by gram-negative bacteria expressing *Pseudomonas syringae* pv. *tomato* avirulence gene *D* (*avrD*) [25, 26]. The *avrD* gene product directs a genotype-specific hypersensitive response (HR) in soybean plants carrying the *R* gene *Rpg4*. It likely encodes an enzyme involved in syringolide biosynthesis.

The intrinsic function of elicitors in the life cycle of phytopathogenic microorganisms often remains elusive. Fungal elicitors of the general type appear to be constitutively present in the cell wall, for example as structural components. In contrast, harpins, a class of bacterial elicitors of the hypersensitive response in nonhost plants as well as in resistant genotypes of some host plants, are produced and secreted only upon con-

Table 1. Elicitors of plant defense responses and their putative receptors.

| Source of elicitor | Elicitor, elicitor type | Biological response stimulated | Characteristics of the elicitor receptor | Reference |
|---|---|---|---|---|
| *Cladosporium fulvum* | 28-mer polypeptide AVR9, race/cultivar-specific | leaf necrosis in resistant tomato cultivars (*CF-9*) | $K_d = 70$ pM | 108 |
| *Phytophthora cryptogea* | 10.2-kDa $\beta$-elicitin cryptogein, general | leaf necrosis in tobacco leaves and SAR | $K_d = 2$ nM | 121 |
| *Phytophthora sojae* | hepta-$\beta$-glucan, general | phytoalexin production in soybean | $K_d = 3$ nM; $K_d = 0.7$ nM | 118, 119 |
| *Phytophthora sojae* | 42-kDa glycoprotein, 13-mer fragment Pep-13, general | furanocoumarin phytoalexin production in parsley | $K_d = 2.4$ nM<br>$M_r = 91$ kDa | 94 |
| *Pseudomonas syringae* pv. *glycinea* | syringolide 1, indirect product of *avrD*, race/cultivar-specific | HR in resistant soybean cultivars (*Rpg4*) | $K_d = 8.7$ nM | 115 |
| *Pseudomonas syringae* pv. *syringae* | $harpin_{Pss}$, HrpZ, general | HR in tobacco leaves | cell wall binding site | 117 |
| *Pseudomonas syringae* pv. *tomato* | Avr Pto | HR in resistant tomato cultivars (*Pto*) | cytoplasmic serine/threonine kinase Pto | 51, 52 |
| *Puccinia graminis* f. sp. *tritici* | 67-kDa peptidoglycan, general | lignification in wheat leaves | $K_d = 2$ μM<br>$M_r = 30$ kDa | 139 |
| *Trichoderma viride* | 22-kDa endoxylanase, general | leaf necrosis in tobacco and tomato, phytoalexin synthesis | $K_d = 6.2$ nM<br>$M_r = 44$ kDa | 122 |
| Yeast | 8-mer glycopeptide fragment from invertase (gp8 c), general | ethylene production, PR gene activation | $K_d = 3.3$ nM | 123 |
| Various fungi | fungal chitin fragments, general | alkalinization response in tomato cells | $K_d = 1.4$ nM | 105 |
| | *N*-acetylchitooligosacharides, general | phytoalexin production in rice | $K_d = 5.4$ nM | 124 |

tact with the plant or under experimental conditions mimicking the apoplastic space of plants [13, 21].

Race-specific elicitors are very often synthesized and secreted only upon infection of the host plant. Therefore a role as pathogenicity factor has been ascribed to race-specific elicitors. A number of bacterial *avr* genes were shown to be indispensible for full virulence in compatible host plants [21, 27–29]. The *avrBs2* gene from *Xanthomonas campestris* pv. *vesicatoria* appears to be important for the pathogen to cause disease on its host, pepper, as deletion of the gene resulted in reduced pathogenicity [30]. Sequence similarity of the *avrBs2* gene product to *Agrobacterium tumefaciens* agrocinopine synthase suggests a role of this protein in pathogen nutrition during infection [31].

Similarly, the *avrNIP1* gene from the barley leaf scald-causing fungus, *Rhynchosporium secalis*, was shown to contribute to virulence of the pathogen on susceptible barley cultivars [32]. A function in pathogenicity may, however, not hold true for all fungal *avr* genes, as deletion did not always reduce virulence of the transformants. A possible role of fungal avirulence genes for development, reproduction or general fitness under field conditions has instead been suggested [33]. Obviously, the role of *avr* genes and their gene products as avirulence determinants is coincidental and disadvantageous for the pathogen. This situation may reflect rather intriguingly the plant's ability to recognize a pathogen and activate its pathogen defense machinery through components that are important (if not indispensible) for the life cycle of the pathogen [6, 15].

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 or general elicitors, respectively, support the idea that highly specific plant receptors mediate nonself recognition in plant-pathogen interactions [3, 9].

