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Colloquium Paper

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Surface signaling in pathogenesis

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ABSTRACT **Surface signaling plays a major role in fungal infection. Topographical features of the plant surface and chemicals on the surface can trigger germination of fungal spores and differentiation of the germ tubes into appressoria. Ethylene, the fruit-ripening hormone, triggers germination of conidia, branching of hyphae, and multiple appressoria formation in *Colletotrichum*, thus allowing fungi to time their infection to coincide with ripening of the host. Genes uniquely expressed during appressoria formation induced by topography and surface chemicals have been isolated. Disruption of some of them has been shown to decrease virulence on the hosts. Penetration of the cuticle by the fungus is assisted by fungal cutinase secreted at the penetration structure of the fungus. Disruption of cutinase gene in *Fusarium solani pisi* drastically decreased its virulence. Small amounts of cutinase carried by spores of virulent pathogens, upon contact with plant surface, release small amounts of cutin monomers that trigger cutinase gene expression. The promoter elements involved in this process in *F. solani pisi* were identified, and transcription factors that bind these elements were cloned. One of them, cutinase transcription factor 1, expressed in *Escherichia coli*, is phosphorylated. Several protein kinases from *F. solani pisi* were cloned. The kinase involved in phosphorylation of specific transcription factors and the precise role of phosphorylation in regulating cutinase gene transcription remain to be elucidated.**

Signaling between pathogenic fungi and plants begins when they meet on the plant surface. If the signals at the plant surface are perceived as favorable by the fungi, conidia germinate and, in some cases, directly penetrate the cuticle, whereas in other fungi the germ tube differentiates into an infection structure called appressorium that produces the infection peg that penetrates into the host. The nature of the plant signals that trigger such a programmed differentiation process is poorly understood. The topographical features of the plant surface or chemical signals at the plant surface could trigger the differentiation process. Experimental evidence obtained in recent years shows that in some cases physical signals and in others cases chemical signals are involved. The most clearly established examples of each will be reviewed. In many cases fungi remain dormant on fruits until the fruit ripens, when the fungus causes major damage. The plant signal that the fungus uses to time its infection to coincide with host ripening will be discussed. Finally, how plant signals are used by the fungus to trigger the expression of the genes necessary to penetrate into the host will be discussed.

Plant Topography as a Signal for Differentiation of Fungal Infection Structure

Topographical signals were shown to induce differentiation into appressorium by the stomatal penetrating rust fungus *Uromyces appendiculatus* (1). When ridges were microfabricated on silicon wafers, 0.5-μm ridges were found to be optimal for induction of differentiation (Fig. 1*A*); on the bean-leaf surfaces the stomatal lip constitutes a similar ridge, which is known to induce appressorium formation in this fungus. Mechanosensitive chemicals that could transduce membrane stress induced by the leaf topography into an influx of ions, including Ca^{2+}, were suggested to trigger the differentiation process (3).

Some of the genes uniquely expressed during the thigmotropic differentiation of *U. appendiculatus* have been cloned. One of them, *INF24*, contains a 450-bp open reading frame that would encode a 16.4-kDa protein with a glycine-rich N terminus that showed homology to a DNA-binding protein (4). Another one that seems to be up-regulated during infection structure formation, *INF56*, contains two open reading frames, one nested in the other (5). The larger one would encode a 14.1-kDa protein rich in glycine, proline, and serine, with homology to mammalian cytokeratin type II. The smaller one would encode a 10.1-kDa proline-rich protein with homology to the cell-surface recognition region of chicken fibronectin. Multiple copies of *INF56* gene were found in the genome (6). The functions of these gene products are not known.

Plant Surface Wax as the Signal That Triggers Germination and Appressorium Formation. The surface of each plant has a characteristic complex mixture of very hydrophobic materials containing very long-chain aliphatic compounds, collectively called waxes (7). Fungal spores that land on plants first meet these surface waxes, and therefore such chemicals could be prime candidates as signals for plant–fungus interactions. The host chemicals that have been reported to induce germination and appressorium formation by fungal pathogens include plant cuticular components (8, 9). Recently such a case was documented when it was found that germination and appressorium formation of spores of *Colletotrichum gloeosporioides* are induced selectively by the surface wax of its host, avocado (2) (Fig. 1*B*). That this signaling was specific was indicated by the observation that other plant waxes could not induce this differentiation in *C. gloeosporioides*, and avocado wax could not induce such developmental processes in other *Colletotrichum* species that are pathogenic to other plants. The fatty alcohol fraction that constituted only 5% of the total wax was found to be most active. Synthetic aliphatic *n*-fatty alcohols with 24 or more carbons were found to induce germination and appressorium formation by *C. gloeosporioides*.

Analysis of the fatty alcohols of avocado wax by combined gas–liquid chromatography and mass spectrometry showed the presence of C_{30} and C_{32} fatty alcohols as the major components (2). Very long chain alcohols of the size that induce appressorium formation are known to be present in many plant waxes (7). Such waxes do not induce appressorium formation prob-

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Abbreviations: CTF1 and CTF2, cutinase transcription factor 1 and 2, respectively.

