Fungal pathogenicity
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Successful penetration of living plant tissue by fungal pathogens is preceded by an exchange of signals between both organisms. Recent mutational approaches revealed the importance of cAMP-dependent signalling pathways for fungal development and virulence on their hosts.

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Abbreviations

cAMP cyclic adenosyl monophosphate
CPKA protein kinase A catalytical subunit
MAC1 Magnaporthe adenylye cyclase 1
MAP mitogen-activated protein
PMK1 pathogenicity MAP kinase 1

Introduction
Fungi are eukaroytic, carbon-heterotrophic microorganisms. To satisfy their need for organic nutrients, most fungal species live a saprophytic lifestyle. A small minority, however, has acquired the capability to develop on living plants, often causing disease in the host. These specialists have found a way to negate the plant defense machinery which consists of a multitude of defense mechanisms (for reviews see [1–3]). Hence, fungal pathogenicity results from the evolution of mechanisms that allow the transition of a saprophyte to a pathogen and that adapt fungal development to their host plants [4–6]. Before the real confrontation between fungi and plants can take place, however, fungi need efficient strategies for invasion of the plant’s outer fortifications (Figure 1). This review will, therefore, focus on signaling aspects of these early stages of pathogenesis.

Spore attachment, germination and plant surface recognition

The interaction of foliar fungal pathogens with plants begins with spore attachment to host surfaces and continues with spore germination, host recognition, formation of infection structures, and penetration of host organs. Active adhesion of fungal spores and infection structures to plant surfaces is regarded as an important mechanism in early pathogenesis [7]. Although the morphology of this process has been described for diverse fungi (for review see [8]), its biochemical basis is not well understood. Secreted material like the spore tip mucilage detected on mature spores of the rice blast pathogen, Magnaporthe grisea, serves for conidial attachment [9]. It contains proteins and lipids as well as α-1,2-mannose disaccharide linked to an unknown non-carbohydrate substituent. Furthermore, extracellular glycoproteins were associated with attachment and also with fungal cellular differentiation [10].

During early stages of pathogenesis including host recognition, plant compounds may serve as signals [11]. The plant surface carries a complex mixture of hydrophobic materials collectively called wax. Wax fractions of the host plant, avocado, induced spore germination and appressorium formation of the avocado pathogen, Colletotrichum gloeosporioides, but not of other Colletotrichum species. The inducers appear to be long-chain aliphatic fatty alcohols that are common to plant waxes. Nevertheless, similar wax fractions from species other than avocado remained inactive, suggesting the co-occurrence of inhibitors of fungal development [12]. These and other data indicate the presence of signaling compounds on the plant surface. However, more detailed investigations of their chemical nature and function are required to properly assess their role in pathogenesis.
The biochemical mechanism of thigmotropic sensing is not fully understood, but changes in the arrangement of the cell’s cytoskeleton appear to be involved [17–19]. In addition, in patch-clamp studies using protoplasts of the cell’s cytoskeleton appear to be involved [17±19]. This was demonstrated on artificial substrates withuredospore germings of the bean rust fungus, Uromyces appendiculatus, that recognize ridges of a height similar to the erected lips of the stomatal guard cells [14]. In response to this recognition, all bean rust races as well as many other rust species [15,16] formed appressoria that were morphologically and functionally similar to those formed in vitro.

The crucial role of cAMP in fungal development [30••] and in the signaling pathway that is initiated by fungal surface attachment [31] was substantiated after isolating the MAC1 gene from M. grisea encoding adenylate cyclase [32••]. Not surprisingly, mac1 mutants showed a pleiotropic phenotype. They were unable to form appressoria on an inductive hydrophobic surface in the absence of exogenous cAMP and failed to penetrate susceptible rice leaves. In addition, they were sterile and showed a reduction in vegetative growth, conidiation, and conidial germination. To further pinpoint the signaling pathway, the CPKA gene encoding the catalytic subunit of protein kinase A, a well-known downstream target of cAMP [33], was cloned [34]. Fungal strains containing different cphA mutant alleles were found to be dramatically reduced in pathogenicity [35••]. This reduction did not appear to be due to a loss of appressorium formation, however. cphA mutants are delayed in appressorium formation, but form appressoria to the same level as wild-type strains. These appressoria are fully melanized, but smaller than wild-type and exhibit variable size; they are dramatically reduced in their ability to penetrate plant cells. cphA mutants, however, can produce infectious hyphae and cause lesion formation when inoculated through wounds. Finally,
Parasitic stages of cutinase isozymes are expressed during saprophytic and biosynthetic regulation may be adapted to the population of hydrolytic enzymes including cutinases, ing germination and penetration, fungi generally secrete mechanisms, enzymatic and mechanical penetration. During this process, fungal phytopathogens have evolved two different mechanisms: enzymatic degradation of cutin, the structural polymer of the plant cuticle, has been postulated to be crucial for fungal pathogenicity and cutinase to be a key player in the penetration process [11].

The recent identification of a mitogen-activated protein (MAP) kinase gene, PMK1, sheds light on the signaling pathways downstream of cAMP [36]. Interestingly, this gene is homologous to the *Saccharomyces cerevisiae* MAP kinase genes *FUS3/KSS1* and can complement the mating defect in a *fus3kss1* double mutant of yeast. *Pmk1* mutants of *M. grisea* did not differ in growth (and mating) from a wild-type strain in culture. Pmk1, therefore, appears to be dispensable for vegetative growth. The *pmk1* mutants are capable of responding both to thigmotropic surface signals and to a cAMP-dependent signal. They failed to penetrate the plant cuticle, however, due to a failure to complete the formation of mature appressoria. Since the mutants are still responsive to cAMP for early stages of appressorium formation it is suggested that *Pmk1* acts downstream of a cAMP-dependent signal [36]. Surprisingly, the α-factor pheromone from *S. cerevisiae* is able to block appressorium formation by *M. grisea* and to protected plants from infection in a mating type-specific manner, probably by affecting unknown signaling processes [37*].