## Products of avirulence genes

Viral coat protein (CP), replicase as well as movement protein have all been shown to be determinants of avirulence in the interaction of tobacco mosaic virus

(TMV) with its host plants, tomato and tobacco [34–36]. Based on the three-dimensional structure of the TMV coat protein specific amino acid substitutions were introduced into different structural areas of the elicitor. This allowed identification of elicitor domains essential for host recognition and subsequent activation of HR in *Nicotiana sylvestris* cultivars carrying the *N′* resistance gene [36, 37]. All mutations that impaired the ability of the mutant protein to cause HR could be located to the right face of the CP's $\alpha$-helical bundle. The amino acid composition of this region is characteristic of recognition surfaces known from various proteins, which suggests binding of the *avr* gene product to a host-encoded receptor. Despite overall sequence divergence among different alleles of CP, this motif was found to be highly conserved in a number of avirulent viral strains tested [38].

More than 30 bacterial avirulence genes, mostly from the genera *Pseudomonas* and *Xanthomonas*, have been cloned and proven to be determinants of incompatibility in the interaction between bacteria and resistant host plant cultivars [39]. Comparison of *avr* gene sequences with sequences present in databases has failed to deduce the biochemical function of the encoded gene products. Bacterial *avr* genes encode hydrophilic, soluble proteins which lack an N-terminal leader sequence. Immunolocalization and biochemical fractionation studies demonstrated cytoplasmic localization of the *X. campestris* pv. *vesicatoria* AvrBs3 and *X. oryzae* pv. *oryzae* AvrXa10 proteins [40, 41]. In addition, when injected into the intercellular space of resistant host plants, these proteins as well as AvrB from *P. syringae* pv. *syringae*, did not induce HR [39, 42, 43]. The site of action of bacterial *avr* gene products has therefore remained uncertain for a long time.

The ability of bacteria to elicit a hypersensitive response in nonhost plants and to cause pathogenicity in susceptible host plant cultivars is controlled by clustered *hrp* genes [44]. HR induction in resistant host plant cultivars not only requires living bacteria [39] as well as bacterial *avr* genes matching particular host plant *R* genes but was also found to be dependent on *hrp* genes [22, 45]. For example, constitutive expression *of X. campestris* AvrBs3 or hyperexpression of *P. syringae* AvrB, respectively, in *hrp*-deficient bacteria did not result in HR induction in resistant host plant cultivars [43, 46]. Sequencing a large number of *hrp* genes from different phytopathogenic bacteria revealed significant homologies of some of these genes to components of a type III protein secretion pathway, recently discovered in animal pathogens of the genera *Yersinia*, *Shigella* and *Salmonella* [22, 47, 48]. In addition, colinearity in the genomic organization of these so-called *hrc* genes (a particular subset of *hrp* genes) [49] and of *Yersinia* genes (*ysc*), encoding components of this protein secretion apparatus, was observed [50]. Our understanding of the mechanism of bacterial phytopathogenicity has since then substantially profited from the discovery that mammalian pathogenic bacteria translocate pathogenicity factors directly into the host cell, employing this *ysc*-encoded protein secretion and delivery apparatus [22]. To prove whether phytopathogenic bacteria would be able to induce resistance upon expression of *avr* genes *in planta* suddenly became the experiment to perform. *Agrobacterium*-mediated transient or stable expression of bacterial *avr* genes in resistant host plant cultivars as well as biolistic transient expression of *avr* genes *in planta* was carried out [13, 22]. Indeed, induction of HR could be demonstrated when *avrPto*, an avirulence gene from *P. syringae* pv. *tomato*, which causes HR in tomato as well as tobacco plants carrying the corresponding *R* gene (*Pto*), and *avrBs3* from *X. campestris*, the determinant of HR induction on pepper cultivars carrying *R* gene *Bs3*, were expressed inside the host plant [42, 51, 52]. Importantly, transgenic *Agrobacterium*-mediated HR induction in host plants was T-DNA-dependent, indicating that expression and recognition by the host occurred inside the infected plant. Stable expression of *avrB* from *P. syringae* pv. *glycinea* and *avrRpt2* from *P. syringae* pv. *tomato*, respectively, confirmed the hypothesis of intracellular recognition of bacterial *avr* genes in host plants [43, 53]. This is intriguing evidence for the plant's ability to trigger endogenous defense mechanisms upon recognition of a part of the aggressive arsenal of attacking pathogens. Translocation of *avr* gene products into host cells also suggests that bacterial avirulence determinants may in the first place act as pathogenicity factors which utilize a sophisticated translocation system to invade host cells.

Bacterial avirulence gene products have not been scrutinized to the same extent as viral or fungal elicitors, respectively, for amino acids essential for recognition by target proteins within the host. However, motifs have been identified within these products that determine specificity in the interaction with host plants. A prominent example is *X. campestris* AvrBs3, which carries an internal region consisting of 17.5 nearly identical 34-amino acid repeats [54]. Deletions of repeats not only rendered *X. campestris* strains virulent on pepper lines carrying *Bs3* but revealed a new resistance specificitiy for an *avrBs3* deletion mutant on a pepper line susceptible to wild-type *avrBs3* [54]. The number of structural repeats may therefore determine specificity in this particular interaction. Furthermore, functional nuclear localization signals have been identified within members of the *avrBs3* family, suggesting specific interaction of the corresponding gene products with constituents of the nuclear import machinery [42, 55].

