INTRODUCTION

Fungi constitute a highly versatile group of eukaryotic carbon-heterotrophic organisms that have successfully occupied most natural habitats. The vast majority of fungi are strict saprophytes; <10% of the ~100,000 known fungal species are able to colonize plants, and an even smaller fraction of these are capable of causing disease. Among the causal agents of infectious diseases of crop plants, however, phytopathogenic fungi play the dominant role not only by causing devastating epidemics, but also through the less spectacular although persistent and significant annual crop yield losses that have made fungal pathogens of plants a serious economic factor, attracting the attention of farmers, plant breeders, and scientists alike.

All of the ~300,000 species of flowering plants are attacked by pathogenic fungi. However, a single plant species can be host to only a few fungal species, and similarly, most fungi usually have a limited host range. The evolution of fungal phytopathogens toward a high degree of specialization for individual plant species may be reflected in the different levels of specialization observed in extant plant–fungal interactions (Scheffer, 1991). The first level may be seen in opportunistic parasites, which enter plants through wounds or require otherwise weakened plants for colonization. These fungal species are usually characterized by a broad host range but a relatively low virulence, that is, they cause only mild disease symptoms. The next level comprises true pathogens that rely on living plants to grow but that under certain circumstances can survive outside of their hosts. Many of the more serious plant pathogens are found at this level; most are highly virulent on only a limited number of host species. Finally, the highest level of complexity is achieved by obligate pathogens, for which the living host plant is an absolute prerequisite to fulfill their complete life cycle.

Therefore, in a simplified view of the evolution toward phytopathogenicity, an ancestral fungus needed first to gain attributes enabling it to live on numerous plant species before refining those traits and/or developing additional devices to increase its virulence on individual plant species, thus gaining an edge on competing pathogens. The strategies pursued by fungal pathogens in this process vary in different types of interactions with their hosts (Keen, 1986). In this review, therefore, I focus on two intriguing questions. First, what are the mechanisms that facilitate the transition of a saprophytic fungus to a plant pathogen? Second, what mechanisms control the degree of virulence on the host once pathogenicity has been established?

ACTIVE PENETRATION OF THE PLANT

To colonize plants, fungal microorganisms have evolved strategies to invade plant tissue, to optimize growth in the plant, and to propagate. Bacteria and viruses, as well as some opportunistic fungal parasites, often depend on natural openings or wounds for invasion. In contrast, many true phytopathogenic fungi have evolved mechanisms to actively traverse the plant’s outer structural barriers, the cuticle and the epidermal cell wall. To gain entrance, fungi generally secrete a cocktail of hydrolytic enzymes, including cutinases, cellulases, pectinases, and proteases. Because these enzymes are also required for the saprophytic lifestyle, they are unlikely to represent the tools specifically developed by fungi to implement pathogenesis, and each individual hydrolytic enzyme may not be absolutely necessary for penetration. This does not, however, preclude the adaptation of their structure or their biosynthetic regulation to the specific needs of a pathogen on a particular host plant.

Experiments addressing the role of cutinase illustrate this point. The cuticle covers the aerial parts of living plants and needs to be pierced before other pathogenetic mechanisms can become effective. Therefore, enzymatic degradation of cutin, the structural polymer of the plant cuticle, has been postulated as crucial for fungal pathogenicity, and cutinase is presumed to be a key player in the penetration process. Indeed, several lines of evidence demonstrate the pivotal role of cutinase during the infection process (reviewed in Kolattukudy, 1985). For example, inhibition of the enzyme by using different chemical inhibitors or cutinase-specific antibodies was shown to prevent infection by Nectria haematococca (Fusarium solani f sp spsi) of pea stem segments with intact cuticle but not of those with mechanically breached cuticle. Further support for the role of cutinase came from the observation that a wound pathogen affecting papaya fruits, Mycosphaerella sp, that does not produce cutinase, was able to penetrate intact
fruits only when transformed with the cutinase gene from *N. haematococca* (Dickman et al., 1989). However, the importance of cutinase for plant penetration has been questioned (Schäfer, 1994) and may vary between fungi, with other mechanisms compensating for the lack of this enzyme. In addition, cutinase may also be involved in prepenetration processes, for example, by altering the adhesive properties of the cuticle and thus facilitating fungal attachment to plant surfaces (Nicholson and Epstein, 1991) or by releasing signal molecules required for early fungal development on the plant (Kolattukudy et al., 1995).

