

Infection-related development in the rice blast fungus *Magnaporthe grisea*

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Recent developments have been made in the identification of signal transduction pathways and gene products involved in the infection-related development of the rice blast fungus, *Magnaporthe grisea*. It has been established that cAMP-dependent and MAP kinase-mediated signaling are both critical for appressorium morphogenesis and function. These signaling pathways may act downstream of hydrophobin-mediated surface sensing by the growing germ tube. Several genes have been identified that are required for invasive growth of *M. grisea* including genes that allow adaptation of fungal metabolism to growth within plant tissues.

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Abbreviation

MAP mitogen-activated protein

Introduction

Fungi form a variety of interactions with plants and have evolved developmental pathways to breach the outer surface of plant cells and colonize living plant tissue. Plant colonization can result in highly mutualistic interactions (e.g. mycorrhiza) or devastating crop diseases (e.g. rice blast). The genetic and molecular tractability of the rice blast fungus, *Magnaporthe grisea*, has provided fascinating insights into the biochemical mechanisms underlying host recognition, colonization and pathogenesis [1]. Here, we review recent discoveries of signal transduction pathways and gene products required for the establishment of disease by *M. grisea*.

Pathogenic differentiation by *M. grisea*

Rice blast disease starts when asexual spores (conidia) land on the plant leaf surface carried in water droplets. They rapidly attach to the hydrophobic cuticle and are able to germinate in free water within two hours. Conidial germ tubes grow for only a short distance before differentiating into a specialized, domed-shaped cell called an appressorium — the infection cell with which the fungus enters the host (Figure 1) [2,3]. The essential features of appressorium formation are the cessation of polarized germ tube growth, synthesis of new cell wall material, formation of adhesives to attach the appressorium to the underlying surface, movement of all cytoplasm from the germ tube into the appressorium, formation of a specialized septum at the

base of the appressorium, and generation of enormous turgor pressure within the appressorium. Once formed, the appressorium represents a terminal stage of cell differentiation as the fungus is unable to reinitiate saprophytic hyphal growth once an appressorium has formed.

The appressorium ruptures the leaf cuticle using predominantly mechanical force and sends a narrow hypha into the underlying epidermal cells. The infecting hyphae swell as they spread within and between plant cells. Within four days, a disease lesion is produced, and the fungus sporulates to spread the disease to adjacent plants. For discussion we consider two major stages of infection-related development: first, the pre-penetration phase, involving spore germination and the differentiation of appressoria, and, second, the plant colonization phase, during which the fungus proliferates rapidly and causes disease.

The pre-penetration phase of development

Appressorium differentiation is favored by hard, hydrophobic surfaces and repressed by hydrophilic surfaces such as agar or glass slides [4,5]. On hydrophilic surfaces, the addition of cutin monomers or cAMP can stimulate appressorium development [6,7]. The fungus might perceive hydrophobic surfaces through a component of the outer fungal cell wall encoded by the *MPG1* gene [8,9]. *MPG1* encodes a hydrophobin, a class of fungal cell-surface proteins that undergo spontaneous polymerization upon encountering air–water or hydrophobic surface interfaces [10**,11]. It has been shown that *mpg1* deletion ($\Delta mpg1$) mutants form fewer appressoria than wild-type strains, and *MPG1* is expressed during appressorium formation. Self-assembly of the MPG1 hydrophobin after secretion from the growing germ tube may increase wettability of the leaf surface, priming it for the action of other adhesives produced by the fungus, and acting as a signal for appressorium formation. Interestingly, hydrophobin self-assembly appears to be the important factor in conditioning appressorium formation, because diverse hydrophobins — which share little homology but are all believed to be able to undergo self-assembly — are able to complement *M. grisea* $\Delta mpg1$ mutants if expressed under control of the *MPG1* promoter [10**]. Consistently, $\Delta mpg1$ mutants can be induced to form appressoria by addition of cAMP demonstrating that *MPG1* acts upstream of signaling pathways for appressorium formation (see below) [8,12].