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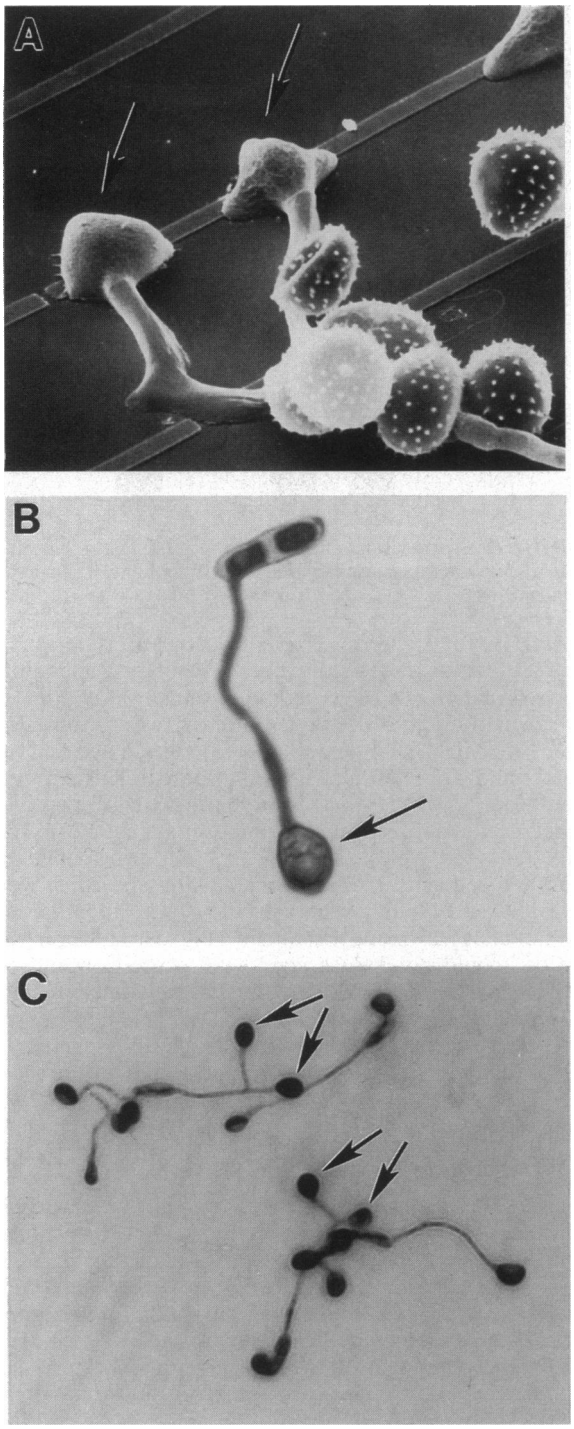

FIG. 1. Induction of appressorium (arrows) formation in *U. appendiculatus* germlings by 0.5-μm-high ridges on polystyrene replicas made from precision ion-etched silicon wafer templates (×780) (*A*) (1), and in *Colletotrichum gloeosporioides* by its host surface wax (4 μg/cm^2) on a glass surface (×960) (*B*) (2), and multiple appressorium formation in *C. gloeosporioides* by ethylene (×430) (*C*). [*A*, reproduced with permission from ref. 1 (copyright American Association for the Advancement of Science, Washington, DC).]

ably because they contain inhibitors of appressorium formation; inhibition of germination and appressorium formation by plant components has been noted (8). In fact, addition of other plant waxes to avocado wax inhibited the ability of avocado wax to induce appressorium formation by *C. gloeosporioides.* The inhibitory molecules have not been identified. Avocado wax components that are more polar than fatty alcohols also showed appressorium-inducing activity. Further chromatographic fractionation and bioassays revealed terpenoid polar components in avocado wax that can induce appressorium formation; (L.M.R. and P.E.K., unpublished work); although these terpenoids have not been identified, authentic urosolic acid and oleanolic acid, two pentacyclic triterpenes found in fruit waxes (8), were found to be potent inducers of germination and appressorium formation by *C. gloeosporioides.* From the available information we conclude that plant surface lipids contain inducers and inhibitors of germination and appressorium formation, and the balance probably is responsible for the selective signaling by the host wax.

Timing of Fungal Infection by Using the Host's Ripening Hormone as the Signal

Although signaling by the surface wax of the host would allow the fungus to enter the fruit in the growing season, many such fungi are known to remain dormant at the surface layer until the fruit ripens, at which time the fungus infects and causes major damage (10, 11). How the fungus times its infection to coincide with the ripening of the fruit remained a mystery. A gaseous material generated by the host specifically at ripening would be an ideal signal for the fungus that rests on the fruit surface. In fact, it was recently discovered that ethylene, the fruit-ripening hormone, at concentrations (≤1 μl/liter) much lower than that normally produced by ripening climacteric fruits, induces both germination and appressorium formation in *C. gloeosporioides* and *Colletotrichum musae* (a banana pathogen) that attack climacteric fruits but not in other *Colletotrichum* species that normally infect nonclimacteric fruits (12). Exposure to ethylene also caused branching of the hyphae and formation of multiple (up to six) appressorium formations from a single spore (Fig. 1*C*).