Alternatively, or in combination with hydrolytic enzymes, some fungi have developed a more complex and sophisticated mechanism to penetrate the cuticle of host plants. In general, phytopathogenic fungi form specialized penetration organs, called appressoria, at the tip of their germ tubes; these organs are firmly attached to the plant surface by extracellular adhesives. As it develops, the porosity of the appressorium wall of mechanically penetrating fungi is markedly reduced by melanin incorporation, allowing high turgor pressure (>8 megapascals; Howard et al., 1991) to build up inside. This pressure is focused effectively on a small area at the base of the appressorium that is kept free of wall material and melanin. From this penetration pore, an infection peg develops and pierces through the cuticle and cell wall, possibly assisted by hydrolytic enzymes (reviewed in Mendgen and Deising, 1993).

Studies of the rice blast fungus *Magnaporthe grisea* have illustrated the importance of melanin for infection peg penetration; melanin-deficient mutants are unable to infect intact plants, but some mutants retain pathogenicity on leaves with abraded (i.e., wounded) epidermis (Chumley and Valent, 1990; Kubo and Furusawa, 1991). Furthermore, melanized appressoria of *M. grisea* were capable of pushing penetration pegs through plastic membranes (Howard et al., 1991). These results suggest that melanin is an essential factor for mechanically penetrating fungi.

Other fungal species, including some rusts, have not evolved a direct penetration mechanism and instead bypass the plant cuticle and outer cell wall by entering through the stomata. These fungi have developed a poorly understood mechanism to locate these openings on the plant surface (Hoch et al., 1987; Correa and Hoch, 1995). Thus, penetration is likely to be controlled by a combination of different factors. In addition to fungal compounds, these factors may include plant surface structures as well as activators or inhibitors of fungal spore germination and germ cell formation.

**IMPAIRMENT OF PLANT FUNCTIONS BY FUNGAL TOXINS**

After penetration, the next step in a fungal strategy to colonize a plant species is often the secretion of toxins or plant hormonelike compounds that manipulate the plant's physiology to the benefit of the pathogen. This interference can consist simply of killing plant cells for the purpose of nutrient uptake or a more subtle redirecting of the cellular machinery (Keen, 1986); often it is achieved through the production of phytotoxins with varying degrees of specificity toward different plants. Some toxins are host selective (see Walton, 1996, in this issue), whereas others are active in a wide range of plant species. Phytotoxins have been identified in a broad spectrum of pathogens, but their actual role in pathogenesis remains poorly understood in most cases. However, in some plant–fungus interactions, genetic and biochemical studies revealed that toxins are the determinants of specificity. In these cases, resistance or susceptibility to the fungus always correlates with insensitivity or sensitivity to the toxins. Consequently, these host-selective toxins, which are produced mainly by species of the fungal genera *Alternaria* and *Cochliobolus*, have attracted much attention (see Walton, 1996, in this issue). In contrast, host-nonselective toxins are active on both host and nonhost species. Although this nonselectivity contradicts a role in host-range determination, these toxins may nevertheless have a crucial function during fungal pathogenesis on a particular host. Alternatively, they may be interpreted as remnants of earlier stages of fungal evolution toward phytopathogenicity whose activity may be obstructed in most plants by detoxification or other mechanisms. Two examples illustrate the potential function of host-nonselective toxins.

Which processes are affected by fungal host-nonselective toxins? The mode of action of only a small portion of these toxins has been elucidated. However, several fungal toxins target the plant plasma membrane–localized H^+-ATPase. This enzyme plays a central role in many cellular functions. For example, in mediating ATP-dependent H^+ extrusion, the enzyme helps to establish an inwardly directed proton electrochemical gradient that is required for a number of “uphill” solute transport processes. H^+-ATPase activity is also involved in various turgor-related processes and in the regulation of the intracellular pH (Briskin and Hanson, 1992; Michelet and Boutry, 1995; see also Gianinazzi-Pearson, 1996, in this issue).

This ATPase is activated by the host-nonselective toxin fusicoacin, the major phytotox metabolite of the peach and almond pathogen *Fusicoacum amygdali* that appears to be generally active in higher plants (Marrè, 1979; Marrè and Ballarin-Denti, 1985). The ensuing increased uptake of K^+ (and other cations), Ca^2+, and water by the stomatal guard cells causes the irreversible opening of the stomata and the disease-typical wilting of leaves (Ballio, 1991). The plasma membrane–localized fusicoacin receptor is a member of the 14-3-3 superfamily of eukaryotic proteins (Korthout and De Boer, 1994; Oecking et al., 1994) that is present in all higher plants (Mayer et al., 1993). Members of the family of 14-3-3 proteins serve a multitude of distinct functions, often through regulating the phosphorylation state of proteins (Aitken et al., 1992). This suggests that fusicoacin may affect a signaling pathway leading to H^+-ATPase stimulation through modulation of protein kinase/phosphatase activity. However, the ubiquitous distribution of the fusicoacin receptor contradicts the limited number of host species of *F. amygdali*. Despite the potential of fusicoacin...
cin, an additional as-yet-unknown factor(s) must be responsible for drastically restricting the fungal host range.