A series of recent studies have established that cAMP-dependent signaling is critical for appressorium morphogenesis. Mutations in genes encoding either a G protein α subunit ($\Delta magB$) or adenylate cyclase ($\Delta mac1$) block appressorium formation and are remediated by

Figure 1



Infection-related development by *Magnaporthe grisea*. A low temperature electron micrograph showing an appressorium forming on the surface of a rice leaf. The appressorium is the dome-shaped cell on the right, adhering strongly to the waxy rice cuticle. The conidium and germ tube from which the appressorium has formed have collapsed and all cytoplasm has been transferred to the infection cell. Once *M. grisea* passes through this developmental stage it is committed to cuticle penetration and plant tissue colonisation. Bar = 5 μ m.

cAMP. These mutations also have pleiotropic effects on growth, mating and sporulation [13^{••},14^{••},15[•]]. In contrast, mutations in *CPKA*, which encodes a catalytic subunit of cAMP-dependent protein kinase A (PKA), are specific for pathogenicity, with Δ *cpkA* mutants showing impaired appressorial penetration, but efficient sporulation and mating [16,17[•]]. Because Δ *cpkA* mutants still form appressoria, another effector of cAMP must be important for early appressorium formation. Consistent with this finding, a mutation in the regulatory subunit of PKA rescues appressorium formation in Δ *mac1* mutants [13^{••}]. Thus, alternate PKA holoenzymes are needed for appressorium differentiation and plant cell penetration (see model in Figure 2).

The cAMP-dependent signal for appressorium morphogenesis cooperates with a mitogen-activated protein (MAP) kinase called Pmk1, which is functionally homologous to the Fus3/Kss1 MAP kinases of the pheromone response pathway in yeast [18]. When stimulated with cAMP or presented with appropriate surfaces, Δ *pmk1* mutants initiate appressorium formation but ultimately fail to form appressoria [16]. The relationship between the pheromone response pathway in yeast and appressorium signaling is more than coincidental. Beckerman *et al.* [19[•]] showed that appressorium development in *M. grisea* could be inhibited in a mating type-specific manner by the *Saccharomyces cerevisiae* α -factor. This remarkable observation may indicate that inappropriate stimulation of the mating pathway in *M. grisea* blocks appressorium morphogenesis.

Appressoria generate high intracellular turgor through the accumulation of molar concentrations of glycerol. Glycerol

is maintained in the appressorium by a wall layer rich in polyketide melanin [20^{••}]. It is not known how appressoria signal glycerol accumulation to such high levels. Deletion of a high osmolarity glycerol response (HOG1)-related MAP kinase for example has no effect on glycerol accumulation (KP Dixon, J-R Xu, NJ Talbot, unpublished data). In contrast, appressoria penetration is blocked in Δ *cpkA* mutants and by deletion of a second MAP kinase-encoding gene *MPS1* [17[•],21]. Mps1 is functionally homologous to the Slr2/Mpk1 MAP kinase from *S. cerevisiae* [21]. In yeast, this kinase is responsible for stimulating cell wall growth during periods of membrane stress. In both Δ *cpkA* and Δ *mps1* mutants, however, reduction in appressorium turgor appears to be minimal, suggesting instead that impairment of penetration in these mutants is due to other defects in appressorium morphogenesis.