The ethylene effect on the developmental changes in the fungus appears similar to the well-characterized ethylene effects on plants (13). For example, just as in plants, propylene, an ethylene analog (but not methane), can substitute for ethylene and induce germination and appressorium formation in fungi. Ag^+ and norbornadiene, known inhibitors of ethylene effects in higher plants, also inhibit the ethylene effect on fungal spores and, just as in plants, the inhibitory effect of norbornadiene can be overcome by higher ethylene concentration.

The ethylene effects first observed on glass surfaces were also found on fruits. When spores of *C. gloeosporioides* were placed on oranges, a nonclimacteric fruit, multiple appressorium formation was not found (12). Exposure to exogenous ethylene caused multiple appressoria formation and infection. On normally ripening tomato fruits, known to be producing ethylene, the fungus formed multiple appressoria and produced infection lesions. On the other hand, on transgenic tomato fruits incapable of producing ethylene the spores did not germinate. Upon exposure to exogenous ethylene the spores germinated, formed multiple appressoria, and produced infection lesions. Thus, the ripening hormone allows the fungus to time the infection and to amplify pathogenicity by allowing each spore to enter at multiple sites on the host.

Signal-Transduction Components in the Induction of Appressorium Formation by Host Wax and Ethylene

We suspected that wax induction of appressorium formation in *C. gloeosporioides* would not be mediated via the ethylene receptor that probably mediates ethylene induction of the differentiation. This hypothesis was supported by the observation that norbornadiene that inhibits the ethylene effect, probably by interacting with ethylene receptor, nearly completely inhibited appressorium formation induced by ethylene without affecting the differentiation induced by wax (12).

Because both signals must ultimately affect the expression of the same set of genes involved in the same final process, they would be expected to share some of the signal-transduction components (Fig. 2). In fact, both ethylene and avocado wax caused phosphorylation of the same set of proteins at ≈29 kDa and at 43 kDa (Fig. 3). It is noteworthy that ethylene caused phosphorylation of some similar-sized proteins also in higher plants (14). That these phosphorylated components are relevant to appressorium formation was suggested by the finding that the protein kinase inhibitor genistein, which inhibited their phosphorylation, also inhibited induction of appressorium formation by the host wax and ethylene (M.A.F. and P.E.K., unpublished work). On the other hand, phosphorylation of these components and appressorium formation caused by the wax were not significantly inhibited by kinase inhibitor H-7. Thus, the H-7-inhibited phosphorylation might be more significant for the ethylene signal-transduction branch not shared by the wax signal-transduction pathway (Fig. 2).

That the phosphorylated 29-kDa and 43-kDa proteins are involved in the differentiation process was further suggested by the observation that treatment of the fungal spores with a phosphatase inhibitor, calyculin A (15), also resulted in the ^{32}P-labeling of the same proteins as those labeled when treated with the host wax and ethylene. Inhibition of dephosphorylation of the 29-kDa and 43-kDa proteins by calyculin A also caused differentiation of the germ tube produced in yeast extract into appressorium (Fig. 4). The calyculin A effect was highly dependent on concentration. Although submicromolar levels caused appressorium formation, slightly higher concentrations caused unusual differentiation processes, such as the formation of an additional appressorium on the infection peg that came out of the first appressorium (Fig. 4). Higher concentrations of calyculin A caused the formation of abnormal elongated appressoria-like structures and other structures that defy identification (Fig. 4). Because phosphorylated signal-transduction components are involved in many differentiation processes (16–18), it is likely that calyculin A-induced formation of unusual structures represent multiple effects of this phosphatase inhibitor.

Genes Involved in Appressorium Formation

The duration of exposure to the signals required to achieve commitment of the spores to undergo differentiation to appressorium was found to be 3–4 hr in the case of all three signals—host wax, ethylene, and calyculin A. Therefore, a 3.5-hr period of wax exposure was chosen to seek genes uniquely transcribed for appressorium formation. Because the host wax induced both germination and appressorium formation, subtracting cDNA for transcripts in the untreated spores

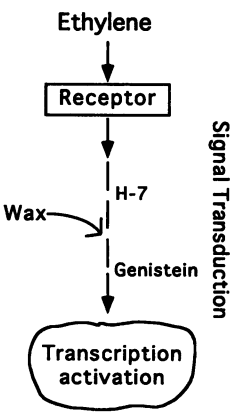

FIG. 2. Schematic representation of the shared signal-transduction elements that lead to transcriptional activation of genes for appressorium formation.