A second fundamental plant process, energy transfer during light-driven photophosphorylation in chloroplasts, is inhibited by a different host-nonselective toxin, tentoxin. In sensitive plants, this cyclic tetrapeptide, which is produced by the broad host range pathogen *Alternaria alternata*, causes seedling chlorosis and the arrest of sensitive plant growth. Different from fusicoccin, however, sensitivity to tentoxin varies between plant species (Durbin and Uchytíl, 1977). The toxin target appears to be the \(\alpha\) subunit complex of the chloroplast coupling factor 1. This is suggested by comparison of the sequences of the \(\beta\) subunit–encoding *atpB* genes from six closely related toxin-sensitive and insensitive *Nicotiana* species. The presence of a glutamate residue at position 83 correlated with tentoxin insensitivity, whereas an aspartate residue correlated with sensitivity (Avni et al., 1992). In addition, tentoxin binding required the presence of the \(\alpha\) subunit (Hu et al., 1993). Despite the identification of a highly specific target for tentoxin action, it has not yet been demonstrated unequivocally that sensitivity of a plant species to the toxin is the only factor responsible for its susceptibility to the pathogen.

In summary, although the mode of action of some host-nonselective fungal toxins has been analyzed in great detail, their contribution to overall pathogenicity of the fungi that produce them remains to be defined. They clearly deserve more attention because their role in pathogenesis may be more significant than generally presumed, but also because they represent valuable tools to study physiological processes in plants (Ballio, 1991).

**ELICITORS AND SUPPRESSORS: FUNGAL HOST RANGE DETERMINANTS**

A fungus capable of actively penetrating plants and of producing a toxin that affects a fundamental biochemical process has the potential to be a universal phytopathogen. Yet such a pathogen does not exist. Instead, all fungal plant pathogens grow preferentially or exclusively on a limited number of hosts. Therefore, additional factors must exist by which the host range of a pathogen is restricted.

Soon after coming into contact with a plant, fungal pathogens are likely to be detected by the plant and confronted with an active defense system. Clearly then, a successful plant defense response must be based on an effective surveillance system that enables an early recognition of the threat and, as a consequence, the activation of defense-specific processes that act to prevent further fungal development. Successful pathogens, in turn, need to neutralize the plant resistance strategy, and so on. The result of such coevolutionary dynamics is seen in the contemporary highly specialized plant–fungus interactions.

The molecular bases for recognition of potential pathogens by plants outside of gene-for-gene systems (see below) are poorly understood. Plants may recognize an aggressor through non-self factors that are present on the fungal surface (e.g., chitin and glucan fragments) or are secreted by the pathogen (e.g., proteins) and/or through self determinants such as plant cell wall fragments (e.g., oligogalacturonates) that are released by an invading pathogen through the activity of hydrolytic enzymes. After recognition of the pathogen, a multitude of plant resistance–associated reactions is initiated: ion fluxes across the plant plasma membrane, the generation of highly reactive oxygen species (the oxidative burst), the phosphorylation of specific proteins, the activation of enzymes involved in strengthening of the cell wall, the transcriptional activation of numerous defense genes, the induction of phytoalexins, localized cell death at the infection sites (the hypersensitive response [HR]), and the induction of systemic acquired resistance in distal plant organs (Baron and Zambryski, 1995; Kombrink and Somssich, 1995, and references therein; see also Bent, 1996; Crute and Pink, 1996; Dangl et al., 1996; Hammond-Kosack and Jones, 1996; Ryals et al., 1996, in this issue).

Although the actual role of particular defense reactions in restricting further fungal progression in specific interactions has only been incompletely unveiled, plant resistance or susceptibility is presumed to be determined after a sequential exchange of signals between pathogen and host. From a number of fungi, molecules have been isolated that trigger most or at least some of these plant defense reactions. These compounds are called elicitors, and several recent review articles have covered various aspects related to their function (Côté and Hahn, 1994; Ebel and Cosio, 1994; Ebel and Scheel, 1996; Knoke, 1996).

Although some of the more general elicitors such as oligo-N-acetylgallosamines and oligogalacturonates are active in several plants, others appear to be species specific. The most extensive data are available for two different elicitors from *Phytophthora sojae*. A glycoprotein derived from culture filtrates of this fungus induces many defense reactions in suspension-cultured cells of the nonhost species parsley but not in the host species soybean. In contrast, a specific heptaglucan elicitor from mycelial walls of the same fungus is active in soybean and other leguminous species but not in parsley (Parker et al., 1988).