While bacterial *avr* gene products are believed to act predominantly intracellularly, fungal *avr* gene products appear to be perceived at the host plant cell surface. In contrast to similar experiments performed with purified bacterial *avr* gene products or total bacterial protein extracts, infiltration of purified fungal race-specific elicitors into the host plant intercellular space resulted in activation of plant defense [22, 32, 56, 57]. It is thus conceivable that plant receptors for race-specific fungal elicitors may preferentially reside in the plasma membrane of host plant cells.

Two *avr* gene products from the tomato pathogen *Cladosporium fulvum* (AVR4 and AVR9, respectively) act as elicitors of hypersensitive cell death on tomato cultivars carrying the matching resistance genes *Cf-4* and *Cf-9*, respectively [56, 57]. Gene complementation and disruption experiments have unequivocally proven the role of the *avr4* and *avr9* gene products as determinants of race/cultivar-specific resistance in this interaction [56–58]. Expression of the *avr4* and *avr9* genes is specifically induced during pathogenesis [56, 57]. However, *avr4* and *avr9* gene products appear to be dispensible for pathogenicity of the fungus [59].

Fungal strains virulent on tomato *Cf-9* cultivars completely lack the *avr9* gene [57], whereas virulence on *Cf-4* cultivars results from single point mutations in the coding region of the *avr4* gene [60]. Transcripts of mutant *avr4* alleles were found in all virulent fungal strains tested upon infection of *Cf-4* tomato cultivars, but elicitor protein was immunologically undetectable. However, infection of *Cf-4* plants with potato virus X hyperexpressing mutant *avr4* alleles resulted in HR induction. Thus, these alleles appear to encode potentially active elicitors. It is thus concluded that instability of AVR4 isoforms produced by virulent *C. fulvum* races *in planta* are crucial factors in circumvention of *Cf-4*-mediated resistance [60].

AVR4 and AVR9 are synthesized as larger precursors [56, 61]. Upon secretion both peptides are proteolytically processed by either fungal or plant proteases, yielding 105-mer and 28-mer polypeptides, respectively. Elicitor activity of both peptides was shown to depend on disulfide bridge formation between cysteine residues. Two-dimensional (2D) $^{1}$H-NMR studies on the secondary structure and global fold of AVR9 revealed a rigid, barrel-like structure containing three antiparallel $\beta$-sheets connected by two loops and three disulfide bridges linking all six cysteine residues in a cystine knot [62]. 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 $\beta$-loop of AVR9 appears to be crucial for necrosis-inducing activity in *Cf-9* tomato cultivars [63].

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* [32, 64]. Proof of avirulence gene function of the *nip1* gene was provided by gene disruption and gene complementation experiments [15, 32]. 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 [32]. 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 [32].

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 [15]. This is corroborated by the nonhost-specific necrosis-inducing activity of this peptide on all barley cultivars as well as on various mono- and dicotyledonous plants [15, 65]. NIP1 exerts its toxic activity partially through indirect activation of the plasma membrane $H^+$-ATPase [65]. 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 nontoxic 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 [32]. NIP1 contains ten cysteine residues whose distribution within the complete amino acid sequence is reminiscent of fungal hydrophobins [66]. 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).

Two elicitor peptides from the rust fungus *Uromyces vignae* that induce hypersensitive cell death in resistant cowpea cultivars have been purified to homogeneity [67]. 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.

Particular races of the rice blast pathogen *Magnaporthe grisea* possess the *avr* gene *AVR2-YAMO*, which renders these races avirulent on rice cultivars carrying the corresponding *R* gene. *AVR2-YAMO* encodes a 223-amino acid protein with homology to neutral $Zn^{2+}$-proteases [15, 68]. Point mutations in the putative protease active site were found in virulent isolates of the fungus. It is therefore tempting to propose a role of AVR2-YAMO in the generation of an active elicitor rather than being itself an elicitor [6]. AVR2-YAMO may therefore resemble AvrD from *P. syringae* pv. *tomato* [25].

