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Mini review

Molecular basis of specificity in host/fungus interactions

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Abstract

Fungal phytopathogens have evolved efficient mechanisms that enable them to exploit the plant nutrient reservoir for the purpose of growth and propagation. These are counteracted by the plants to arrest fungal development. Two general principles control the specificity of host/fungus interactions. In several cases, the interplay between fungus-produced toxins and either plant toxin targets or detoxification mechanisms determine the outcome of the interaction. An analogous principle appears to be operative in the opposite direction; deposition by plants of fungitoxic compounds that can be detoxified by pathogenic fungi. Presumably of more general importance is the recognition-based plant defense system. The ensuing resistance is frequently controlled by single genes in both interacting organisms. Originally observed in many crop plants at the sub-species level, it has recently also been described in wild plants and at the species level. The structures of disease resistance genes cloned to date from different plants allow the conclusion that the plant protective system against pathogens is based on a general principle that appears to be as effective as the animal disease protection system.

Introduction

Genetic analyses employing many different pathosystems have led to the observation that the outcome of plant/fungus interactions below the species level is frequently controlled by single genes. Many studied cases involve crop species with a more or less extensive breeding history. However, recent results obtained with wild plant species provide good evidence that single-gene control can also be found in unbred plants. The factors that determine whether a pathogen is able to colonize a given plant or whether the plant is resistant are envisaged to originate from a process of mutual genetic adaptation of both interacting organisms. Fungal pathogens pursue very divergent strategies to implement the host state in a plant (Keen, 1986) and thus, require different complementary plant protection mechanisms. The available data can be condensed into two groups of counteracting general principles that control the interaction; (1) toxin production versus detoxification and, (2) recognition versus non-recognition or, alternatively, suppression of recognition versus defense response. Consequently, the compatible or the incompatible interaction may be specific.

Many fungal pathogens secrete phytotoxins to influence the physiology of plant cells in their favor or to kill the cells. If the plant's sensitivity is based on the presence of a specific target molecule, specificity of the interaction would reside in compatibility. In contrast, specific incompatibility may arise if the plant is capable of inactivating a toxin through an efficient detoxification mechanism. Analogously, detoxification of fungitoxic compounds by the fungus would also result in specific compatibility. An alternative and presumably more general mechanism to generate specificity is provided through pathogen recognition by the plant. Here, specific incompatibility may be the consequence of an induction of plant defense reactions through interaction of plant receptors with distinctive fungal ligands, whereas neutral-

ization of recognition through the release of fungal suppressors of the defense response would result in specific compatibility. Depending on the availability of the different compatibility and incompatibility factors, the host range of a pathogen can be broad or narrow (Keen, 1990). The goal of the present review is to outline recent results involving different plant/fungus interactions that provide examples for these specificity determinants and their genetic control.

Specificity through fungal host selective toxins and plant detoxification mechanisms

Host selective toxins are mainly known from two fungal genera, *Cochliobolus* and *Alternaria*. *Cochliobolus (Helminthosporium)* spp. have been the cause of a number of devastating epidemics of cereal crop plants. Consequently, a great deal of research interest was devoted to the interaction of these pathogens with their hosts. Different from *Alternaria* spp. that belong to the Imperfect Fungi, *Cochliobolus* spp. are Ascomycetes, thus enabling genetic analyses in addition to molecular studies. The closely related maize pathogens, *C. heterostrophus* and *C. carbonum*, and the oat pathogen, *C. victoriae*, each produce different, potent host selective toxins (for review see Walton and Panaccione, 1993). Best studied is the interaction of *C. carbonum* and maize that will therefore serve in the following to exemplify the genetic and molecular basis of toxin/detoxification-based specificity.

C. carbonum, race 1, produces HC-toxin, a cyclic tetrapeptide (D-prolyl-L-alanyl-D-alanyl-L-Aeo; Aeo = 2-amino-9,10-epoxy-8-oxodecanoic acid) which is toxic to maize that is homozygous recessive at the *Hm1* locus (Walton and Panaccione, 1993). By inhibiting a ubiquitous enzyme, histone deacetylase, the toxin appears to interfere with reversible histone acetylation and, thus, with the control of fundamental cellular processes such as formation of chromatin structure, cell cycle progression and gene expression (Brosch et al., 1995). If the inhibition of defense gene expression in response to fungal attack is included in the consequences of toxin activity, HC-toxin may also be regarded as a specific suppressor (Walton et al., 1994a; see also below).