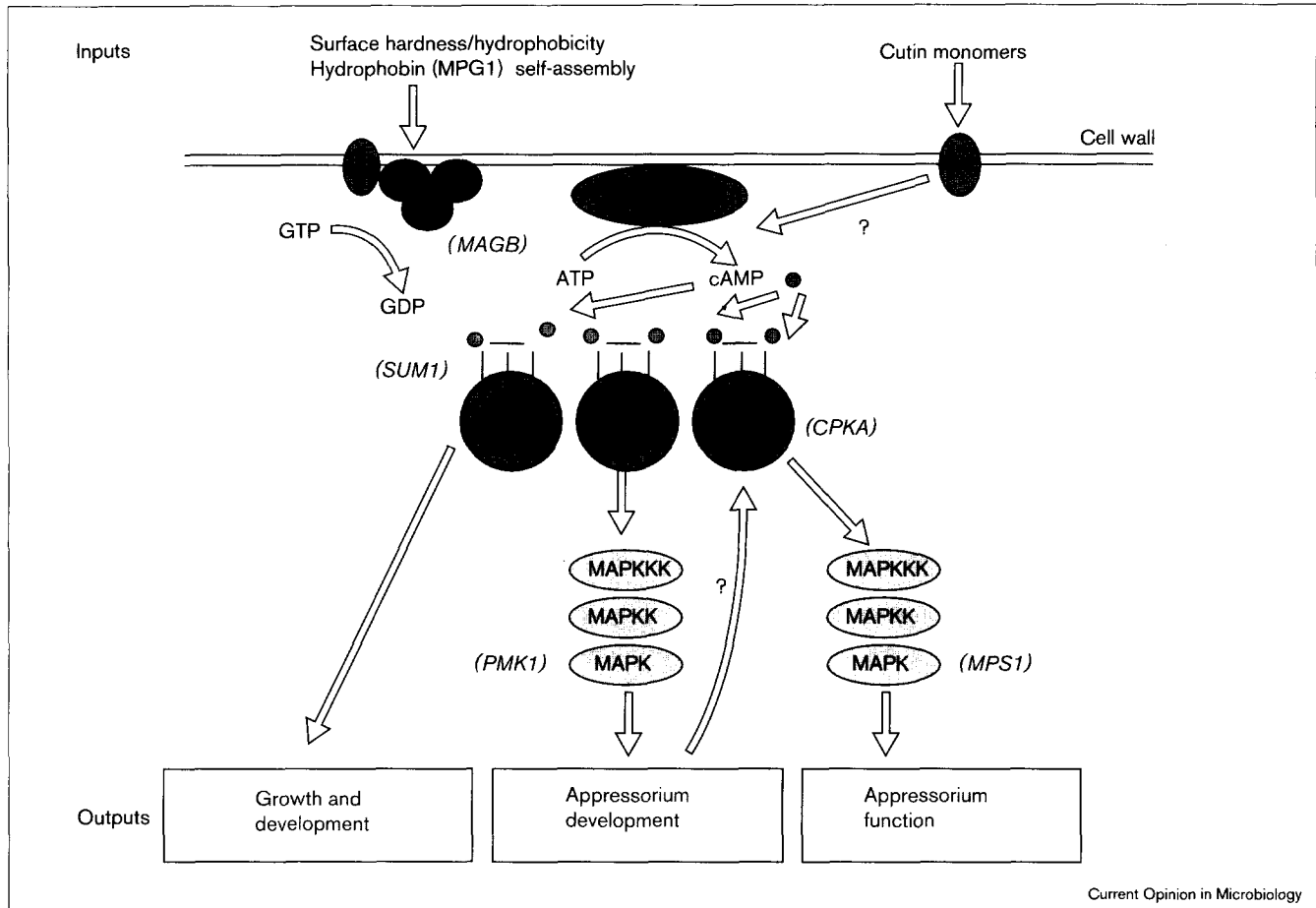
Plant tissue colonization

Studies of the early stages of rice blast disease reveal little or no plant cell necrosis following penetration of susceptible plants [22], suggesting that *M. grisea* is unlikely to produce an array of phytotoxic compounds early in the disease. Indeed, infections on barley and some rice hosts generate 'green islands' — sites of intense chlorophyll pigmentation surrounding infection sites — indicating maintenance of active photosynthesis in the host. Fungal proliferation is rapid; fungal biomass can account for up to 10% of the infected leaf area after only three days of infection [9]. The swift colonization of plant tissue is also apparent by the high level expression of *M. grisea* genes associated with ribosomal and mitochondrial biogenesis [23,24].