## General elicitors

Gram-negative phytopathogenic bacteria of the genera *Erwinia*, *Pseudomonas* and *Ralstonia* produce and secrete proteinaceous elicitors, collectively termed harpins, in an *hrp*-dependent manner. Harpins have been identified from *E. amylovora* ($harpin_{Ea}$) [69], *E. chrysanthemi* ($harpin_{Ech}$) [70], *P. syringae* pv. *syringae*, *tomato* and *glycinea* ($harpin_{Pss, Pst, Psg}$) [71, 72], and from *R. solanacearum* (PopA1 and PopA3) [73]. Although quite dissimilar in sequence, harpins commonly induce a hypersensitive response upon infiltration into nonhost plants, such as tobacco or in some cases in resistant cultivars of host plants [13]. Harpins are therefore most likely not determinants of host range in plant-bacteria interactions. $Harpin_{Pss}$ has also been shown to trigger systemic acquired resistance (SAR) in cucumber [74] as well as expression of a number of *hrp*-dependently induced HR-associated tobacco genes (*hin* genes) [75]. Harpin-induced HR in tobacco appears to be an active plant response that depends on gene transcription and protein synthesis [71, 76]. At present, the endogenous function of harpins remains unclear [13]. Mutations in the genes encoding $harpin_{Ea}$ or $harpin_{Ech}$, respectively, significantly reduced pathogenicity of the mutant strains, whereas in similar experiments with PopA1 no reduction in pathogenicity was observed [69, 70, 73]. It remains to be seen to which extent structurally unrelated harpins contribute either directly or indirectly to host colonization in compatible plant-pathogen interactions. Undoubtedly, phytopathogenic bacteria may take selective advantage of harpins. Otherwise, the genes encoding harpins most likely would have been eliminated during evolution.

When infiltrated into tobacco leaves, $harpin_{Pss}$ elicits necrosis which is indistiguishable from the HR elicited by bacteria [71]. Furthermore, HR-inducing activity of various harpins is heat-stable and may therefore not be due to enzyme activity [13]. This rules out generation by harpins of plant-derived elicitors such as oligogalacturonides, which have been shown to trigger a series of defense responses in tobacco cells [77]. Harpins are highly hydrophilic proteins, and thus are unlikely to cross the plant plasma membrane. In addition, harpins activate a number of rapidly induced defense responses that are known to be receptor-mediated in other systems. Taken together, these arguments strongly suggest a direct action of harpins at the plant cell surface. The carboxy-terminal 148-amino acid portion of $harpin_{Pss}$ was identified to be neccessary and sufficient for elicitor activity [71]. Within this moiety two directly repeated sequences were defined which, when individually deleted, rendered the recombinant product elicitor-inactive. This apparent signal specificity suggested a receptor/ligand-like interaction of harpin with its putative plasma membrane target site. In a similar approach nonoverlapping, His-tagged portions (N-terminal 109 amino acids and C-terminal 216 amino acids, respectively) of the $harpin_{Pss}$-encoding gene, *hrpZ*, were expressed in *E. coli* [78]. Surprisingly, infiltration of the purified recombinant proteins in tobacco plants resulted in HR induction with either product. This led the authors to conclude that elicitor activity resides in multiple regions of HrpZ, a concept that is rather difficult to reconcile with a true ligand/receptor interaction.

Since the first barrier invading fungi have to overcome is the plant cell wall, fungal endohydrolytic enzymes have been suggested to act as elicitors of the general type [12]. 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 [79]. On the other hand, research on fungal 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 activating pathogen defense is very likely to function in many plant-pathogen interactions. Most plants possess a cell wall polygalacturonase-inhibiting protein (PGIP) which 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 [12].

Elicitins constitute a family of highly conserved, nonglycosylated 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 [80–82]. Elicitins stimulate HR-like leaf necrosis in tobacco, other *Nicotiana* spp. and apparently in a cultivar-specific manner in some radish and turnip cultivars [80, 83–85]. HR-like necrosis is accompanied by systemic protection of the plant against subsequent infection with virulent *Ppn* isolates or the unrelated pathogen, *Sclerotinia sclerotiorum* (SAR) [85, 86]. Induction of SAR, however, is not dependent on the presence of elicitins in leaves remote from the site of elicitor application [86]. Recently, a low-molecular weight diffusible signal was found to be released from cultured tobacco cells treated with the *P. cryptogea* elicitin, cryptogein [87]. This compound is capable of triggering activation of the same defense genes as cryptogein, but in cells which are not in intimate 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 [83, 88]. 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 $\alpha$-elicitins, such as capsicein (from *P. capsici*), are 100-fold less toxic than basic $\beta$-elicitins, such as cryptogein. Similarly, basic elicitins are 10–50-fold more active than acidic elicitins in inducing SAR in tobacco [85]. 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. [89] 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 [89]. Only very few residues were identified as key determinants accounting for much of the observed difference in necrotic activity of the two elicitin types [90]. Six conserved cysteine residues form three disulfide bridges crucial for necrotic activity [80, 91]. X-ray crystallography of cryptogein revealed a complex structure of six $\alpha$-helices, an antiparallel two-stranded $\beta$-sheet, and an $\Omega$-loop. This motif is assumed to be a recognition site for a putative receptor [92].

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 [93, 94]. This response can be also observed in parsley seedlings upon infection with zoospores of the fungus [95]. HR induction and callose apposition observed in fungus-infected parsley seedlings could not be detected in elicitor-treated cell cultures. Single plant cells may therefore utilize different signals as well as nonoverlapping signal transduction pathways to trigger activation of subsets of the overall defense response.

