Genetic control of infection-related development in Magnaporthe oryzae
Guotian Li¹,2, Xiaoying Zhou² and Jin-Rong Xu¹,2

Diseases caused by various pathogenic fungi pose a serious threat to global food security. Despite their differences in life cycles, fungal pathogens use well-conserved genetic mechanisms to regulate different developmental and infection processes. This review focuses on the key signaling pathways and recent advances in Magnaporthe oryzae, which is a model for studying fungal–plant interactions. In addition to the core components, a number of upstream genes and downstream targets of the cAMP–PKA and mitogen-activated protein (MAP) pathways have been identified. Recent advances in studies with cytoskeleton organization, effector biology, and ROS signaling in M. oryzae and future directions are also discussed.

Addresses
¹ Purdue-NWAFU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China
² Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

Corresponding author: Xu, Jin-Rong (jinrong@purdue.edu)

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Introduction
Fungal pathogens normally initiate infection by attaching dispersing propagules to plant surfaces. In the rice blast fungus Magnaporthe oryzae, a model for studying fungal–plant interactions, specialized infection structures called appressoria are formed at the tip of germ tubes. Turgor pressure generated in melanized appressorium is used to puncture through plant cuticle and cell wall. After penetration, invasive hyphae grow biotropically in host cells. In late infection stages, infectious growth of the pathogen results in plant cell death and lesion development [1,2]. In the past two decades, various genetic mechanisms regulating different infection processes have been characterized in M. oryzae.

Cyclic AMP signaling regulates surface recognition and pathogenesis
The cyclic AMP-protein kinase A (cAMP-PKA) pathway is known to regulate morphogenesis and pathogenesis in a number of fungal pathogens (Table 1) [3]. In M. oryzae, appressorium formation requires the attachment of germ tubes to hydrophobic surfaces but can be induced with cAMP or cutin monomers on hydrophilic surfaces. Molecular characterization of the MAC1 adenylate cyclase and CPKA catalytic subunit of PKA genes (Figure 1) further confirmed the role of cAMP signaling in surface recognition [1]. Recently, the CAP1 gene was shown to be involved in association with the actin cytoskeleton and Mac1 activation [4]. Several components of heterotrimeric G-proteins, including Ga MagA or MagB, Gb MgB1, and Gγ Mgg1, that function upstream from the cAMP–PKA pathway also have been characterized [5]. Furthermore, Rgs1 (Regulator of G-protein signaling) negatively regulates MagA-dependent adenylate cyclase activity. The rgs1 mutant had an elevated intracellular cAMP level and formed appressoria on hydrophilic surfaces [6], which was similar to the phenotype of mutants deleted of the PDE10 cAMP phosphodiesterase gene [7]. Among seven additional RGS-like genes recently characterized in M. oryzae, three of them, RGS3, RGS4, and RGS7, also were required for full virulence [8]. PTH11 encodes a putative G-protein-coupled receptor (GPCR). The defects of ph11 mutants in appressorium formation and pathogenesis were restored by cAMP or DAG treatment, indicating that Pth11 may function as a receptor for cAMP signaling [9].

Transcription factors that may function downstream from the cAMP–PKA pathway in M. oryzae include Mstu1, Som1, and Cdf1. The mstu1 mutant was reduced in appressorium formation and virulence. It was delayed in the mobilization of lipid bodies and glycogens to appressoria, which is regulated by cAMP signaling [10]. Som1 and Cdf1 are two novel transcription factors important for sporulation and appressorium formation [11]. Som1 interacted strongly with Mstu1 and Cdf1 but weakly with CpkA in yeast two-hybrid assays. The expression levels of SOM1 and CDTF1 was reduced in both macl and cpkA mutants. Ph12 also may be functionally related to cAMP signaling because exogenous cAMP induced appressorium formation in the ph12 mutant [12].