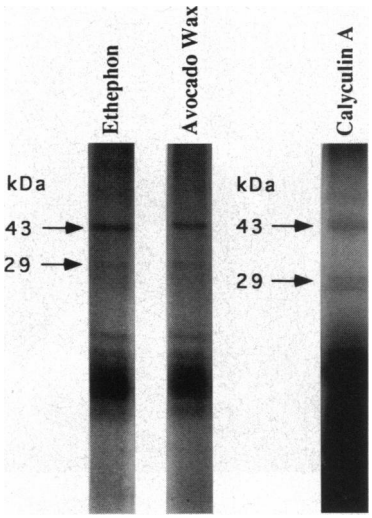

FIG. 3. Phosphorylation of 29-kDa and 43-kDa proteins in *C. gloeosporioides* conidia caused by ethylene (ethephon), host wax, and calyculin A.

from cDNA for transcripts from the appressorium-forming spores left cDNA representing both germination and appressorium formation. To select only those involved in appressorium formation, the subtracted library was differentially probed with cDNA for ungerminated, germinated (in yeast extract), and appressorium-forming spores. This approach yielded four cDNA clones representing transcripts found uniquely in the appressorium-forming spores (19, 20). These cDNAs and the genes encoding the four transcripts were cloned and sequenced. Two of them, *cap3* and *cap5*, would code for 26- and 27-amino acid cysteine-rich polypeptides with some homology to metallothioneins (Fig. 5). However, Cu^{+} and Cd^{2+} could not induce *cap3* gene expression and only weakly induced *cap5* expression. Thus, we tentatively conclude that *cap3* and *cap5* are developmentally regulated to express during appressorium formation, although the functions of these proteins in appressorium formation remain unknown. These normally developmentally regulated genes may be recruited when the organism needs to respond to environmental stress involving heavy metals because of the ability of the gene products to bind metals.

A gene (*MPG1*) for another type of cysteine-rich hydrophobic protein in the hydrophobin class was found to be expressed during appressoria formation by the rice blast fungus *Magnaporthe grisea* (21). This gene was also expressed during conidiation and in mycelial cultures during starvation for nitrogen or carbon. The gene appeared to be expressed at much higher levels in the plant than in culture. Disruption of this gene caused a decreased frequency of appressorium formation and reduced pathogenicity on rice.

One of the genes (*cap22*) uniquely expressed during appressorium formation by *C. gloeosporioides* would encode a 22-kDa protein that showed limited homology to a variety of surface glycoproteins. This protein contains two consensus N-glycosylation sites (19). To immunologically examine whether the protein encoded by *cap22* is produced during appressorium formation, the open reading frame was expressed in *Escherichia coli*, and the recombinant CAP22 protein of 22 kDa was isolated. Immunoblot analysis of the proteins from *C. gloeosporioides* with antibodies prepared against this protein showed that an immunologically cross-reacting protein was present only in the appressorium-forming spores but not in nongerminating or germinating spores. However, the strongly cross-reacting protein was at 43 kDa, whereas the protein expressed in *E. coli* was a 22-kDa protein. Most probably the higher-molecular-weight form represents the glycosylated form. A

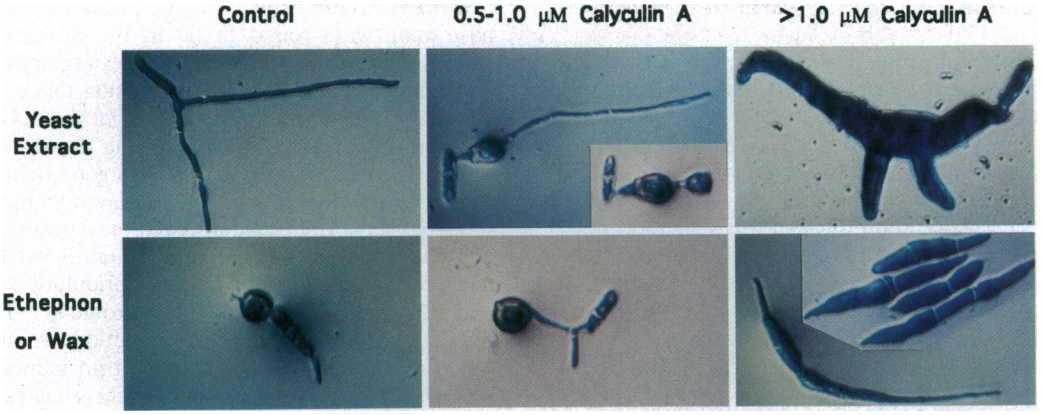

FIG. 4. Effect of the phosphatase inhibitor calyculin A on conidia of *C. gloeosporioides* exposed to ethylene (ethephon), avocado wax, or yeast extract. Spores were exposed to 0.8% yeast extract, 2 μM ethephon, or avocado wax at 1 μg/ml with the indicated concentration of calyculin A. Spores were stained with cotton blue/lactophenol before photography.

time-course study showed that the immunologically cross-reacting material appeared in significant amounts at 4 hr of exposure to the host wax and remained at high levels at least until 28 hr. At all times the 43-kDa form was the dominant one, and some cross-reacting material at even a higher-molecular-mass area was found, possibly representing more highly glycosylated forms. Immunogold electron microscopic examination of appressoriated fungal spores showed that CAP22 protein is associated with appressorial wall. Thus, CAP22 is probably a glycoprotein secreted into the appressorial walls.