A single Mendelian locus, *TOX2*, seemed to control the production of HC-toxin in *C. carbonum*. The locus contains 2 copies of a 22-kb region of DNA that includes the *HTS1* gene encoding a multifunctional cyclic peptide synthetase, HC-toxin synthetase, flanked by two other genes and that is completely absent from toxin non-producing strains (Panaccione et al., 1992; Scott-Crait et al., 1992). Disruption of both *HTS1* copies led to the loss of toxin formation and to non-pathogenicity of the mutants. In contrast, mutants in which only one copy was disrupted retained both the ability to produce the toxin and pathogenicity (Panaccione et al., 1992). However, transfer of the complete 22-kb DNA region into a wild-type tox^- strain failed to generate HC-toxin producing transformants indicating that additional genes are required for toxin biosynthesis (Walton et al., 1994b). Consequently, other regions of tox^+-unique DNA were detected that may control the biosynthesis of the unusual amino acid, Aeo (Panaccione et al., 1992; Walton et al., 1994a). These data reveal that *TOX2* of *C. carbonum*, race 1, is in fact not a simple Mendelian locus since it contains more than one copy of *HTS1* as well as additional genes. However, since there is no equivalent in *C. carbonum*, race 2, it segregated as a single locus in crosses between these races (Walton et al., 1994b). Even an entire chromosome, such as the dispensable chromosome of *Nectria haematococca* (VanEtten et al., 1994a; see below), may appear to segregate as a single locus in fungal crosses if the other parent lacks the chromosome. Therefore, genetic co-segregation studies with plant pathogenic fungi may not always be sufficient to interpret the actual molecular situation.

Resistance of maize to race 1 of *C. carbonum* and insensitivity to HC-toxin are based on a single dominant gene, *Hm1*, encoding HC-toxin reductase (HCTR), that became the first plant resistance gene to be cloned (Johal and Briggs, 1992; Meeley et al., 1992). HCTR inactivates HC-toxin by NADPH-dependent reduction of the 8-keto group of the functionally essential amino acid Aeo (Kim et al., 1987; Meeley and Walton, 1991, 1993). Consequently, in the interaction of *C. carbonum*, race 1, and maize incompatibility is the specific interaction. As indicated above, this is not a clean gene-for-gene relationship, since *TOX2* encodes more than one gene and *HST1* is necessary but not sufficient for HC-toxin production. However, specific resistance of maize is due to the presence of a single feature, the *Hm1*-controlled inactivation of HC-toxin. Interestingly, HCTR activity was found in other monocots such as wheat, barley, oat and sorghum, but not in dicots (Meeley and Walton, 1993), indicating that the enzyme may also contribute to host species specificity.

Specificity through plant fungitoxins and fungal detoxification mechanisms

A strategy analogous to that of toxin-producing fungal pathogens is also followed by plants. Antifungal toxic compounds affecting the interaction and thus influencing the host rang of a pathogen are produced by many plants. If accumulated in unchallenged plants they are termed phytoanticipins, while phytoalexins are primarily synthesized upon infection (VanEtten et al., 1994b). The steroid-like saponins that occur in more than 100 plant families represent an example for phytoanticipins. Their ability to form complexes with membrane sterols, thereby causing a loss of membrane integrity, is thought to be responsible for the toxicity of these antibiotics (Bangham and Horne, 1962). However, by saponin detoxification several fungi have gained an effective resistance mechanism. This was recently shown for the interaction of the root-infecting causal agent of the take-all disease of cereals, *Gaeumannomyces graminis* (Bowyer et al., 1995). Most oat species accumulate the glycosylated triterpenoid saponin, avenacin A-1, in the epidermal cells of their roots, while one oat species, *Avena longiglumis*, and also wheat are saponin non-containing plants. Wheat isolates of the fungus (*G.g.* var. *tritici*) are non-pathogenic on saponin-containing oats and sensitive to avenacin A-1. In contrast, oat isolates (*G.g.* var. *avenae*) are insensitive due to the production of an enzyme, avenacinase, that detoxifies the saponin through removal of terminal glucose residues. When the avenacinase gene in *G.g.* var. *avenae* was disrupted, transformants were obtained that were no longer able to infect oat, however, without loosing their pathogenicity to wheat. Therefore, the interaction of *G. graminis* and different cereals provides an example for the role of plant antifungal compounds that restrict the host range of a pathogen species. In addition, this interaction illustrates a speciation strategy for a fungal pathogen. By gaining a new function, a detoxifying enzyme, the fungus obliterates an important plant defense strategy and opens a plant species for colonization.