A well-conserved MAP kinase (MAPK) pathway is required for appressorium formation
Although cAMP signaling controls surface recognition, late stages of appressorium formation are regulated by Pmk1, a MAPK orthologous to yeast Fus3/Kss1. The pmk1 deletion mutant failed to form appressoria but still recognized hydrophobic surfaces. Several upstream
### Table 1

Major functions of different pathways in plant pathogenic fungi

<table>
<thead>
<tr>
<th></th>
<th>Pmk1</th>
<th>Mps1</th>
<th>Osm1</th>
<th>cAMP-PKA</th>
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</thead>
<tbody>
<tr>
<td>Magnaporthe oryzae</td>
<td>Appressorium formation, pathogenicity, and invasive growth</td>
<td>Pathogenicity, penetration, cell wall integrity, and conidiation</td>
<td>Osmoregulation, stress response, and fungicide sensitivity</td>
<td>Pathogenicity, surface recognition, and turgor generation</td>
</tr>
<tr>
<td>Cochliobolus heterostrophus</td>
<td>Appressorium formation, virulence, conidiation, and melanin biosynthesis</td>
<td>Virulence, cell wall integrity, and conidiation</td>
<td>Virulence, osmoregulation, and stress response</td>
<td>N/A</td>
</tr>
<tr>
<td>Colletotrichum orbiculare</td>
<td>Appressorium formation, pathogenicity, and spore germination</td>
<td>Appressorium formation, pathogenicity, and conidiation</td>
<td>Osmoregulation and fungicide sensitivity</td>
<td>Virulence, penetration, and spore germination</td>
</tr>
<tr>
<td>Ustilago maydis</td>
<td>Virulence, appressorium formation, penetration, and mating (Kpp2 and Kpp6)</td>
<td>Cell wall integrity, stress response, and cell cycle regulation</td>
<td>N/A</td>
<td>Pathogenicity, mating, and filamentous growth</td>
</tr>
<tr>
<td>Bipolaris oryzae</td>
<td>Pathogenicity, conidiation, and vegetative growth</td>
<td>N/A</td>
<td>Osmoregulation, stress response, and fungicide sensitivity</td>
<td>N/A</td>
</tr>
<tr>
<td>Pyrenophora teres</td>
<td>Appressorium formation, pathogenicity, and conidiation</td>
<td>N/A</td>
<td>Virulence, pigmentation, and conidiation</td>
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<td>Cryphonectria parasitica</td>
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</tr>
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<td>Fusarium oxysporum</td>
<td>Pathogenicity, root attachment, and invasive growth</td>
<td>N/A</td>
<td>N/A</td>
<td>Virulence, root attachment, and conidiation</td>
</tr>
<tr>
<td>Fusarium graminearum</td>
<td>Pathogenicity, female fertility, and deoxynivalenol biosynthesis</td>
<td>Pathogenicity, fertility, cell wall integrity, and DON biosynthesis</td>
<td>Virulence, fertility, osmoregulation, and DON biosynthesis</td>
<td>Virulence, fertility, colony morphology, and DON biosynthesis</td>
</tr>
<tr>
<td>Fusarium verticilloides</td>
<td>Pathogenicity, fumonisin biosynthesis, and conidiation</td>
<td>Virulence, penetration, conidiation, and sclerotium formation</td>
<td>N/A</td>
<td>Virulence, conidiation, and stress response</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>Pathogenicity, penetration, and spore germination</td>
<td>Virulence, penetration, conidiation, and sclerotium formation</td>
<td>Virulence, penetration, osmoregulation, and conidiation</td>
<td>Virulence and vegetative growth</td>
</tr>
<tr>
<td>Verticillium dahlia</td>
<td>Pathogenicity, conidiation, and sclerotium formation</td>
<td>N/A</td>
<td>N/A</td>
<td>Virulence and conidiation</td>
</tr>
<tr>
<td>Mycosphaerella graminicola</td>
<td>Pathogenicity, pycnidium formation, and stoma penetration</td>
<td>Pathogenicity, cell wall integrity, invasive growth, and pycnidium formation</td>
<td>Pathogenicity, osmoregulation, fungicide sensitivity, and dimorphism</td>
<td>Virulence, pycnidium formation, and filamentation</td>
</tr>
<tr>
<td>Stagonospora nodorum</td>
<td>Virulence, conidiation, melanin biosynthesis, and stress response</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Alternaria brassicicola</td>
<td>Virulence, conidiation, and hydrolytic enzyme production</td>
<td>Virulence, cell wall integrity, and camalexin sensitivity</td>
<td>Virulence, stress response, camalexin and fungicide sensitivity</td>
<td>N/A</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>Virulence, penetration, conidiation, and melanin biosynthesis</td>
<td>Virulence, cell wall integrity, conidiation, and toxin biosynthesis</td>
<td>Virulence, osmoregulation, and stress response</td>
<td>Virulence, toxin biosynthesis, and vegetative growth</td>
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*Not assayed.*
components of the Pmk1 pathway have been identified, including MEKK Mst11, MEK Mst7, and an adaptor protein Mst50 [1]. Mst50 interacted with Mst11 and Ras proteins (Ras1 and Ras2). While the ras1 mutant was normal in pathogenesis, RAS2 appeared to be an essential gene in *M. oryzae*. Expression of a dominant active RAS2G12V (RAS2DA) allele in the wild type induced appressorium formation on aerial hyphae and hydrophilic surfaces. Ras2 may function upstream from both the Pmk1 and cAMP signaling pathways (Fig. 1) because RAS2DA transformants had increased Pmk1 phosphorylation and intracellular cAMP levels. Recently, the GAP1 RasGAP and RGF1 RasGEF genes that regulate Ras2 activities also have been identified (Zhou and Xu, unpublished).