Characterization of corresponding complementary DNA (cDNA) clones revealed that the gene encodes a 57-kDa precursor protein [96]. 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 [94–96]. The amino acid sequences of the oligopeptide and the intact protein elicitor did not show any significant homology to known sequences [96]. Substitution analysis, in which individual amino acids of Pep-13 were progressively replaced by alanine, identified only two residues critical for activity. $^1$H-nuclear magnetic resonance (NMR) studies revealed a random-coil-like structure of the oligopeptide in aqueous solution (J. Vervoort, personal communication).

Branched (1-3, 1-6)-$\beta$-glucans from the mycelial wall of the same fungus, *P. sojae*, were identified to stimulate phytoalexin production in the host plant soybean in a non-race/cultivar-specific manner [9, 97]. Unlike the cell wall of other fungi the cell wall of *Phytophthora* species consists largely of $\beta$-glucans instead of chitin [98]. In a search to elucidate the minimal structural motif required for elicitor activity a branched hexa($\beta$-D-glucopyranosyl)-D-glucitol was identified [99, 100]. Within that molecule the branched trisaccharide at the nonreducing end as well as a characteristic spacing between two branch points were found to be essential for elicitor activity [101].

Interestingly, phytoalexin production in parsley cells could exclusively be induced by proteinaceous components of a crude elicitor preparation from this fungus. In contrast, glucan fractions of the same elicitor preparation, but not proteinaceous components, activated the same response in soybean plants [102]. In both plants elicitor treatment triggers transcriptional activation of genes encoding enzymes of phytoalexin biosynthesis [103]. To test whether different signals from one pathogen may trigger similar signaling cascades in both plants, a reporter gene was fused to the promoter of a

glucan elicitor-inducible soybean gene, encoding such an enzyme. Protein elicitor treatment of parsley protoplasts transiently expressing this construct strongly stimulated reporter gene activity [104]. This finding points towards the existence of highly conserved signaling cascades operational in both plants.

Other oligosaccharide elicitors of various plant defense responses comprise fungal oligochitin fragments, oligochitosan and plant-derived oligogalacturonides [9]. Precisely defined chitooligosaccharide structures were again required for elicitor-mediated activation of plant defense responses, suggesting interaction of the ligand with a specific receptor site [105, 106].

## Plant resistance genes: structure and function

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 signal recognition and initiation of an intracellular signal transduction cascade mediating activation of multifaceted defense reactions, both in host and nonhost incompatible plant-pathogen interactions. Numerous plant elicitor receptors have been biochemically characterized, and kinetic parameters of ligand/receptor interactions have been determined (table 1).

It is hypothesized that host plant resistance genes encode receptors for ligands encoded either directly or indirectly by *avr* genes [6, 8]. Fundamental mechanistic differences in the site (and mode?) of recognition of viral/bacterial and fungal pathogens appear to exist. Phytopathogenic viruses enter host plants directly through wounds, and intercellular spread is mediated through trafficking of viral components via plasmodesmata [107]. A contact-dependent transfer mechanism appears to mediate delivery of *avr* gene products from phytopathogenic bacteria directly into host plant cells, where av*r* gene products are assumed to interact directly with corresponding plant *R* gene products [13, 22]. The molecular architecture of such perception systems for fungal *avr* gene products, however, appears to be different [59, 108]. Race-specific elicitors encoded by fungal *avr* genes appear to interact with receptor-like structures at the host cell surface [6, 15]. There is yet no evidence for internalization of fungal signals. Signal perception may instead trigger subsequent generation of an intracellular signal that initiates pathogen defense [6]. Recognition of fungal *avr* gene products may thus be mediated by integral plasma membrane receptors, whereas viral and bacterial elicitors are perceived intracellularly by possibly soluble receptors.

A key question remains whether plant *R* genes indeed encode receptors for pathogen-derived signals. There is overwhelming genetic evidence for plant *R* genes being specificity determinants in plant-pathogen interactions, but the biochemical function of the encoded proteins is often uncertain. Plant genes conferring resistance to the major classes of plant pathogens have now been isolated from various, taxonomically unrelated plant species by either positional cloning or transposon mutagenesis [2, 14, 16]. According to common structural motifs plant *R* genes can be divided into six classes [14]. A compilation of cloned plant resistance genes and their most prominent structural features is given in table 2. Domain similarities are observed in plant *R* genes conferring resistance against pathogens as diverse as viruses, bacteria, fungi and root-parasitizing nematodes. Thus, plant pathogen defense against a broad spectrum of pathogens may depend on common molecular mechanisms of signal perception and signal transduction. Moreover, it appears likely that signal transduction pathways triggered by different *R* gene products converge at some point downstream of signal perception. This possibility has recently been proven by the isolation of an *Arabidopsis* gene, *ndr1*, that is required for resistance against both bacterial and fungal pathogens, and may thus integrate different signals to activate a common defense response [109]. Several of these genes have been demonstrated to exist in plants and to be important for disease resistance [14, 18].