A homologous mechanism to determine the host species range of a pathogen was found in the tomato pathogen, *Septoria lycopersici*. This fungus produces an enzyme, β_2-tomatinase, which detoxifies the tomato steroidal alkaloid saponin, α-tomatine, by removal of a single glucose residue to yield β_2-tomatine (Osbourn et al., 1995; Sandrock et al., 1995). However, when the non-pathogenic fungus, *Aspergillus nidulans*, was transformed with the β_2-tomatinase gene, the transformants were still sensitive to α- and β_2-tomatine. This indicates that β_2-tomatinase expression is not the only mechanism to cope with α-tomatine and that *S. lycopersici*, but not *A. nidulans*, possesses additional non-degradative tolerance mechanisms (Sandrock et al., 1995).

A detoxification strategy can also be envisaged for the inactivation of phytoalexins which have been associated with restricting the spectrum of fungal pathogens of a given plant species. For a long time it was believed that detoxification of the pea phytoalexin, pisatin, by pisatin demethylase (PDA) from *Nectria haematococca* constitutes the decisive step in the colonization of this plant, since all naturally occurring isolates that lacked PDA and all Pda^- progeny were essentially non-pathogenic on pea. However, Pda^- mutants created by disruption of the *PDA* gene retained virulence on pea. It turned out that the *PDA* gene is located on a small dispensable chromosome and only if this chromosome is lost, the fungal isolates become non-pathogenic. This indicates that the chromosome in addition to *PDA* carries other genes needed for pathogenicity and that naturally occurring Pda^- isolates lack the entire chromosome (VanEtten et al., 1994a). Although the original hypothesis of a phytoalexin-detoxifying enzyme being a compatibility factor does no longer hold, the findings point to another possible specification strategy of phytopathogenic fungi. Fungal dispensable chromosomes may be analogous to bacterial plasmids which allow these microorganisms to colonize different habitats. The dispensable chromosome of *N. haematococca* contains genes that permit individual isolates of this broad host range pathogen to occupy specific biological niches, *i.e.* host plants (VanEtten et al., 1994a).

Specificity based on elicitor recognition and active defense

Phytoalexin biosynthesis is only one part of the plant's multifaceted active defense response (Kombrink and Somssich, 1995). Prerequisite for its onset is the recognition of an invading pathogen. The molecules that have been shown to trigger all or at least most of the plant defense reactions are termed elicitors. Many of these compounds originate from fungal mycelial walls or culture filtrates (exogenous elicitors). In addition, there are endogenous elicitors such as oligogalacturonates that are released from host cell walls

by the activity of fungal hydrolytic enzymes. Elicitor origin, chemistry, specificity and membrane binding have been covered in several recent reviews (Côté and Hahn, 1994; Ebel and Cosio, 1994; Ebel and Scheel, 1996; Knogge, 1996). In the context of the present review, it suffices to mention that some fungal elicitors appear to act in a family- or species-specific manner. For example, a glycoprotein elicitor from *Phytophthora sojae* induces defense reactions in suspension-cultured cells of the non-host species, parsley, but not in the host species, soybean. In contrast, a heptaglucan elicitor from the same fungus is elicitor-active in soybean (and other leguminous species; Honée and N[urnberger, 1995) but not in parsley (Parker et al., 1988). In addition, an as yet limited number of protein elicitors was shown to exert cultivar specificity (see below).