### Penetration

For the next step in pathogenesis, the invasion of plant tissues, fungal phytopathogens have evolved two different mechanisms, enzymatic and mechanical penetration. During germination and penetration, fungi generally secrete a mixture of hydrolytic enzymes including cutinases, cellulases, pectinases, and proteases. Although these enzymes are also required by saprophytes, their structures and biosynthetic regulation may be adapted to the specific needs of pathogens. For instance, different cutinase isozymes are expressed during saprophytic and parasitic stages of *Alternaria brassicicola* [38]. Many fungal genes encoding various hydrolytic enzymes have been cloned. Usually, however, the infection phenotype of gene disruption/replacement mutants does not differ from wild-type [39].

In particular, enzymatic degradation of cutin, the structural polymer of the plant cuticle, has been postulated to be crucial for fungal pathogenicity and cutinase to be a key player in the penetration process [11]. Conflicting results were published, however, on the pathogenicity of cutinase-deficient mutants of *Nectria haematococca*, a pathogen of pea and other plants [39,40]. In first experiments, a cutinase disruption mutant displayed the same infectivity as wild-type strains [40]. Later, however, a significant decrease in virulence was observed. More detailed microscopical analyses attributed the remaining virulence mainly to a different way of fungal penetration through host stomata, thus by-passing the plant cuticle [41]. Cutinase disruption mutants of *M. grisea* [42] and, recently, of *Botrytis cinerea* [43] also did not show a modified infection phenotype. Interestingly, in a recent report a different function of cutinase was described [44]. The lipolytic activity of cutinase purified from the apple scab pathogen, *Venturia inaequalis*, was able to protect bean leaves from infection by *Rhizoctonia solani*. The role of this enzyme in host penetration, therefore, remains controversial and appears to vary in different fungi [45].

Alternatively, or in addition to hydrolytic enzymes, some fungi have developed a mechanism to mechanically penetrate the host cuticle. After firm appressorial attachment to the plant surface the porosity of the appressorium wall is drastically reduced by melanin incorporation followed by the establishment of a turgor pressure in excess of 8 MPa [46]. This pressure is focused to a small area at the base of the appressorium which is kept free of wall material and melanin [9]. From this penetration pore, a fine infection hypha, usually called a penetration peg, develops and pierces through the plant cuticle and cell wall (reviewed in [8,9]).

An intriguing question, generated by this work, was what is the nature of the solute responsible for generating such tremendously high hydrostatic pressure? It was recently shown that glycerol levels rise sharply during turgor generation in appressoria of *M. grisea* [47*]. The mean concentration was estimated to be >3.2 M which would account for an osmotic potential of around ~6 MPa. Protein kinase A is known to play a role in the mobilization of storage polysaccharides in fungi and other organisms. The *CPKA* gene, therefore, may have a role in regulating glycerol synthesis. The failure of *cpkA* mutants to penetrate plant cells may be due to their impaired ability to synthesize the high glycerol levels needed from glycogen for sufficient turgor.

The other key player in mechanically penetrating fungi is melanin. In *M. grisea*, single gene mutations at loci encoding melanin biosynthetic enzymes resulted in non-melanized appressoria that are unable to generate turgor and that are non-pathogenic [9,48]. Conversely, pathogenicity of a melanin non-producing albino mutant of the cucumber pathogen, *Colletotrichum lagenarium*, could be restored by transformation with a melanin biosynthetic gene [49]. In addition, appressoria from melanin mutant strains of *M. grisea* as well as wild-type appressoria after treatment with a melanin synthesis inhibitor displayed much lower glycerol levels [47*]. Thus, glycerol appears to be the major compound generating the turgor pressure and melanization to be required for efficient build-up of turgor by rendering the appressorial walls impermeable to glycerol [47*].

### Conclusions

Fungal penetration of living plants is a process controlled by a combination of many factors. In addition to fungal
compounds, these factors also include physical and chemical plant surface features that affect fungal spore germination and appressorium formation. Unraveling these very early stages of fungus-plant interactions clearly deserves further investigations. In particular, mutational approaches to dissect the cAMP-dependent signaling pathways are expected to lead to the identification of those fungal traits that are specifically required for pathogenicity.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


This excellent review summarizes recent evidence for the importance of cAMP signaling in morphogenesis and virulence of those four plant pathogenic fungi that are best studied in this respect. Highly recommended.


This paper convincingly supports the direct role of cAMP and, in particular, of adenylate cyclase in fungal development and presents a model of the signaling system regulating appressorium formation.


This very careful analysis of the phenotype of cAMP-dependent protein kinase A mutants supplements the results in [33], thus providing strong evidence for the role of several cAMP-dependent protein kinase cascades in surface sensing and appressorium function.


This article is the first to report on the surprising interference of pheromone binding with signalling towards infection structure formation; a result that opens up an entirely new strategy to control plant disease.


This report provides convincing evidence for the mechanism that is responsible for generating the highest turgor pressure observed in living organisms.
