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Genetic control of infection-related development in *Magnaporthe oryzae*

Guotian Li[1,2], Xiaoying Zhou[2] and Jin-Rong Xu[1,2]

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 also are 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 appressoria is used to puncture through plant cuticle and cell wall. After penetration, invasive hyphae grow biotrophically 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*.

cAMP 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 Gα MagA or MagB, Gβ 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 *PDEH* 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 *pth11* 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 Cdtf1. 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 Cdtf1 are two novel transcription factors important for sporulation and appressorium formation [11•]. Som1 interacted strongly with Mstu1 and Cdtf1 but weakly with CpkA in yeast two-hybrid assays. The expression levels of *SOM1* and *CDTF1* was reduced in both *mac1* and *cpkA* mutants. Pth12 also may be functionally related to cAMP signaling because exogenous cAMP induced appressorium formation in the *pth12* 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

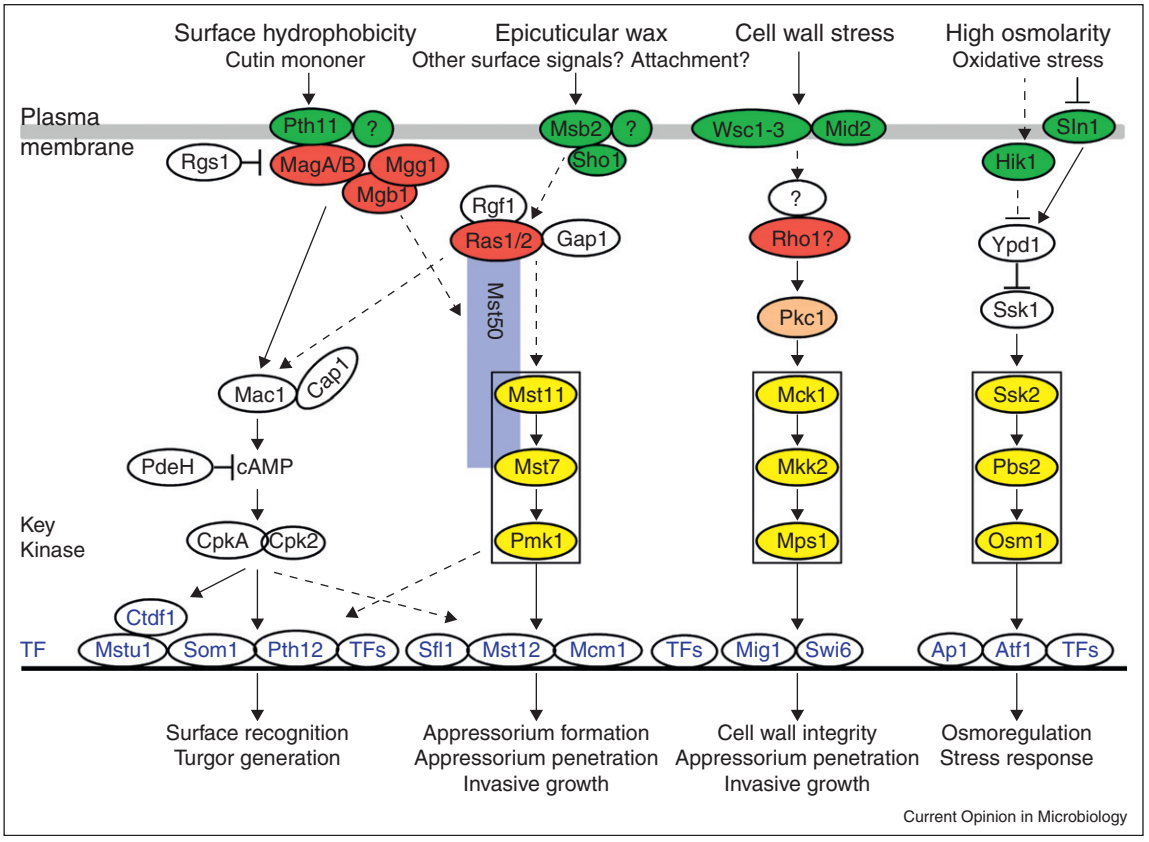
Table 1

Major functions of different pathways in plant pathogenic fungi

	Pmk1	Mps1	Osm1	cAMP–PKA
Magnaporthe oryzae	Appressorium formation, pathogenicity, and invasive growth	Pathogenicity, penetration, cell wall integrity, and conidiation	Osmoregulation, stress response, and fungicide sensitivity	Pathogenicity, surface recognition, and turgor generation
Cochliobolus heterostrophus	Appressorium formation, virulence, conidiation, and melanin biosynthesis	Virulence, cell wall integrity, and conidiation	Virulence, osmoregulation, and stress response	N/A[a]
Colletotrichum orbiculare	Appressorium formation, pathogenicity, and spore germination	Appressorium formation, pathogenicity, and conidiation	Osmoregulation and fungicide sensitivity	Virulence, penetration, and spore germination
Ustilago maydis	Virulence, appressorium formation, penetration, and mating (Kpp2 and Kpp6)	Cell wall integrity, stress response, and cell cycle regulation	N/A	Pathogenicity, mating, and filamentous growth
Bipolaris oryzae	Pathogenicity, conidiation, and vegetative growth	N/A	Osmoregulation, stress response, and fungicide sensitivity	N/A
Pyrenophora teres	Appressorium formation, pathogenicity, and conidiation	N/A	N/A	N/A
Cryphonectria parasitica	Virulence, conidiation, and vegetative growth	N/A	Virulence, pigmentation, and conidiation	N/A
Fusarium oxysporum	Pathogenicity, root attachment, and invasive growth	N/A	N/A	Virulence, root attachment, and conidiation
Fusarium graminearum	Pathogenicity, female fertility, and deoxynivalenol biosynthesis	Pathogenicity, fertility, cell wall integrity, and DON biosynthesis	Virulence, fertility, osmoregulation, and DON biosynthesis	Virulence, fertility, colony morphology, and DON biosynthesis
Fusarium verticilloides	Pathogenicity, fumonisin biosynthesis, and conidiation	N/A	N/A	Virulence, conidiation, and stress response
Botrytis cinerea	Pathogenicity, penetration, and spore germination	Virulence, penetration, conidiation, and sclerotium formation	Virulence, penetration, osmoregulation, and conidiation	Virulence and vegetative growth
Verticillium dahlia	Pathogenicity, conidiation, and sclerotium formation	N/A	N/A	Virulence and conidiation
Mycosphaerella graminicola	Pathogenicity, pycnidium formation, and stoma penetration	Pathogenicity, cell wall integrity, invasive growth, and pycnidium formation	Pathogenicity, osmoregulation, fungicide sensitivity, and dimorphism	Virulence, pycnidium formation, and filamentation
Stagonospora nodorum	Virulence, conidiation, melanin biosynthesis, and stress response	N/A	N/A	N/A
Alternaria brassicicola	Virulence, conidiation, and hydrolytic enzyme production	Virulence, cell wall integrity, and camalexin sensitivity	Virulence, stress response, camalexin and fungicide sensitivity	N/A