In *Phytophthora* spp., a group of small extracellular proteins, collectively termed elicitins, was detected (Ricci et al., 1989, 1993; Yu, 1995). These proteins induce necrosis and systemic resistance in tobacco and other solanaceous species (Pernollet et al., 1993) as well as in some cultivars of two Brassicacean species, *Raphanus sativus* and *Brassica campestris*. In addition, highly virulent tobacco isolates of *P. parasitica* were found to not secrete elicitins (Kamoun et al., 1994), while elicitin-producing isolates were less virulent (Bonnet et al., 1994). Therefore, these proteins appear to be genus-specific determinants of resistance in the *Solanaceae* and cultivar-specific elicitors of necrosis in the *Brassicaceae* (Kamoun et al., 1993b). After cloning of the elicitin gene, *par A1*, from a tomato isolate of *P. parasitica*, it was demonstrated that elicitins are encoded by a multiple gene family (Kamoun et al., 1993a). Since elicitin non-producing isolates were also found to carry elicitin genes, loss of formation of these proteins may arise from mutations in regulatory processes. However, further molecular analyses are required to unravel the role of elicitins in determining host/fungus specificity.

While basic resistance of many plant species may originate from the triggering of active defense reactions by general elicitors (Heath, 1981), it has been proposed that basic susceptibility may result from the activity of fungus-derived suppressors of the plant defense (Bushnell and Rowell, 1981). Elicitor activity is presumed to be mediated through binding to specific plant receptors. In contrast, different mechanisms can be envisaged for the mode of action of suppressors. They may interfere with elicitor binding, with signal transduction, with defense gene activation or with the activity of defense-related compounds from the plant. First evidence for the existence of suppressors came from the observation that plant tissues can be conditioned towards general susceptibility. Successful infection by virulent fungal races frequently allows the infection by normally avirulent fungi (Heath, 1982). However, suppressors have been described for only a few phytopathogenic fungi. Best characterized are those from the pea pathogen, *Mycosphaerella pinodes*, termed supprescins A and B (Shiraishi et al., 1992). These compounds are mucin-type glycopeptides with the structures (A) α-Gal*N*Ac-*O*-Ser-Ser-Gly and (B) β-Gal(1$\rightarrow$4)-α-Gal*N*Ac-*O*-Ser-Ser-Gly-Asp-Glu-Thr. Since the A structure is included in the B structure, both compounds appear to originate from the same biosynthetic pathway. Treatment of pea leaves with a mixture of both supprescins increased the infection frequency of several usually non-pathogenic fungi (Shiraishi et al., 1978, 1991). *In vitro*, supprescin B inhibits the plasma membrane H^+-ATPase in isolated vesicles from pea, but also from non-host species. In contrast, electron microscopical studies *in situ* revealed a host-specific inhibition of the enzyme indicating that the *M. pinodes* suppressors may indeed be determinants of species specificity (Knogge, 1996; Oku et al., 1980; Shiraishi et al., 1991).

Species-specific fungal avirulence genes

The gene-for-gene hypothesis, originally worked out for the sub-species interaction of flax and *Malampsora lini* (Flor, 1955, 1971), has been substantiated with data from many pathosystems. However, as has been previously pointed out (Heath, 1991), there is no reason to assume that a similar type of interaction cannot be found at the species level. Consequently, single gene-inherited avirulence was recently identified in the interaction of the blast fungus, *Magnaporthe grisea*, and different grasses (Valent and Chumley, 1991, 1994). The *PWL* (pathogenicity to weeping lovegrass) genes from this fungus encode glycine-rich, hydrophilic proteins with putative secretory signal sequences that appear to trigger the defense response in different grasses.