Another gene uniquely expressed during appressorium formation was *cap20*, which would encode a 20-kDa protein with no detectable primary sequence homology to any other known protein (20). Using recombinant CAP20 protein expressed in *E. coli*, antibodies were prepared against this protein. Immunoblots with these antibodies showed that CAP20 protein was produced mainly during appressorium formation starting at ≈4 hr of exposure to the signal that triggers the differentiation. Immunogold electron microscopy revealed that CAP20 protein was associated with the appressorial walls. To determine whether *cap20* is expressed during a real infection, *C. gloeosporioides* spores were placed on ripening tomato fruits, infection was allowed to proceed, and various layers excised from the infected area and underlying tissue were examined for cap20 transcripts by reverse transcription–PCR. During the first 2 days cap20 transcript was detected in the outermost 1-mm layer. In 6 days, as infection proceeded deeper into the fruit, no cap20 transcript was found in the outer layer or in the underlying layer through which the fungus had already passed. Deeper in the fruit at the infection front cap20 transcript was found, and at this internal region some appressorium-like structures were found (M.A.F. and P.E.K., unpublished work). Even though the appressorium is thought to be a structure formed outside the plant for initial penetration, appressorium-like structures were recently observed in an internal plant tissue (22). With our finding of an appressorium-associated cap20 transcript at the infection front in the internal tissue, it appears possible that appressorium-like infection structures are also used for penetrating internal host barriers.