Several classes of genes are required for invasive growth of *M. grisea*. One class of genes is that required for host-specific nutrition. *PTH3* encodes a protein involved histidine biosynthesis (imidazole glycerol phosphate dehydratase) and *pth3* mutants are histidine auxotrophs and nonpathogenic on either rice or barley [25^{••}]. *M. grisea* cells are particularly sensitive to histidine levels, because leaky *pth3* mutants — which have some residual gene activity — are able to produce small lesions in host plants. Similarly a methionine auxotroph is also reduced in pathogenicity suggesting that the fungus probably cannot scavenge sufficient methionine from the plant and perhaps synthesizes its own methionine from inorganic sulfate (PV Balhadère, NJ Talbot, unpublished data). In the future, judicious gene knockout experiments may be used to distinguish other metabolic requirements that cannot be met by the plant host.

Another class of genes necessary for plant tissue colonization may be required to adapt *M. grisea* metabolism to allow efficient growth within plant cells. *M. grisea* strains with insertional mutations in the *PTH2* gene are nonpathogenic [25^{••}]. *PTH2* encodes an *M. grisea* homolog of carnitine acetyltransferase, which is an enzyme required to allow activated fatty acids to traverse the mitochondrial membrane for oxidation. Thus, fatty acid oxidation must be an important energy source for pathogenic growth and consequently one

Figure 2



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Model depicting the possible signaling pathways regulating infection related development of *Magnaporthe grisea*. The developing germ tube responds to cues from the rice leaf surface including hydrophobicity [4,5] and cutin monomers [7]. Hydrophobicity may be perceived by self-assembly of the MPG1 hydrophobin [8,9,10**,12]. R is an unidentified receptor for inductive signals, including MPG1 self-assembly, which interacts with the heterotrimeric G-protein leading to transmission of signal for appressorium morphogenesis. The hydrophobicity inductive signal appears to be transmitted via a heterotrimeric G-protein (G α , G β and G γ - encoded by *MAGB*) [14**], and through adenylate cyclase (encoded by *MAC1*) [13**,15*].

Appressorium development is regulated by cAMP signaling involving a cAMP-dependent protein kinase A holoenzyme (PKA II) and MAP kinase signaling cascade involving the product of the *PMK1* gene [18]. Appressorium function may be triggered as a developmental consequence of appressorium formation and is known to involve the cAMP-dependent PKA III catalytic subunit, encoded by *CPKA* [16,17*], and the MAP kinase encoded by *MPS1* [21]. Pleiotropic effects of $\Delta mac1$ mutation on growth and development [13**,15*] suggest that cAMP signalling has a number of other important roles in *M. grisea*, perhaps regulated by further PKA holoenzymes. *SUM1* encodes the regulatory subunit of cAMP-dependent PKA.

can surmise that *M. grisea* cells may be limited for more preferable carbon sources in the plant cell environment. Other genes with moderate pathogenicity phenotypes such as *PTH9*, which encodes a neutral trehalase, suggest that mobilization of stored carbohydrates within the mycelium also contributes to the ability of the fungus to generate disease symptoms [25**].

PTH1, which has homology to the yeast glucose regulatory gene *GRR1*, would also seem to belong to this class of genes that adapt *M. grisea* metabolism during plant tissue colonization [25**]. Unlike the yeast *grr1* mutants, however, *pth1* mutants have no observable defects in glucose regulation (D Ebbole, personal communication). Rather

pth1 mutants form abnormal appressoria that exhibit defects in maintaining turgor. Thus, the role played by *pth1* in appressorium morphogenesis is unclear but may be similar to the role played by *GRR1* in yeast in linking nutrient sensing with control of bud morphogenesis [26].