The host range of *M. grisea* includes more than 50 grass species. However, the fungus exists in a number of genetically distinct, asexually reproducing populations that colonize different species. When a weeping lovegrass (*Eragrostis curvula*) isolate was crossed with a finger millet (*Eleusine coracana*) isolate, a single

segregating gene, *PWL1*, was identified. Another gene, *PWL2*, was found in a cross between two laboratory parental strains that both infect rice, but only one infecting weeping lovegrass (Valent and Chumley, 1991, 1994). *PWL2* was isolated by map-based cloning (Sweigard et al., 1995), *PWL1* by its homology to *PWL2* (Kang et al., 1995). Southern analysis revealed a *PWL* gene family with homologs in many fungal strains isolated from different host species. Two additional *PWL* homologs were cloned, *PWL3* from a finger millet pathogen and *PWL4* from a weeping lovegrass pathogen (Kang et al., 1995). While *PWL3* and *PWL4* map to the same locus and, thus, appear to be allelic, *PWL1* and *PWL2* are localized at different genomic positions. Transformation experiments revealed that *PWL1* and *PWL2*, but not *PWL3* and *PWL4*, have an all-or-nothing effect on the ability of *M. grisea* strains to infect weeping lovegrass without loosing pathogenicity to other hosts. The inactivity of *PWL4* appears to be caused by its improper expression, since it became functional when under the control of the *PWL1* or *PWL2* promoters, while *PWL3* remained non-functional. Furthermore, the pathogenic allele *pwl2-2* was found to code for a protein with a single aspartate $\rightarrow$ asparagine exchange resulting in the loss of function (Sweigard et al., 1995). Therefore, non-transcription or introduction of point-mutations into the primary sequence appear to be ways to retain pathogenicity in *PWL*-expressing fungal strains.

Analysis of *PWL* sequences and comparison of mutation frequencies with the rest of the genome strongly indicate that the *PWL* gene family has rapidly diverged (Kang et al., 1995). In addition, while only one field isolate was found to lack the *PWL2* gene, spontaneous *PWL2* deletions did not affect fungal development under laboratory conditions. Therefore, these genes appear to have an alternative, as yet unknown intrinsic function that may be necessary for fungal fitness in the field. There were also quantitative polygenic factors identified in *M. grisea* that are likely to control compatibility towards rice (Valent and Chumley, 1991). This suggests that after establishment of basic compatibility (Heath, 1981) between the fungal pathogen and the different host species, plant resistance is controlled by recognition events specific for a particular host species. Thus, the *PWL* genes function at the species level in a way very similar to classical avirulence genes at the sub-species level.

Cultivar-specific fungal avirulence genes

The elicitor/receptor model explains the gene-for-gene hypothesis by presuming an avirulence gene to encode a race-specific elicitor of the plant defense response that interacts with a resistance gene-encoded receptor (Keen, 1990). However, interactions involving host selective toxins can also give genetic patterns that are identical to recognition-based gene-for-gene patterns showing specificity in incompatibility. Binding of HC-toxin to HCTR can be regarded as a recognition process that is followed by a single-gene controlled defense reaction, the inactivation of the toxin by the resistance gene product. In contrast, recognition of fungal avirulence gene products by plant receptors is ensued by the triggering of a multiple-gene controlled defense response.

Currently, the most advanced molecular data on fungal avirulence genes come from the tomato leaf mould pathogen, *Cladosporium fulvum*, from the barley leaf scald pathogen, *Rhynchosporium secalis*, and from the rice blast pathogen, *M. grisea*.Different experimental strategies were employed in the cloning of avirulence genes. *C. fulvum* and *R. secalis* produce race-specific elicitors of plant defense reactions. The elicitor-encoding genes were isolated based on the amino acid sequences of the gene products and subsequently demonstrated to be genuine avirulence genes. In contrast, an avirulence gene from *M. grisea* was isolated by map-based cloning.

From *C. fulvum*, two avirulence genes, *Avr4* and *Avr9*, were cloned (Joosten et al., 1994; Van Kan et al., 1991; Van den Ackerveken et al., 1992). Both encode elicitors of the hypersensitive response on tomato cultivars carrying the complementary resistance genes, *Cf-4* and *Cf-9*. The gene products, cysteine-rich pre-pro-proteins, are processed by fungal and/or plant proteases to yield active proteins of 105 (AVR4) and 28 (AVR9) amino acids, respectively (Joosten et al., 1994; Van den Ackerveken et al., 1993). Analogous to the tox^+-unique *TOX2* DNA of *C. carbonum*, the *Avr9* gene is only present in fungal races that are avirulent on *Cf-9* tomato plants, but is lacking in all virulent races (Van Kan et al., 1991). In contrast, all fungal races contain the *Avr4* gene. However, while this gene is identical in all races avirulent on *Cf-4* tomato plants, the virulent races carry alleles with single nucleotide alterations frequently affecting cysteine residues, and, in one race, with a frame-shift mutation leading to a truncated gene product. All races transcribe the *Avr4* gene, but the products of the mutated alleles were

not detectable indicating that these proteins are either unstable or not secreted (De Wit, 1995; Joosten et al., 1994).