To test for functional roles for *cap20*, mutants in which *cap20* was disrupted and therefore was unable to produce CAP20 protein were produced (20). Spores of such mutants germinated and formed normal-looking appressoria without any gross abnormality. To test whether lack of CAP20 affected the function of the appressoria, spores of wild-type and *cap20* gene-disrupted mutants were placed on avocado and tomato fruits, and infection was allowed to proceed until the wild-type spores caused obvious lesions. The mutants failed to produce lesions (Fig. 6); some growth of mycelia on the surface was sometimes observed, but no penetration of mutants into the fruits could be detected. Thus *cap20* gene expression must be somehow necessary for functional appressoria formation, although the precise function of the *cap20* gene product remains to be elucidated.

```
                1                                  27
MT-1(Mouse)      MDPNCSCSTGGSCTCTSSCACKNCKCT...
                 1   | |      | |   | | |   |   26
MT(N.crassa)       MGDCGCSGASSCNCGSGCSCSNCGSK
                 1|  |||      | ||  | | |   |  |26
CAP3 protein       MSGCGCASTGTCHCGKDCTCAGCPHK

                1                                  27
CAP5 protein     MAPCSCKSCGTSCAGSCTSCSCGSCSH
                      32|||    |   |  || |48
MT(Xenopus)          ...KSCCSCCPAECSKCSQG...
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FIG. 5. Comparison of CAP3 and CAP5 proteins with metallothioneins (MT). *N. crassa, Neurospora crassa.*

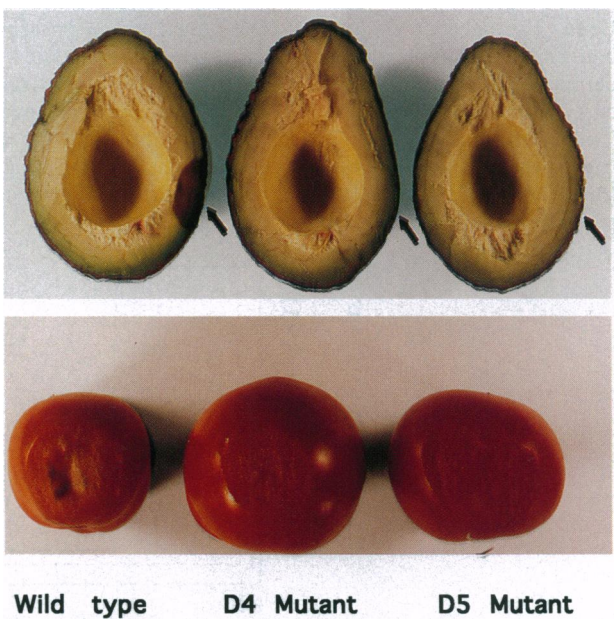

FIG. 6. Tests for pathogenicity of the wild-type and *cap20* gene-disrupted mutants of *C. gloeosporioides* on avocado (*Upper*) and tomato (*Lower*) fruits. Conidia (10^4) of the wild-type or the mutants were placed in a 1-cm^2 area of the fruit surface and incubated under high humidity until the wild type caused obvious lesion formation. The cross-sections of avocado fruits through the inoculation area (arrows) are shown. The top layer of the tomato fruits was removed to expose internal tissue.

Penetration of Cuticular Barriers by Using Host Signals

Role of Cutinase in Infection. Entry of the fungal infection peg, whether it is the initial germ tube or that which emerges from an appressorium, involves penetration through the cuticular barrier. The major structural component of the plant cuticle is cutin, a biopolyester composed of interesterified hydroxy, and epoxy hydroxy C_{16} and C_{18} fatty acids (7). Whether the fungal penetration through this structural polymer is an enzyme-assisted process has been debated for the better part of a century (23, 24). The confusion has been, at least in part, because the penetration process was often viewed as purely enzyme-assisted or solely a physical process for all fungi, whereas, in reality, the process most probably involves a combination of physical force of growth and enzyme-assisted breakdown of the barrier, and the relative role of the two probably varies a great deal among host–pathogen systems.

Cutinase, the enzyme postulated to be involved in cuticular penetration by fungi, was first purified and characterized from *Fusarium solani pisi* grown on cutin as the sole carbon source—obviously, a saprophytic growth condition (25, 26). That the fungus, during infection of its host (pea), produces a cutinase that is at least immunologically related to this enzyme was demonstrated by using immunoelectron microscopy (27). The essential role of cutinase in infection by several different fungi that infect leaves, fruits, stems, and flowers was strongly suggested by complete protection of intact host tissue by specific inhibition of cutinase by monoclonal (28) and polyclonal (29, 30) antibodies and by a variety of cutinase-directed inhibitors (29, 31–33), including suicide inhibitors (J. Sebastian and P.E.K., unpublished work). Cutinase-deficient mutants were found to have drastically reduced virulence on intact host, and virulence could be restored by the addition of exogenous cutinase (34, 35). Cutinase gene introduced into a wound pathogen made it capable of infecting the host without a wound (36). Disruption of cutinase gene in *F. solani pisi* was found to drastically reduce its virulence on intact host (37).

Plant Signal That Triggers Expression of Fungal Cutinase Gene. The mechanism by which a fungal spore perceives contact with a plant and consequently triggers expression of cutinase gene was elucidated. Spores of highly virulent strains carry cutinase that releases cutin monomers when the spores contact the host surface. These unique hydroxy fatty acids trigger expression of cutinase gene in the germinating fungus (38), allowing the fungus to target the secretion of the enzyme to the infection structure (39).