Sequence homology of *R* gene products to yeast and animal proteins of known function suggests a role for these proteins in signal perception and subsequent signal transduction. Isolated plant *R* genes share common sequence motifs such as transmembrane domains, nucleotide binding sites and imperfect repetitions of approximately 25 amino acids, termed leucine-rich repeats (LRR) [2, 14]. In particular, LRRs are thought to mediate protein-protein interactions and ligand binding in eukaryotic signal-transducing proteins [110]. LRRs are found in various proteins that differ in their function and cellular location. *R* genes carrying LRR but no membrane anchor (myristoylation site) or transmembrane domain may encode intracellular, soluble receptors (for examples see table 2). In contrast, *R* genes harboring LRR and transmembrane domains, such as tomato *Cf*-genes and the rice gene *Xa21* (which confers resistance against all strains of the phytopathogenic bacterium *X. oryzae* pv. *oryzae*), may encode (plasma) membrane receptors. A role of the Xa21 protein as plasma membrane receptor, however, is hard to reconcile with *hrp*-dependent injection of the avrXa21 protein into the host plant cell. Xa21 may therefore represent an intracellular membrane receptor. In general, determination of the cellular location of *R* gene products will definitely be crucial to elucidating the role of *R* gene products in signal perception and intracellular signal transduction. *Xa21* as well as the tomato *Pto* gene

Table 2. Structural features of isolated plant resistance genes. Classification of *R* genes was adapted from ref. 14.

| Class | Plant *R* gene | Pathogen *avr* gene | Predicted functions, structural features and location of the plant *R* gene product | Reference |
|---|---|---|---|---|
| I | maize *HM1* | *Cochliobolus carbonum* race1 | NADPH reductase | 112 |
| II | tomato *Pto* | *Pseudomonas syringae* pv. *tomato avrPto* | intracellular serine/threonine kinase | 140 |
| III a | *Arabidopsis RPS2* | *Pseudomonas syringae* pv. *tomato avrRpt2* | LZ/LRR/NBS, intracellular | 141, 142 |
| | *Arabidopsis RPM1* | *Pseudomonas syringae pv. maculicola avrRpm1/avrB* | LZ/LRR/NBS, intracellular | 143 |
| | tomato $I_2$ | *Fusarium oxysporum* f.sp. *lycopersicon* | LZ/LRR/NBS, intracellular | 144 |
| III b | tobacco *N* | tobacco mosaic virus replicase (?) | LRR/NBS/Toll, intracellular | 2, 145 |
| | flax *L6* | *Melampsora lini AL6* | LRR/NBS/Toll, intracellular | 146 |
| | flax *M* | *Melampsora lini AM* | LRR/NBS/Toll, intracellular | 147 |
| | *Arabidopsis RPP5* | *Peronospora parasitica* | LRR/NBS/Toll, intracellular | 148 |
| IV | tomato *Cf-9* | *Cladosporium fulvum avr9* | intracellular LRR/TM | 149 |
| | tomato *Cf-2* | *Cladosporium fulvum avr2* | intracellular LRR/TM | 150 |
| | tomato *Cf-4* | *Cladosporium fulvum avr4* | intracellular LRR/TM | 151 |
| | tomato *Cf-5* | *Cladosporium fulvum avr5* | intracellular LRR/TM | 14 |
| V | rice *Xa-21* | *Xanthomonas oryzae* pv. *oryzae* (all races) | extracellular LRR/TM, cytoplasmic kinase domain | 136 |
| VI | barley *mlo* (recessive) | *Erysiphe graminis* f.sp. *hordei* (all races) | six membrane-spanning helices | 114 |

Abbreviations used are: LZ, leucine zipper domain; LRR, leucine-rich repeat; NBS, nucleotide binding site; Toll, proteins with sequence homology to *Drosophila* Toll protein; TM, transmembrane domain.

(conferring resistance against *P. syringae* pv. *tomato*) possess a structural motif that is characteristic of serine/threonine protein kinases. This suggests a role of Xa21 in activating an intracellular signal transduction cascade in a receptor kinase-like manner. In contrast, Pto appears to be a soluble, cytoplasmic serine/threonine kinase with proven autophosphorylating as well as substrate-phosphorylating activity [111].

As stated earlier, biochemical evidence for receptor function of plant *R* genes has very rarely been presented. A reason for that may be that only in some systems have the corresponding *avr* genes and/or *avr* gene products been isolated [6]. The only plant/pathogen system in which physical interaction of the products of an *avr* gene and a plant *R* gene has been demonstrated is the *P. syringae* pv. *tomato*/tomato interaction [51, 52]. Taking advantage of the yeast two-hybrid system, the authors proved direct interaction of the *avrPto* and *Pto* gene products. Importantly, gene products of susceptible *pto* alleles lacked both the ability to induce pathogen defense and to bind to AvrPto protein, thus validating the specificity of this interaction. Since AvrPto is delivered into the host plant cell in an *hrp*-dependent manner [52], intracellular interaction of AvrPto and Pto may reflect the physiological situation.