The *nip1* gene from *R. secalis* encodes an 82 amino acid pro-protein with a 22 amino acid secretory signal peptide. The mature protein is also cysteine-rich and elicits defense reactions in barley plants possessing the resistance gene, *Rrs1*, in a manner very similar to an avirulent fungal race (Hahn et al., 1993). A hypersensitive response is not included in the response of barley to *R. secalis* infection (Lehnackers and Knogge, 1990) or NIP1 treatment. All avirulent races carry the *nip1* gene, whereas virulent races either appear to lack the gene or contain an allele with a single nucleotide exchange resulting in elicitor-inactivity of the gene product (Rohe et al., 1995).

In *M. grisea*, in addition to the *PWL* genes, a second gene family was identified that is involved in gene-for-gene relationship with rice at the sub-species level (Valent and Chumley, 1994). The *AVR2-YAMO* gene determining avirulence on rice cultivar Yashiro-mochi encodes a 223 amino acid protein that contains a sequence motif homologous to the active center of a neutral Zn^{2+}-protease (De Wit, 1995). Virulence on Yashiro-mochi can result from DNA insertion into *AVR2-YAMO* or from deletions in the gene. In addition, in some isolates point mutations were identified in the putative protease active site indicating that an enzymatically active gene product may be required for avirulence expression. Although direct evidence for protease activity of AVR2-YAMO is still missing, this avirulence gene product itself may not be an elicitor but function by releasing an active elicitor from a plant or fungal precursor molecule.

The avirulence genes from *C. fulvum* and *M. grisea* appear to be dispensable when the fungi are grown under laboratory conditions since their disruption (or in the case of *Avr4*, the frame-shift mutation) does not reduce virulence on susceptible plants (Joosten et al., 1994; Marmeisse et al., 1993; De Wit, 1995). In contrast, a *nip1* disruption mutant of *R. secalis*, that gained virulence on plants carrying the *Rrs1* resistance gene, shows a lower level of virulence on Rrs1- as well as rrs1-plants as compared to $nip1^+$ races on susceptible plants (Knogge, unpublished data). This phenotype appears to be similar to that of wild-type $nip1^-$ races indicating that the gene product plays a role in virulence expression. This was substantiated by the finding that NIP1, although at higher concentrations as required for elicitor activity on Rrs1 plants, is a non-specific toxin (hence the name, necrosis-inducing protein) that is also active in other mono- and several dicot plants (Wevelsiep et al., 1991; Knogge, unpublished data). At least in part, the toxic activity appears to be based on a stimulation of the plasma membrane-localized H^+-ATPase (Wevelsiep et al., 1993). In this case, the host plant, barley, apparently utilizes a fungal virulence factor in the recognition process that leads to resistance (Knogge, 1991).

Plant disease resistance genes

Neither from rice nor from barley have the complementary resistance genes been cloned to date. However, the tomato resistance genes, *Cf-4* and *Cf-9* as well as *Cf-2* and *Cf-5*, were isolated by map-based cloning and transposon tagging, respectively (Jones et al., 1994; Dixon et al., 1996). *Cf-4* and *Cf-9* that had been introgressed into tomato from *Lycopersicon hirsutum* and *L. pimpinellifolium*, respectively, map essentially to the same position on chromosome 1 (Balint-Kurti et al., 1994, 1995), while *L. pimpinellifolium*-derived *Cf-2* is located on chromosome 6 (Dickinson et al., 1993). These *Cf* genes encode proteins with putative secretory signal sequences, single transmembrane domains and short cytoplasmic tails indicating their membrane-anchored extracellular localization (Staskawicz et al., 1995).