*PMK1* orthologs are required for appressorium formation in several other appressorium-forming fungi (Table 1) [2,13]. In *M. oryzae*, Msb2 and Sho1 have been reported to function as upstream sensors of the Pmk1 pathway for recognizing surface chemical signals. The msb2 mutant barely formed appressoria on artificial hydrophobic surfaces but were normal in appressorium formation on plant surfaces or surfaces coated with plant epicuticular waxes [14*]. In *Ustilago maydis*, Msb2 and Sho1 also function upstream from Kpp2 and Kpp6, two MAPKs with overlapping functions in pathogenesis but the latter being more critical for appressorium formation [15*]. In *Fusarium oxysporum*, msb2 mutants had similar defects with *mik1* mutants in cellophane penetration and in planta growth [16*].

**Pmk1 is also important for appressorium penetration and invasive growth**

The *pmk1* mutant was defective in the degradation of glycogen and lipid bodies for turgor generation and failed to infect through wounds. Expression of the MEK inhibitor HopA11 during *in planta* growth with the *MIR1* or
Several genes regulated by Pmk1 also have been functionally characterized, including GAS1 and GAS2 [20]. Expression profile analysis has identified 481 and 146 genes that had reduced expression levels in the pmk1 and mst12 mutants, respectively [21]. Several of them are known virulence factors, such as MoHox7 and Pth11. Two Pmk1-interacting proteins Pic1 and Pic5 were identified by yeast two-hybrid assays. Pic1 is a nuclear protein with one putative MAP kinase phosphorylation site and Pic5 has two functionally unknown CTNS (cystinosin/ERS1p repeat) motifs. Whereas disruption of Pic1 had no detectable phenotype, the Pic5 mutant was defective in appressorium formation, penetration, and pathogenesis [22]. Orthologs of PMK1 are also known to regulate plant penetration and infectious growth in other fungal pathogens (Table 1). For example, Pmk1 orthologs regulates the expression of genes encoding cell wall degrading enzymes and secreted lipases in F. oxysporum and Fusarium graminearum.