An interesting group of small proteinaceous elicitors, termed elicins, are secreted by species of *Phytophthora* that cause diseases on various plants (Picci et al., 1989; Yu, 1995). Because elicins were also found to be produced by another Oomycete, *Pythium vexans*, they may be ubiquitous in this fungal class (Huet et al., 1995). The purified proteins induce necrosis and other defense reactions at the site of application but also distally after their translocation, thus mimicking the effects of fungal infection. In addition, they trigger SAR in tobacco and other solanaceous species. On tobacco, the virulence of *P. parasitica* is inversely correlated with elicitin secretion (Bonnet et al., 1994; Kamoun et al., 1994, implying
that elicitors may be genus-specific determinants of resistance in this (and other solanaceous) species. In contrast, elicitors may be cultivar specific in some *Brassica* species because they have been shown to function as elicitors of necrosis only in a few cultivars of *Raphanus sativus* and *Brassica campestris* (Kamoun et al., 1993b, 1994).

Molecular analyses using a cloned elicitin-encoding gene, *parA1*, from a tomato isolate of *P. parasitica* (Kamoun et al., 1993a) revealed that elicitin genes occur as a multigene family in this fungus. Interestingly, isolates that do not produce elicitors are virulent on tobacco were found to have retained a set of elicitin genes. This indicates that the virulence of these isolates may be the result of mutations within loci that regulate the transcription of the entire elicitin gene family.

The production of elicitors and the ensuing recognition by the plant are counterproductive for fungal pathogenesis. Therefore, fungi must develop mechanisms to elude recognition by the host or to interfere with plant defense mechanisms. One strategy could include the secretion of suppressors of the defense response (Bushnell and Rowell, 1981). In the most common model, elicitor activity is explained by binding to a specific cell surface–localized plant receptor that initiates a defense-related signal transduction cascade. By comparison, suppressors may interfere directly with elicitor binding, signal transduction, gene activation, or the activity of defense factors from the plant.

Evidence for the actual existence of suppressors came from the observation that successful infection by virulent fungal races frequently renders plant tissues more susceptible to normally avirulent fungi, indicating that plant tissue can be conditioned toward susceptibility (Heath, 1982). To date, suppressors have been described for only a few phytopathogenic fungi (Shiraishi et al., 1991b), those from the pea pathogen *Mycosphaerella pinodes* being the best characterized.

The structurally related glycopeptides supprescin A and B were purified from germination fluid of *M. pinodes* (Shiraishi et al., 1992). Treatment of pea leaves with a mixture of both supprescins increased the infection frequency of several otherwise nonpathogenic fungi (Shiraishi et al., 1978, 1991b). Furthermore, suppressor specificity coincided with the host range of *M. pinodes*. The fungus infects different leguminous species to varying degrees. When species that are highly susceptible to *M. pinodes* were treated with supprescins, they became highly susceptible to the nonpathogen *A. alternata*. Conversely, after supprescin treatment, *A. alternata* infection levels stayed lower in species that are less susceptible to *M. pinodes*. This observation indicates that the supprescins may be the determinants of host species specificity for *M. pinodes* (Oku et al., 1980; Shiraishi et al., 1991b).

How do these suppressors allow the fungus to escape the host resistance mechanism? A polysaccharide elicitor also present in fungal germination fluid induces the accumulation of the pea phytoalexin pisatin as well as of two biosynthetic enzymes, phenylalanine ammonia-lyase and chalcone synthase. In the presence of supprescins, this response is delayed (Yamada et al., 1989). Furthermore, the elicitor-activated transcription of the phenylalanine ammonia-lyase gene was found to be rapidly deactivated upon suppressor treatment of pea tissue (Wada et al., 1995). In vitro studies demonstrated that supprescin B inhibits the pea plasma membrane H⁺-ATPase in a noncompetitive manner (Kato et al., 1993). However, inhibition also occurred in isolated membranes from four nonhost species. By contrast, after treating leaves with the suppressors, cytochemical observations employing the lead precipitation technique in combination with electron microscopy revealed that the ATPase was inhibited only in the host plant, pea (Shiraishi et al., 1991a). Taken together, these data indicate that the suppressors may not function simply by inhibiting elicitor binding to a receptor in pea membranes but rather by affecting the signaling pathway that leads to the activation of the resistance response.

In addition to suppressor formation, several alternative fungal strategies to counter or to avoid the plant defense response can be envisaged. For example, if a recognized elicitor is essential for pathogenesis, the pathogen could develop mechanisms to increase its ability to tolerate the plant defense reactions. If the elicitor component is not crucial, deletion of the respective gene may lead to increased virulence (see below).