However, unequivocal, direct evidence for the receptor function of a plant *R* gene product has yet to be presented. Alternative functions of plant *R* gene products in signal perception and intracellular signal transduction are discussed in the following section.

Two notable exceptions exist among isolated plant *R* genes, suggesting functions of the encoded proteins apart from signal perception and transduction. The maize *HM1* gene encodes an NADPH reductase which catalyzes degradation of a host-selective toxin produced by the phytopathogenic fungus *Cochliobolus carbonum* [112]. Detoxification of this pathogenicity factor renders the interaction of the fungus and otherwise susceptible maize cultivars incompatible. Recessive alleles of the barley *mlo* gene confer nonrace-specific resistance to the fungus *Erysiphe graminis* f. sp. *hordei* [113]. This finding is compatible with a negative control function of the Mlo protein in the onset of pathogen defense; absence of Mlo primes the responsiveness for the onset of multiple defense functions. The only sequence motif deducible from the gene sequence suggests formation of six membrane-spanning helices [114].

## Elicitor receptors

Tremendous experimental effort has been undertaken to characterize the interaction between AVR9, the product of the *C. fulvum avr9* gene, and tomato cultivars with various resistance specificities. A saturable high-affinity binding site for AVR9 ($K_d = 0.07$ nM) was indistinguishably detectable in all tomato cultivars, including those lacking a functional *Cf-9* resistance gene [108]. Moreover, all solanaceous plant species tested possessed this binding site, whereas nonsolanaceous plants did

not. The presence of the AVR9 binding site correlated 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 [108]. Membrane preparations from transgenic *Arabidopsis* plants expressing the *Cf-9* gene did not show detectable binding of $^{125}$I-AVR9, suggesting that under these conditions (expression and proper assembly of Cf-9 were not investigated in these transgenics) Cf-9 does not bind AVR9 [6]. Furthermore, 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 representing the receptor for the race-specific elicitor AVR9. Instead, the *Cf-9* gene product may be recruited by the AVR9-bound-receptor into a heteromeric complex to facilitate subsequent signal transduction and *Cf-9*-specific induction of plant defense, or may function downstream of signal perception.

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. This would be consistent with the above-described findings, and propose a role of the Cf-9 protein as the AVR9 receptor. Alternatively, two types of AVR9 receptors with differing ligand affinities may exist [6]. A low-affinity binding site encoded by *Cf-9* would mediate cultivar-specific induction of plant defense, whereas the high-affinity binding site detected in kinetic studies [108] would serve another so far unknown purpose. To date, it remains open whether Cf-9 is implicated in AVR9 recognition or may constitute a downstream signaling element at an early rate-limiting step in the signal transduction cascade [6].

A similar situation can be envisaged in the case of syringolides which are glycolipid elicitors produced by *P. syringae* pv. *glycinea* expressing avirulence gene *avrD* [25]. The syringolides induce pathogen defense only on soybean cultivars carrying the *Rpg4* resistance gene. A proteinaceous, soluble, high-affinity binding site for $^{125}$I-syringolide 1 ($K_d = 8.7$ nM) was detected in all soybean cultivars tested irrespective of their resistance specificities [115]. Using a series of structural derivatives of syringolide 1 in binding assays demonstrated a direct correlation between binding affinity to the soluble fraction and elicitor activity. Thus, the binding site fulfills several criteria of a bona fide receptor, but may not be the product of the *Rpg4* gene. Recently, a 34-kDa protein that accounted for syringolide 1-binding activity was isolated and shown to share homology with thiol proteases [116].

Harpin$_{Pss}$ from *P. syringae* pv. *syringae* elicits HR in the nonhost, tobacco [21]. Binding of this molecule to the periphery of cultured tobacco cells was detected but was undetectable in tobacco protoplasts [117]. Binding was dependent on extracellular $Ca^{2+}$, as EGTA-treatment of cultured cells abolished binding. However, specificity of the binding by competition experiments could not be demonstrated under the experimental conditions used. These results suggest that the cell wall is crucial for induction of HR in tobacco cells.

High-affinity receptors for fungal elicitors of the general type have been reported to reside in the plasma membrane of plant cells [9, 24]. 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) [94]. A binding site recognizing a hepta-$\beta$-glucan elicitor from the same fungus has been characterized in plasma membranes from soybean [118, 119] and other fabaceae [120]. Membrane binding sites of very similar ligand affinities have been reported for the *P. cryptogea* $\beta$-elicitin cryptogein [121] and for the *T. viride* endoxylanase [122] in tobacco, for yeast invertase glycopeptide fragment (gp8c) and chitin fragments in tomato [123], and *N*-acetylchitooligosaccharides in rice cells [124]. 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 nonproteinaceous general elicitors [9]. 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 [94]. 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 demonstration of a functional link between signal perception and elicitation of a physiological response in parsley or tomato cells, respectively [94, 123]. The dual function of receptors, that is 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 [123]. Thus, ligand domains responsible for signal recognition and intracellular signal generation can be structurally separated from each other in this molecule.