The cell wall integrity MAPK pathway and calcium signaling

Studies have shown that the cell wall integrity MAPK pathway is well conserved for pathogenesis in plant and human pathogens (Table 1) [2]. In M. oryzae, the MPS1 pathway is dispensable for appressorium formation but essential for cell wall integrity, appressorium penetration, and invasive growth [23,24]. Interestingly, Mps1 regulates the accumulation of alpha-1, 3-glucan, a component of the outer cell wall layer that may provide protection against chitinases during plant infection [25].

Mps1 interacts with two of its putative downstream transcription factors, Mig1 and MoSwi6 [26,27]. MIG1 is required for overcoming plant defense responses and invasive growth. The mig1 mutant formed invasive hyphae-like structures in heat-killed but not live plant cells [26]. Deletion of MoSwi6 resulted in increased sensitivity to cell wall and oxidative stresses and reduced virulence. Interestingly, the Moswi6 mutant had higher chitin content than the wild type and upregulated expression levels of several chitin synthase genes.

In yeast, protein kinase C (PKC) and calcium signaling are known to function upstream from the Slt2 pathway. Systematic characterization of proteins related to calcium signaling, including the Mocch1 Ca2+ channel, calmodulin, and PMG1 Ca2+ pump, confirmed its role in infection-related morphogenesis in M. oryzae [28]. The MoplCi1, MoplCi2, and MoplCi3 phospholipase C genes and both catalytic (MCNA) and regulatory (MCNB) subunits of calcineurin also have been functionally characterized in M. oryzae [29]. Cyclosporin A forms a complex with Cyp1 cyclophilin [30] to inhibit calcineurin. The interaction of MCNA with Morz1, the major downstream transcription factor of calcineurin, was enhanced by Ca2+. Morz1 is involved in cell wall integrity, turgor pressure generation, penetration, and virulence [31]. In ChIP–chip assays, 140 genes were identified as targets of Morz1 [29], including Chsi1, Chsi3, PMCI, LHSI1, PDE1, and MgAPT2 [32*].

The osmoregulation pathway and stress response

In M. oryzae, OSM1 is essential for response to hyperosmotic stress but dispensable for appressorium turgor generation and plant infection [33]. Genes required for turgor generation, such as TPS1 and BUF1, must be independent of Osm1 regulation during appressorium formation. Deletion of the HIK1 (OS-1 ortholog) histidine kinase or Mossk1 gene resulted in similar phenotypes [34]. However, the Mosln1 and Moskn7 mutants were reduced in virulence, suggesting that these genes may have downstream targets other than Osm1 [35,36].

In a number of fungi, the osmoregulation pathway also is involved in response to oxidative species and resistance to phenylpyrrole and dicarboximide fungicides [13]. In general, mutants blocked in the osmoregulation pathway became more sensitive to oxidative species but were resistant to phenylpyrrole and dicarboximide fungicides. Like in other fungi, MoAtf1 is a downstream target of Osm1 in M. oryzae for regulating response to ROS. The Moatf1 mutant was hypersensitive to oxidative stress and reduced in the expression of several extracellular peroxidase and laccase genes. Its defects in plant infection were suppressed by treatments with ROS scavenging compounds [38].

While Osm1 is dispensable for pathogenicity in M. oryzae and Colletotrichum lagenarium, its orthologs are important for plant infection in a number of plant and human pathogens (Table 1), including Mycosphaerella graminicola and Cryptococcus neoformans. In addition to pathogenesis, this MAPK also plays species-specific roles in conidiation.
survival structure formation, sexual reproduction, and secondary metabolism [2,39].

**Effector delivery mechanisms**

To date, over a dozen effectors have been characterized in *M. oryzae*, including Avr-Pita, AvrPiz-t, and a LysM domain-containing protein Spl1 [40,41*]. Like in other ascomycete pathogens, mechanisms for effector translocation and cell-to-cell trafficking are not clear in *M. oryzae*. The effectors that have been characterized to date lack common or conserved motifs similar to RXLR in oomycete effectors. Microscopic examinations have revealed that some effectors such as Pwl2 were delivered into plant cells via the biotrophic interfacial complex (BIC), a distinct structure consisting of host cytoplasm membrane [42]. Other effectors such as Bas4 were accumulated in the space between invasive hyphae and enveloping host membrane. Interestingly, fungal effectors were able to move to neighboring plant cells ahead of invasive hyphae, possibly by cell-to-cell trafficking through plasmodesmata [40,43*]. It will be important to determine the mechanism regulating BIC localization, which appears to be independent of protein sequences.