**TRIGGERING OF PLANT RESISTANCE BY FUNGAL AVIRULENCE GENE PRODUCTS**

Genetic analyses of races of fungal pathogens and cultivars of host species demonstrated that pathogen recognition is often determined by the interaction of plant resistance genes with single avirulence genes of the pathogen (Flor, 1955, 1971; see also reviews in this issue by Alfano and Collmer, 1996; Crute and Pink, 1996). This gene-for-gene hypothesis may be interpreted in biochemical terms as the interaction of a race-specific pathogen elicitor with either a cultivar-specific plant receptor or alternatively with a cultivar-specific signal transduction compound (Keen, 1982, 1990). In other words, resistant plant cultivars are capable of utilizing specific features of pathogen races to trigger their defense response.

During the past few years, results obtained with a number of pathosystems, mainly involving bacterial pathogens, have corroborated the gene-for-gene complementarity at the molecular level. The small genome size of bacteria and the availability of efficient molecular biology techniques enabled the cloning of many avirulence genes from bacterial pathogens by genetic complementation. A shotgun strategy is, however, not practical for fungi because of their larger genomes and the fact that transformation protocols do not exist for many phytopathogenic fungal species, in particular for most obligate biotrophs. In addition, even if fungi can be transformed, transformation frequencies are usually low. The alternative, a map-based cloning strategy, is not applicable to the Imperfect Fungi, which lack a sexual stage and hence cannot be
Table 1. Cloned Fungal Avirulence Genes

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Avirulence Gene</th>
<th>Gene Producta</th>
<th>Activityb</th>
<th>Intrinsic Functionb</th>
<th>Specificity Level</th>
<th>Gain of Virulencec</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fulvum</td>
<td>Avr4</td>
<td>135 (105)</td>
<td>Elicitor</td>
<td>?</td>
<td>Cultivar</td>
<td>m</td>
</tr>
<tr>
<td>C. fulvum</td>
<td>Avr9</td>
<td>63 (28)</td>
<td>Elicitor</td>
<td>?</td>
<td>Cultivar</td>
<td>d</td>
</tr>
<tr>
<td>R. secalis</td>
<td>nip1</td>
<td>82 (60)</td>
<td>Elicitor</td>
<td>Toxin</td>
<td>Cultivar</td>
<td>d, m</td>
</tr>
<tr>
<td>M. grisea</td>
<td>AVR2-YAMO</td>
<td>223</td>
<td>Protease?</td>
<td>?</td>
<td>Cultivar</td>
<td>d, l, m</td>
</tr>
<tr>
<td>M. grisea</td>
<td>PWL1</td>
<td>147</td>
<td>?</td>
<td>?</td>
<td>Species</td>
<td>d?</td>
</tr>
<tr>
<td>M. grisea</td>
<td>PWL2</td>
<td>145</td>
<td>?</td>
<td>?</td>
<td>Species</td>
<td>d, m</td>
</tr>
</tbody>
</table>

a Number of amino acids in the primary translation products and in the processed proteins (in parentheses).
b ?, unknown.
c m, point mutation; d, deletion; i, insertion.

Many serious plant pathogens belong to this group of fungi; the identification of their avirulence genes depends on the purification of race-specific elicitors of plant defense responses. To date, avirulence genes have only been cloned from a few fungal species. These include the tomato leaf mold pathogen Cladosporium fulvum and the barley leaf scald pathogen Rhynchosporium secalis, which are both Imperfect Fungi. In addition, a more thorough discussion, see Bent, 1996, in this issue).

In apoplastic fluids from C. fulvum-infected susceptible tomato leaves, two race-specific elicitors, AVR4 and AVR9, were identified (Scholtens-Toma and De Wit, 1988; Joosten et al., 1994) that trigger the HR in tomato cultivars carrying the resistance genes Cf-4 and Cf-9 (see Bent, 1996; Hammond-Kosack and Jones, 1996, in this issue). Characterization of the cloned Avr4 and Avr9 genes (Van Kan et al., 1992; Joosten et al., 1994) revealed that the gene products are cysteine-rich preproproteins that are processed by fungal and/or plant proteases to yield active proteins of 105 (AVR4) and 28 (AVR9) amino acids, respectively (Van den Ackerveken et al., 1993; Joosten et al., 1994). An- other race-specific elicitor, NIP1, was isolated from culture filtrates of R. secalis (Figure 1). The 82–amino acid product of the nip1 gene is processed to yield a 60–amino acid mature protein that is also cysteine rich (Rohe et al., 1995). In barley plants with the resistance gene Rrs1, this elicitor triggers several defense reactions (Hahn et al., 1993). However, the HR is not involved in the resistance of barley to R. secalis (Lehnackers and Knogge, 1990).