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 [125]. Similarly, a 75-kDa soybean plasma membrane protein representing (part of ?) the receptor for the *P. sojae* hepta-$\beta$-glucan elicitor was detected by means of photoaffinity labelling [126]. Very recently, purification of this soybean plasma membrane protein was reported [127, 128]. A cDNA encoding this protein was isolated and used for production of a recombinant receptor in *E. coli*, cultured tobacco cells [127] or baculovirus-infected insect cells (A. Mithöfer and J. Ebel, personal communication). Evidence for the recombinant protein representing the functionally intact hepta-$\beta$-glucan elicitor, however, is lacking. Current efforts are directed towards isolating elicitor receptors and cloning cDNAs encoding these molecules. Unfortunately, the apparent lack of specific plant target cells or tissues, in which the respective elicitor receptor is abundantly expressed, significantly hampers isolation of elicitor receptors.

At present no elicitor receptor has been isolated nor has receptor function directly been proven for any of the cloned plant resistance genes conferring resistance to microbial 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 [14].

## Elicitor receptor-mediated intracellular signal generation

Different molecular genetic and biochemical strategies are currently pursued to identify and characterize signal transduction components mediating activation of plant pathogen defense [3, 14]. Targeted loss of function by constitutive sense or antisense suppression, or transposon tagging of particular genes, may eliminate individual elements of the multicomponent defense response of infected plants. This will help determine the contribution of these factors to overall resistance. Alternatively, random mutagenesis of resistant plants and screening for disease-sensitive mutants will lead to the identification of genes other than *R* genes, important for plant disease resistance. A general limitation to loss-of-function experiments, however, is functional redundancy in the targeted pathways or lethal effects of mutations. Negative regulators of plant defense responses may be identified by re-mutagenesis of mutants impaired in disease resistance (gain-of-function). T-DNA-activation tagging may prove similarly effective in identifying potential elements of signal transduction cascades in plant defense as in hormone signaling [129, 130]. Mutagenesis of transgenics homozygous for defense gene promoter-driven reporter genes may yield both mutants insensitive to pathogen-derived signals as well as mutants expressing reporter gene activity constitutively in the absence of any stimulus. Numerous genes encoding putative signal transduction elements have been identified this way, which are now being analyzed for their biochemical function. Experimental systems of reduced complexity, such as plant cell suspensions, have proven extremely valuable in characterizing very rapidly induced defense-associated plant responses. In addition, pharmacological dissection of signaling pathways involved in plant defense has extensively been carried out using cell cultures. This would not have been possible to the same extent in intact plants. Nevertheless, results obtained with cell cultures should be verified using intact plant tissue wherever possible [3].

Receptor function comprises signal perception and subsequent activation of downstream elements of signal transduction. 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). 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 [3, 9, 131–134]. 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 (RLKs) structurally resemble receptor protein kinases of animal cells containing large extracytoplasmic domains, single transmembrane spanning segments and cytoplasmic kinase domains [135]. 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 RLKs are involved in many physiological processes, such as sporophytic self-incompatibility, plant growth and organogenesis, and cell differentiation [135]. The rice gene *Xa21* conferring resistance to the bacterial pathogen *Xanthomonas oryzae* encodes a receptor-like protein kinase [136]. It remains to be seen whether plant cells employ

functional RLKs to recognize phytopathogen-derived signals and trigger pathogen defense.

Cytoplasmic receptors for strain-specific bacterial elicitors have been identified for *P. syringae* pv. *tomato* AvrPto and *P. syringae* pv. *glycinea* syringolides, respectively [51, 115]. Pto, a cytoplasmic serine/threonine protein kinase, has been shown to physically interact with AvrPto [51], suggesting activation of an intracellular signaling cascade via protein phosphorylation of specific substrates. Using the yeast two-hybrid system, different putative substrates for Pto have been identified (Ptis), one of them, Pti1, being itself a serine/threonine kinase [137, 138]. Expression of a *Pti1* transgene in tobacco plants enhanced the hypersensitive response to a *P. syringae* pv. *tabaci* strain carrying the avirulence gene *avrPto*. These findings indicate that Pti1 is involved in a Pto-mediated signaling pathway, probably by acting as a component downstream of Pto in a phosphorylation cascade. Another three putative substrates of Pto identified in yeast two-hybrid screenings comprise proteins resembling transcription factors with homology to the tobacco ethylene-responsive element-binding proteins (EREBPs) [138]. Using a gel mobility-shift assay, these newly identified proteins were shown to specifically recognize and bind to a DNA sequence that is present in the promoter region of a large number of genes encoding PR proteins. Since expression of several *PR* genes and a tobacco *EREBP* gene is specifically enhanced upon AvrPto-Pto recognition in tobacco, these observations establish a direct connection between a disease resistance gene and the specific activation of plant defense genes. Intriguingly, Pto-mediated phosphorylation of transcription factors that trigger transcriptional activation of *PR* genes may thus represent a complete signal transduction cascade involved in activation of a subset of the plant defense arsenal.

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