Genetic studies have shown that *LHS1* and *MgAPT2* may be involved in effector folding or secretion because their mutants failed to elicit HR response on resistant cultivars [44]. Lhs1, an endoplasmic reticulum (ER) luminal protein required for penetration and biotrophic growth may be important for ER secretion and protein translocation. The MgApt2 P-type ATPase is important for Golgi apparatus development and secretion of extracellular enzymes via exocytosis [44].

**Dynamic organization of cytoskeleton during plant penetration**

Earlier studies have shown that appressorium pore area of emerging penetration pegs mainly contains actin cytoskeleton and Pls1 tetraspanin [45] is essential for peg differentiation. Although the relationship between Pls1 and actin is not clear, the cortical cytoskeleton is known to be associated with tetraspan-enriched microdomains. In *M. oryzae*, MoRac1 GTPase directly interacted with Chm1 PAK kinase [46] and the activation of Chm1 regulated actin organization and polarized growth [47]. The *Mgac1* mutant produced only a few deformed conidia and was defective in appressorium formation.

Appressorium formation requires one round of mitosis and the development of a septum to delimit appressoria from the rest of germ tubes. Blocking the initiation of DNA replication inhibited appressorium formation [48]. Recently, septins were shown to interact with the F-actin network to form a ring surrounding the appressorium pore. The septin ring formation requires Cdc42, Chm1, and MAPK signaling components Mps1 and Mst12. All the septin (*sep3–sep6*) mutants were defective in plant infection and the organization of F-actin network in appressoria. Septins were required for directing membrane curvature proteins to the center of appressorium pores and emergence of penetration pegs [49*].

**ROS and pathogenesis**

In *M. oryzae*, ROS are involved in conidium germination and appressorium formation [50]. The *nox1* and *nox2* NADPH oxidase mutants were defective in ROS generation, conidiogenesis, cuticle penetration, and invasive growth. *MoRAC1* interacted with Nox1 and Nox2 in yeast two-hybrid assays and *Morac1* mutants were impaired in ROS generation [47].

Response to ROS involves a wide variety of proteins. Many mutants were hypersensitive to oxidative stress in *M. oryzae*. For some of them, such as the *des1*, *abc3*, and *Moorf1* mutants, their defects in plant infection were partially recovered by treatments with ROS inhibitors [38,51]. In *Saccharomyces cerevisiae*, the Hry1 thiol peroxidase activates the bZIP transcription factor Yap1 upon ROS induction, which in turn activates a suite of anti-oxidant genes. In *M. oryzae*, *MoHYR1* is important for *H2O2* tolerance and virulence [52]. *MoAP1* also plays critical roles in stress response and pathogenicity [53]. Among the genes regulated by MoAp1, *MoSSADH* and *MoACT* were shown to be important for plant infection.

**Conclusions**

In fungal pathogens, the core components of key signaling or regulatory pathways are well conserved. However, various fungi differ significantly in the upstream signals and sensors. In plant pathogenic fungi, multiple sensors may be responsible for the recognition of different physical and chemical signals and activation of various signaling cascades. For example, yeast has only one HK and three GPCR genes but there are over 10 HK and 50 GPCR-like genes in many filamentous fungi. Also, the involvement of one pathway in various developmental and infection processes may be executed through different downstream transcription factors and target genes. More importantly, different mechanisms involved in infection-related morphogenesis must be coordinated or integrated during plant infection. It will be important to identify and characterize the networks of transcription factors and other regulatory genes that control various infection processes and fungal–plant interactions.

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References and recommended reading

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