Genetic complementation and gene disruption were used to analyze the role of these elicitors during pathogenesis. After transfer of the genes into virulent fungal races, transformants were isolated that are avirulent on plants carrying the respective resistance genes (Van den Ackerveken et al., 1992; Joosten et al., 1994; Rohe et al., 1995). In addition, replacement by nonfunctional genes in avirulent races through homologous recombination (Marmeisse et al., 1993; W. Knogge, unpublished data) yielded virulent fungi. Similarly, in a virulent C. fulvum isolate, a frame-shift mutation was detected in Avr4, leading to a truncated gene product (Joosten et al., 1994). These results demonstrate that the genes Avr4, Avr9, and nip1 are sufficient and necessary to condition avirulence in combination with the corresponding plant resistance genes and thus by definition are avirulence genes.

The intriguing questions now are whether the avirulence gene products bind to specific plant receptors and whether these receptors are encoded by the corresponding resistance genes. Several tomato resistance genes including Cf-9 have been cloned and found to encode proteins with putative secretory signal sequences, single transmembrane domains, and short cytoplasmic tails, indicating their membrane-anchored extracellular localization. In addition, a role of the gene products in recognition is suggested by the occurrence of leucine-rich repeats in the putative extracellular domain (for a more thorough discussion, see Bent, 1996, in this issue). Studies with AVR9 revealed high-affinity binding sites on plasma membranes isolated from Cf-9 plants. However, binding was also detected to membranes from Cf-0 plants lacking resistance to C. fulvum and from other solanaceous species, all carrying Cf-9 homologous genes (Jones et al., 1994; Kooman-Gersmann et al., 1996). Therefore, the question of whether the Cf proteins interact directly with the fungal avirulence gene products is still open for debate (see Hammond-Kosack and Jones, 1996, in this issue).

Which mechanisms allow races of these fungal pathogens to grow on host plants carrying resistance genes? The Avr9 gene is unique to races of C. fulvum that are avirulent on Cf-9 tomato plants but is absent from all virulent races (Van Kan et al., 1991). In contrast, all fungal races contain the Avr4 gene (Joosten et al., 1994). However, whereas this gene is identical in all races avirulent on Cf-4 tomato plants, the virulent races carry alleles with single nucleotide alterations that frequently affect cysteine residues or, in one case, with a frame-shift mutation. All races transcribe the Avr4 gene, but the products of the alleles from virulent races were not detectable in the apoplastic compartment of susceptible host cultivars, indicating that the mutated AVR4 proteins are either unstable or not secreted (Joosten et al., 1994; De Wit, 1995; see also Hammond-Kosack and Jones, 1996, in this issue).
In addition to factors required for pathogenicity, the fungus secretes a number of virulence factors, including NIP1, but also additional toxic proteins such as NIP2 and NIP3.

(A) Compatible interaction. In susceptible host cultivars lacking the resistance gene Rrs1, these factors mediate cell death through stimulation of the plant plasma membrane H+-ATPase (NIP1 and NIP3) as well as through additional unknown mechanisms (NIP2).

(B) Incompatible interaction. In resistant cultivars, the Rrs1 gene product is the decisive component in the signal perception and transduction machinery that enables the activation of host defense genes upon specific interaction with one of the virulence factors, NIP1. As a consequence, fungal development is arrested.

R. secalis races that are avirulent on Rrs1 barley carry two classes of nip1 alleles, both of which encode elicitor-active proteins that differ in three amino acid positions. In contrast, most virulent races lack the nip1 gene. In addition, not only a highly virulent race but also a race avirulent on an rrs1 cultivar secrete nip1 gene products that are elicitor inactive. These proteins carry a fourth amino acid alteration at two different positions (Rohe et al., 1995).

The avirulence genes from the imperfect fungi C. fulvum and R. secalis were isolated after the identification of their products. In contrast, cultivar-specific avirulence genes were isolated from the Ascomycete M. grisea by map-based cloning. The AVR2-YAMO gene that prevents infection of the rice cultivar Yashiro-mochi resides near the tip of a fungal chromosome. It encodes a 223-amino acid protein that shares a short stretch of amino acid similarity with the active site of neu-
tral Zn\(^{2+}\)-proteases (Valent and Chumley, 1994; De Wit, 1995).