The idea that the plant cell environment may be limiting for particular carbon or nitrogen sources has led to a search for genes that might play a role in pathogenicity and nitrogen or carbon catabolite repression. In fungi, nitrogen catabolic pathways are controlled by a member of the GATA-factor family of transcriptional regulators [27]. Although the GATA-factor type regulator for nitrogen metabolism in *M. grisea*, *NUT1*, is dispensable for pathogenicity [28], novel

mutations in two unlinked genes, *NPR1* and *NPR2*, are required for nitrogen regulation and pathogenic growth [29]. In addition, limiting nitrogen levels elevate transcript levels of *MPG1* and lead to the production of low molecular weight compounds that enhance leaf desiccation [9,30]. Nitrogen limitation appears to be an important signal for various fungal morphogenesis and pathogenesis pathways [31] and the effect of these mutations on appressorium function (see above) is consistent with this idea.

Disease symptom expression

To date there have been no genes identified that play an active role in plant cell destruction and attempts to demonstrate a role in pathogenicity for plant cell-wall degrading enzymes have (as in many other pathosystems) been unsuccessful [32,33]. A plethora of low molecular weight compounds have been identified in the germination fluids of *M. grisea* cells that may play roles in pathogenicity as phytotoxins but strong genetic and biochemical evidence for a specific toxin in rice blast disease is lacking.

Rice and almost all other plants produce low molecular weight compounds with demonstrable antimicrobial activity, called phytoalexins [34]. Plants are also capable of producing an array of 'pathogenesis-related proteins' some of which have antifungal activity [35]. It seems likely that these host defenses must be overcome if fungal infections are to be successful. One mechanism for this is the production of low molecular weight compounds that suppress the onset of the plant defense response. Although low molecular weight compounds with such activity have been detected in other fungal pathogens [36], none have yet been identified in *M. grisea*. *M. grisea* may possess genetic mechanisms that act in a counter defensive manner to inhibit plant defense activity. One gene from *M. grisea* that may possess this type of activity is *ABC1* [37], which encodes an ABC-transporter highly similar to the multidrug resistant transporters from yeast. These transporters use ATP to drive the efflux of metabolic poisons from the cell and have been implicated in antifungal drug resistance [38]. *ABC1* was identified by an insertional mutation in the regulatory region of the *ABC1* promoter that results in a nonpathogenic phenotype. The mutation prevents high level expression of the *ABC1* transcript in response to metabolic poisons. An *ABC1* null mutant is similarly reduced in pathogenicity but neither the insertional mutant or the null mutant showed increased sensitivity to any of the metabolic poisons tested so far. The *ABC1* transcript is up-regulated by exposing *M. grisea* cells to a rice phytoalexin, suggesting that *ABC1* may be involved in providing resistance to a narrow group of antimicrobial compounds produced by rice.

Conclusions

Studies of fungal phytopathogenicity have been conducted with a typical microbial pathogenesis paradigm, that is pathogens encode gene products that are responsible for disease symptoms. Apart from a narrow group of fungi that

produce host-specific toxins [39], this paradigm is probably insufficient to explain the many diseases caused by fungi. Pathogenicity in *M. grisea*, and many other fungal pathogens, may be viewed as an intricate developmental program that responds to environmental and host-specific cues by eliciting changes in cellular morphogenesis and metabolism in response to the host environment. Fungi are well adapted to growth on complex carbon and nitrogen sources and, thus, it may be that the ability to differentiate certain specialized cells (appressoria) and counter-act host defensive measures (e.g. *ABC1*) may go a long way to explaining some aspects of disease.

So far, almost all pathogenicity genes identified in *M. grisea* have homologs in nonpathogens, although genes with a direct role in causing disease symptoms remain to be identified. Insertional mutagenesis screens and candidate gene knockouts still offer a powerful approach to identifying such pathogenicity genes. Comparative genome sequencing of related pathogenic and nonpathogenic fungi should also make it far simpler to identify an array of pathogen-specific genes and test directly their roles in pathogenesis. These new genomic approaches, coupled with the classic genetics and cell biology approaches, are already being applied to *M. grisea* and new insights into fungal pathogenesis will shortly emerge.

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