Cutinase Gene Promoter. The promoter elements of cutinase gene were found to be in the 5′-flanking region of cutinase gene (40), and 360 bp of this region was found to be sufficient to cause inducible expression that is suppressed by glucose (41), as previously found *in vivo* (42). A detailed promoter analysis was done by using transformants carrying the 5′-flanking segment of cutinase gene or its deletion and substitution mutants, fused to a chloramphenicol acetyltransferase gene (43). Such studies revealed a positive acting G-rich Sp1-like element between −310 and −360 bp, a silencer element that can function in either orientation between −249 and −287 bp, and a GC-rich palindrome at −159 to −172 bp that is absolutely essential for induction by cutin monomers. Upon deletion of the G-rich region no inducible expression was seen because of the presence of the silencer (Fig. 7). Upon removal of this negative element, inducible expression is recovered but with a higher level of constitutive expression, suggesting that the silencer keeps the constitutive expression extremely low. Upon removal or mutation of the GC-rich palindrome, no inducible expression could be found.

Transcription Factors Involved in Induction of Fungal Cutinase Gene by the Plant Signal. Nuclear protein binding to the promoter region was detected. Gel retardation revealed that nuclear proteins from glucose-depleted cultures of *F. solani pisi* contained protein(s) that bind to the GC-rich palindrome essential for induction (Fig. 8*A*); this protein was designated cutinase transcription factor 1 (CTF1). Another protein(s) that binds to a region closer to the transcription initiation site was also revealed. This protein was designated cutinase transcription factor 2 (CTF2). Methylation interference analysis identified the specific promoter regions involved in binding CTF1 and CTF2 (Fig. 8*B*) (43).

To identify clones of the transcription factors the cognate oligonucleotides were concatamerized, labeled with ^{32}P, and used as probes to screen an expression library. This approach yielded clones that presumably encode proteins that bind the regulatory elements in the cutinase promoter (D.L. and P.E.K., unpublished work). The putative *CTF1* clone was completely sequenced to reveal an open reading frame that would code for a 49,847-Da protein that has characteristics of a transcription factor. This sequence has a nuclear localization signal and a putative zinc finger motif that shares homology with DNA-binding domains of vertebrate GATA factors (44–46) and fungal (47–49) and yeast transcription factors (50, 51). It also showed many putative casein kinase II-specific phosphorylation sites, protein kinase C sites, and protein kinase A

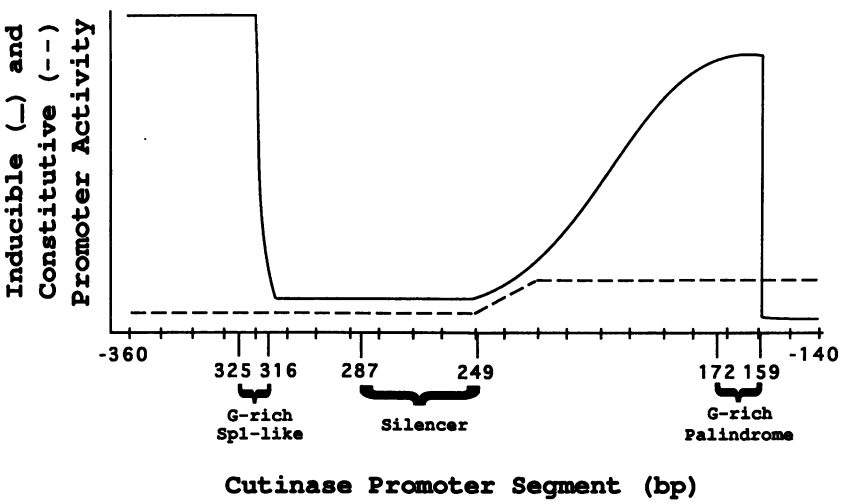

FIG. 7. Inducible and constitutive expression of the chloramphenicol acetyltransferase gene by the different segments of the 5′-flanking region of cutinase gene. Transformants containing the deletion constructs were assayed as described (43).

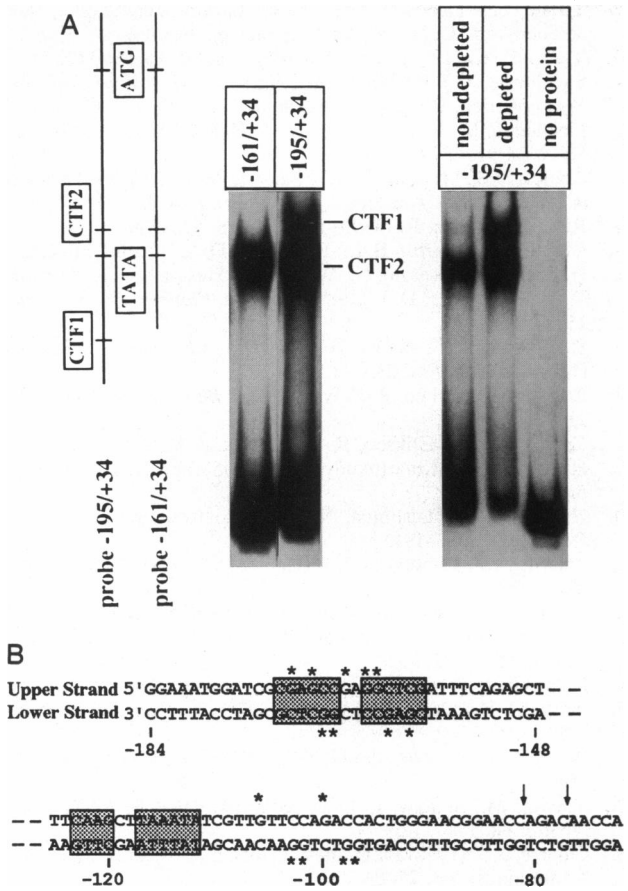

FIG. 8. (*A*) Gel retardation of ^{32}P-labeled 5′ segments of *F. solani pisi* cutinase gene by nuclear extract from glucose-depleted or non-depleted cultures of *F. solani pisi* grown on glucose. (*B*) The region of the cutinase promoter involved in binding cutinase transcription factor 1 (CTF1) (*Upper*) and cutinase transcription factor 2 (CTF2) (*Lower*) as determined by methylation-interference analysis (43). The guanine residues whose methylation interferes with binding are shown by stars.

sites (Fig. 9). CTF2 sequence suggests that it might be a general transcription factor.

Possible Role of Phosphorylation in Cutinase Induction

To elucidate the mechanism by which hydroxy fatty acids trigger the expression of cutinase gene, an isolated nuclear preparation was developed that would transcribe cutinase gene only when hydroxy fatty acids and a fungal protein factor were added to it (52). The position and number of hydroxyl groups in the fatty acid required to give optimal transcription of cutinase gene reflected those normally found in the major unique cutin monomers. The protein factor that gave maximal

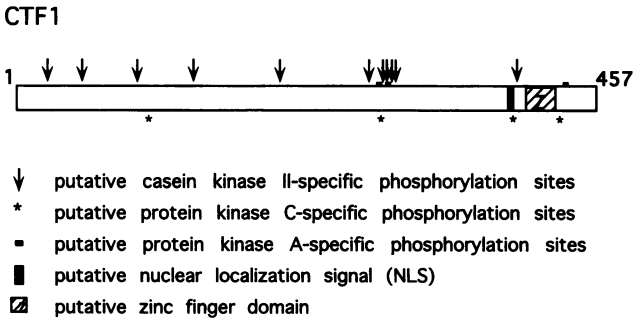

FIG. 9. Consensus motifs identified in the cloned palindrome-binding protein.