Mutant analysis revealed that gain of virulence can result from DNA deletion as well as from DNA insertion at the chromosomal tip. In addition, in some virulent isolates, point mutations were identified in the putative protease motif (De Wit, 1995). Although direct evidence for protease activity of AVR2-YAMO is still missing, this avirulence gene product may be functionally different from the C. fulvum and R. secalis avirulence proteins. The latter are presumably the actual ligands in the resistance-causing signal perception process, whereas the AVR2-YAMO gene product may function by releasing an active elicitor from a plant or fungal precursor molecule.

The PWL gene family from M. grisea exemplifies the concept that resistance at the plant species level can also be controlled by single fungal genes that function in a way very similar to cultivar-specific avirulence genes (Heath, 1991). These genes encode glycine-rich, hydrophilic proteins with putative secretory signal sequences and prohibit pathogenicity on weeping lovegrass (Eragrostis curvula). The host range of M. grisea includes >50 grass species. The fungus exists in a number of genetically distinct, asexually reproducing populations, only one of which favors rice (Valent and Chumley, 1991). The single species-specific avirulence genes PWL1 and PWL2 were isolated from a finger millet (Eleusine coracana) and a rice isolate (Kang et al., 1995; Sweigard et al., 1995), respectively.

PWL homologs have been detected in many fungal strains isolated from different host species. However, no correlation has been found between the presence of PWL gene sequences in fungal strains and their inability to infect weeping lovegrass, indicating that not all members of this gene family function as avirulence signals. This was substantiated by the finding that two apparently allelic PWL homologs, PWL3 from a finger millet pathogen and PWL4 from a weeping lovegrass isolate, did not affect the ability of transformed M. grisea strains to infect weeping lovegrass. The inactivity of PWL4 appears to be caused by improper expression of the gene, because it becomes functional when put under the control of the PWL1 or PWL2 promoters. By contrast, PWL3 remained inactive in these experiments, indicating that the gene product is nonfunctional (Kang et al., 1995). In addition, as with the avirulence genes from C. fulvum and R. secalis, pathogenicity can also be restored by deletion of particular PWL genes or be retained in PWL-expressing M. grisea strains by single base pair changes (Sweigard et al., 1995).

**INTRINSIC FUNCTIONS OF FUNGAL AVIRULENCE GENES**

If deletion of avirulence genes is advantageous for fungi to overcome recognition by resistant host plants, why is this strategy not always followed by pathogens? Clearly, the role of these genes as avirulence determinants is coincidental and a function of the plant defense mechanism. But what are the genuine gene functions? In the case of the PWL genes, sequence comparison and mutation frequencies higher than those in the rest of the M. grisea genome strongly indicate that this gene family is highly dynamic and rapidly evolving (Kang et al., 1995). In addition, although spontaneous PWL2 deletions occurred in fungal strains without affecting the fitness under laboratory conditions, only one field isolate was found to lack PWL2-related DNA (Sweigard et al., 1995). Therefore, one can speculate that the PWL genes have a function that may be required for fungal fitness in the field.

The C. fulvum Avr4 gene is induced in plants and expressed during pathogenesis in compatible interactions. In addition, the occurrence of Avr4 alleles in virulent races suggests a function in virulence. However, a frame-shift mutation detected in the Avr4 allele from a natural isolate had no visible effect on fungal development in the plant, indicating that the gene is dispensable (Joosten et al., 1994). The Avr9 gene is activated after fungal hyphae have passed the stomata; it is highly expressed in hyphae growing in the vicinity of vascular tissue. In vitro, Avr9 expression requires nitrogen-limiting conditions (Van den Ackerveken et al., 1994), possibly reflecting the situation found in the apoplastic of tomato leaves. The Avr9 promoter contains several copies of a sequence motif that was identified as the recognition site of regulatory proteins in Neurospora crassa (Caddick, 1992) and Aspergillus nidulans (Fu and Marzluf, 1990; Marzluf et al., 1992). Deletion of a number of these elements from the Avr9 promoter abolishes the inducibility of the gene under low nitrogen conditions, suggesting that they are functional in the transcriptional regulation of the C. fulvum gene (De Wit, 1995). Nevertheless, under laboratory conditions, the Avr9 gene appears to be dispensable for fungal development in the plant, as indicated by the lack of the gene in races virulent on Cf-9 tomato and by disruption mutants. As with the PWL genes, a possible role of the gene for fungal development under field conditions has been discussed (De Wit, 1995).