How do the Cf proteins interact with the fungal avirulence gene products to trigger the plant resistance response? A characteristic structural feature of the deduced proteins is a domain of varying length of conserved leucine-rich repeats (LRRs; Dixon et al., 1996; Jones et al., 1994). The LRR motif is known to be involved in ligand recognition or protein/protein interactions in many intra- and extracellular proteins (Kobe and Deisenhofer, 1994). Studies with AVR9 revealed a similar high-affinity binding to plasma membranes isolated from Cf-9 plants and from Cf-0 plants lacking resistance to *C. fulvum* as well as to membranes from other solanaceous species (Honée et al., 1994; Kooman-Gersmann et al., 1996). Furthermore, several *Cf-9* homologs were detected in DNA from Cf-9 as well as Cf-0 plants (Jones et al., 1994). Based on the elicitor/receptor model of the gene-for-gene hypothesis, at least two alternative hypotheses may serve to interpret these findings. (1) The products of the *Cf-9* gene as well as of its homologs from both resistant and susceptible plants interact with AVR9. However, they differ in those domains that are involved in signal transmission into the cytoplasm. (2) AVR9 binds to a

receptor – not encoded by *Cf-9* – which then interacts specifically only with the functional *Cf-9* gene product of resistant plants. In this case, Cf-9 would be downstream of the initial recognition event. In addition, the observed non-specific binding may reflect that AVR9 has an additional, as yet unknown intrinsic function in virulence expression, similar to NIP1 from *R. secalis*. This may be mediated through a receptor that is present in all solanaceous plants. Which of these alternatives is valid needs to be unravelled in future experiments including the analysis of membranes from *Cf-9* transgenic non-solanaceous plants that originally do not bind AVR9.

Additional clues for the function of resistance genes may be provided by comparing several others of these genes that have meanwhile been isolated from different plants. Based on various sequence motifs of the deduced proteins they fall into different structural classes. PTO from tomato (Martin et al., 1993) appears to be a cytoplasmic serine/threonine protein kinase. RPS2 (Bent et al., 1994; Mindrinos et al., 1994) and RPM1 (Grant et al., 1995) from *Arabidopsis thaliana* are also putative cytoplasmic proteins with multiple LRRs, nucleotide binding sites and leucine zipper-type dimerization domains. Other cytoplasmic proteins are N from tobacco (Whitham et al., 1994) and L^6 from flax (Lawrence et al., 1995), both characterized by LRRs, nucleotide binding sites and, in addition, by homologies to domains found in the *Drosophila* Toll protein (Hashimoto et al., 1988) and the mammalian interleukin-1 receptor (Sims et al., 1989). Finally, Xa21 from rice appears to be a transmembrane protein with extracellular LRRs and an intracellular serine/threonine kinase domain (Song et al., 1995). Taken together, this suggests a signal transduction pathway that is based on protein/protein interactions and phosphorylation/dephosphorylation analogous to pathways in animal cells (Heguy et al., 1992; Staskawicz et al., 1995). While several of the resistance gene products may interact with intracellular, as yet unknown pathogen- (or possibly plant-) derived ligands, the extracellular proteins such as Cf-9 may through their cytoplasmic tail activate a PTO-type kinase. Alternatively, Cf-9 may dimerize with an Xa21-type receptor kinase for signal transmission.

Conclusion

The last two years have seen a major breakthrough in molecular phytopathology through cloning of a number of disease resistance genes from different plants. One of these genes controls the inactivation of a host-selective toxin, thus explaining a very specific plant/fungus interaction. However, of more general importance appears to be the identification of resistance genes encoding putative components of the defense-related signal perception and transduction pathway of plants. More of these genes as well as additional genes that are required for resistance gene function will soon become available. Analysis of the interaction of their products with specific ligands such as avirulence gene products as well as of their function in triggering plant resistance will contribute decisively to unveiling the molecular basis of plant/pathogen specificity. Furthermore, although biochemical data are still missing to date, the current interpretations on resistance gene structures suggest that the general principles ruling the enormously effective plant protective system against microbial pathogens are on the verge of being uncovered. It can be expected that the next few years will see tremendous progress in this exciting field of plant biology.

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