stimulation of cutinase transcription appeared to be a 100-kDa protein of nuclear origin (53). Cutinase transcription in this nuclear preparation required a preincubation of the preparation with the cutin monomer and the protein factor for ≈30 min (52). That this period was required to phosphorylate transcription factors essential for cutinase transcription was strongly suggested by the observations that the presence of kinase inhibitor H-7 or genestein during this period (but not if added after the initial preincubation) inhibited cutinase transcription. With [^{32}P]ATP, labeling of a 50-kDa protein during this period was detected, and this phosphorylation required the presence of cutin monomer. The involvement of phosphotyrosine suggested by genistein inhibition of transcription was supported by the detection of phosphorylation of tyrosine that also required cutin monomer (Fig. 10) (K. Podila and P.E.K., unpublished work). That this tyrosine phosphorylation is required for cutinase transcription was further supported by the inhibition of cutinase transcription in the nuclear preparations by the addition of antiphosphotyrosine antibodies during the initial preincubation of the nuclear preparation with cutin monomer—but no inhibition occurred when the antibodies were added after this preincubation period. The involvement of phosphorylation in cutinase transcription suggested by all these observations was strongly supported by the finding that preincubation of nuclear extract with immobilized phosphatase abolished its binding to cutinase promoter (41). With such a strong indication that the hydroxy fatty acids cause phosphorylation of a 50-kDa transcription factor that binds to the promoter in the phosphorylated form, probably as the dimer, we tested whether the cloned CTF1 represents such a phosphorylatable transcription factor. The CTF1 open reading frame was expressed in *E. coli* yielding a 50-kDa protein that specifically bound to the palindromic element in the cutinase gene promoter (D.L. and P.E.K., unpublished work). Incubation of the recombinant protein with [^{32}P]ATP yielded labeled CTF1. This phosphorylation process and its function in the transcription process remain to be elucidated.

Catabolite repression of cutinase gene expression (42) probably involves cAMP-dependent phosphorylation. Glucose depletion of *F. solani pisi* resulted in 3-fold increase in the cAMP level, and addition of exogenous cAMP to nonglucose-

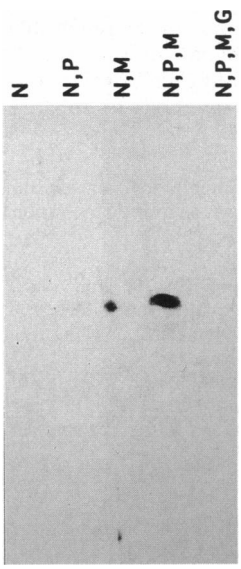

FIG. 10. Immunoblots with antiphosphotyrosine antibodies revealing tyrosine phosphorylation in the nuclear preparations from *F. solani pisi* caused by cutin monomer and a fungal protein factor previously found necessary to induce cutinase gene transcription in the nuclear preparation (52). N, nuclear preparation; P, protein factor; M, cutin monomer; G, genistein.

depleted cells with cutin hydrolysate resulted in cutinase gene transcription (U. Kämper and P.E.K., unpublished work); without exogenous cAMP cutin hydrolysate induces cutinase gene only in glucose-depleted cells. The fungal protein factor that stimulated cutinase gene transcription in the fungal nuclear preparation (52) also catalyzed cAMP-dependent phosphorylation of a 50-kDa protein (L.M.R. and P.E.K., unpublished work). Whether the protein kinase A sites found in CTF1 are involved in this process is yet to be determined.

Protein Kinases from *F. solani pisi*

With the strong indication that phosphorylation of transcription factors is involved in the induction of the fungal cutinase gene by the plant signal a search was made for kinase transcripts that might be involved in this process. Reverse transcription–PCR with degenerate primers based on consensus sequences yielded several kinases (D.L. and P.E.K., unpublished work). Nucleotide sequences of the three kinases so far examined showed the following homologies: *Fusarium* kinase PTK10 to yeast KCR8, a probable serine/threonine kinase (54, 55), as well as to human (56) and frog (57) *cdk2* and human *cdk3* (58); *Fusarium* kinase 26 to *Schizosaccharomyces pombe* RAN1, negative regulator of sexual conjugation and meiosis (59), as well as to certain cell-division-control proteins—CC21 from peas (60), CC22 from rice (61), and CC2 from alfalfa (62). The third *Fusarium* kinase, PTK23, showed a high degree of homology to *Candida albicans* extracellular signal-regulated kinase 1 (ERK1) (63), human ERK2 (64, 65), and mitogen-associated kinases from *Saccharomyces cerevisiae* (KSS1) (66, 67), *Sch. pombe* (SPK1) (68), *Caenorhabditis elegans* (Sur-1) (69), and rat (ERT1) (70–72). Whether any of these kinases are, in fact, involved in phosphorylation of transcription factors that bind cutinase promoter and the role of such phosphorylation steps in the regulation of transcription of cutinase gene remain to be determined.

Conclusion

It should be apparent from the above discussions that the exploration of the molecular aspects of surface signaling between plants and fungi is only the beginning. The consequences of such signaling have a major impact on the production of food, fiber, and other renewable resources. Understanding of the signaling process could reveal a means of intervention that could yield novel methods to protect plants against diseases and methods to enhance beneficial interactions.

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