In contrast to these avirulence genes, an actual function is suggested for the nip1 gene from R. secalis. This gene appears to encode a factor that, in addition to its role in determining avirulence, is essential for the expression of virulence (Figure 1). Evidence comes from a fungal nip1 disruption transformant that displays a level of virulence that is reduced compared with the parental nip1" race on rrs1 barley. This phenotype is, however, similar to that of wild-type nip1" races, regardless of the presence or absence of the Rst1 gene in the host (W. Knogge, unpublished data). A contribution of NIP1 to fungal virulence is further substantiated by the finding that it is a necrosis-inducing protein in all barley cultivars tested, although at higher concentrations than those required for elicitor activity. Moreover, it is toxic to other mono- and several dicotyledonous plants (Wevelsiep et al., 1991; W. Knogge, unpublished data). At least in part, this host-nonspecific toxic activity appears to be based on an indirect stimulation of the plasma membrane H\(^+\)-ATPase (Wevelsiep et al., 1993). This observation now raises the question whether the dual functions of NIP1 are mediated through the same plant receptor or whether the elicitor receptor (the one triggering resistance) is distinct from the toxin receptor (the one conditioning disease).
The avirulence (elicitor) function of NlPl is depicted on the left, and the virulence (toxin) function of NlPl is depicted on the right in each panel.

(A) Avirulence activity of NlPl is mediated through the product of barley resistance gene Rrs1, whereas toxic activity results from the interaction with a different plasma membrane receptor. The product of the recessive rrs1 allele in susceptible plants may not allow efficient binding of NlPl. Alternatively, signal transduction upon binding may be impaired.

(B) Both avirulence function and toxicity of NlPl are mediated through the same receptor that is encoded by the Rrs1 locus. Again, differences between the products of the Rrs1 and rrs1 alleles in the efficiency of NlPl binding or in signal transduction may determine whether the plant defense response is triggered.

(C) A single NlPl receptor mediates the toxic activity. However, it is not encoded by the Rrs1 locus and requires an interaction with the product of the rrs1 gene to initiate the signal transduction pathway leading to the plant defense response. The rrs1 gene product may interact too poorly with the NlPl receptor to trigger the defense response. Alternatively, transmission of the signal may be inefficient. In this model, the resistance gene encodes a signal transduction component that may or may not be localized in the plasma membrane.
Further experimentation is also required to determine whether the elicitor receptor is encoded by the resistance gene Rrs1 (Figure 2).

Current research in several laboratories focuses on the isolation of elicitor receptors from plants. For the heptaglucan elicitor as well as for a 13-amino acid fragment of the glycoprotein elicitor from P. sojae, specific binding sites were detected on plasma membranes from soybean and parsley, respectively (Cosio et al., 1992; Nürnberger et al., 1994, 1995). Isolation and cloning of these elicitor receptors should be successful in the near future (Honée and Nürnberger, 1995) and will give further insight into the defense-related signal perception systems of plants. Furthermore, isolation and characterization of the receptor(s) for AVR9, NIP1, and additional avirulence gene products will help to answer the question whether elicitor receptor genes are structurally related to and encoded by plant resistance genes (see Bent, 1996; Hammond-Kosack and Jones, 1996, in this issue).

CONCLUDING REMARKS

Many questions concerning fungal infection of plants remain unanswered. However, research in this field obtains its significance from the fact that these microorganisms are major pathogens of many crop species. An understanding of fungal pathogenicity will not only afford insights into the evolution of fungi but also into the highly dynamic process of their coevolution with plants. In addition, the various factors fungi developed to manipulate the physiology of their hosts to optimize the parasitic lifestyle represent valuable tools to study the affected plant processes. This is clearly demonstrated by the impact of fusicoccin and other phytotoxins on unraveling the roles of the plasma membrane H+-ATPase in plant cells.

Furthermore, the compounds of fungal origin that are utilized by their hosts to initiate the defense machinery offer the potential for fine-tuned analyses of signaling pathways in plant cells under microbial attack. These analyses, in turn, impinge on cellular functions in healthy plants. Because disease resistance is a response of plant tissues, not only of single cells, one such function is cell-to-cell communication. A major applied goal of this research is to develop strategies to counter the effect of fungal pathogens on crop species. One approach aims to place fungal avirulence genes under the control of defined pathogen-responsive plant promoters (De Wit, 1992). Transformation-mediated combination of such constructs with the complementary plant resistance genes should provide transgenic plants with both components of the switch required to turn on the resistance response. Therefore, experimentation in the coming years is likely not only to deliver answers to the questions raised in the present article but also to utilize the results in molecular breeding approaches to improve resistance of plants to disease.

ACKNOWLEDGMENTS

Drs. Thorsten Nürnberger and Imre E. Somssich are gratefully acknowledged for critical discussions of the manuscript. The work in my laboratory was supported by grant No. 0136101A from the German Ministry for Research and Technology and by grants Kn 325/3 from the Deutsche Forschungsgemeinschaft.

REFERENCES