Alternaria alternata	Virulence, penetration, conidiation, and melanin biosynthesis	Virulence, cell wall integrity, conidiation, and toxin biosynthesis	Virulence, osmoregulation, and stress response	Virulence, toxin biosynthesis, and vegetative growth

[a] Not assayed.

Figure 1



The key signaling pathways involved in infection-related morphogenesis in *M. oryzae*. The receptor/sensor, trimeric or small GTPase, and MAP kinase cascade genes are shaded in green, red, and yellow, respectively. All putative downstream transcription factors (TFs) are in blue texts. Two genes encoding catalytic subunits of PKA (*CPKA* and *CPK2*) may have overlapping functions in cAMP signaling for surface recognition and plant infection. Multiple upstream signals may converge on Mst50, an adaptor protein of the Pmk1 pathway, for regulating appressorium formation, penetration, and invasive growth. Cross talking between the cAMP signaling and Pmk1 pathway may occur at the upstream G-proteins or downstream TFs. The Mps1 pathway also is important for appressorium penetration and invasive growth. Although its upstream components are not well studied, PKC, calcium signaling, and related sensor genes are likely conserved in *M. oryzae* for the cell wall integrity MAPK pathway. Osm1 is dispensable for plant infection but plays a critical role in responses to hyperosmotic and other stresses and possibly survival in nature.

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 $RAS2^{G18V}$ ($RAS2^{DA}$) 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 $RAS2^{DA}$ 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 *fmk1* 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 HopAI1 during *in planta* growth with the *MIR1* or

BAS4 promoter also blocked lesion development (Liu and Xu, unpublished). In addition, mutants deleted of *MST12*, one of the downstream targets of Pmk1, still formed melanized appressoria but failed to penetrate and infect plant cells [17].

Because *mst12* mutants still form appressoria, other transcript factors must function downstream from Pmk1 for appressorium formation. Sfl1 was identified in *in vitro* phosphorylation assays with a protein microarray of over 500 *M. oryzae* transcription factors [18•]. It has putative MAPK docking and MAPK phosphorylation sites. However, the *sfl1* deletion mutant was normal in appressorium formation although it was reduced in virulence. MoMcm1 was isolated as one of the Mst12-interacting proteins in *M. oryzae* by affinity purification. The *Momcm1* mutant produced long, deformed germ tubes on hydrophobic surfaces and was defective in appressorium formation and invasive growth [19].

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 Ca^{2+} channel, calmodulin, and *PMC1* Ca^{2+} pump, confirmed its role in infection-related morphogenesis in *M. oryzae* [28]. The *MoPLC1*, *MoPLC2*, and *MoPLC3* 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 *MoCRZ1*, the major downstream transcription factor of calcineurin, was enhanced by Ca^{2+}. *MoCRZ1* is involved in cell wall integrity, turgor pressure generation, penetration, and virulence [31]. In ChIP–chip assays, 140 genes were identified as targets of *MoCRZ1*, including *CHS1*, *CHS3*, *PMC1*, *LHS1*, *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 [37], 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 Slp1 [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) lumenal 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 *Mgrac1* 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 *Moatf1* mutants, their defects in plant infection were partially recovered by treatments with ROS inhibitors [38,51]. In *Saccharomyces cerevisiae*, the Hyr1 thiol peroxidase activates the bZIP transcription factor Yap1 upon ROS induction, which in turn activates a suite of antioxidant genes. In *M. oryzae*, *MoHYR1* is important for H_2O_2 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

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wilson RA, Talbot NJ: **Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae***. *Nat Rev Microbiol* 2009, **7**:185-195.

2. Zhao X, Mehrabi R, Xu JR: **Mitogen-activated protein kinase pathways and fungal pathogenesis**. *Eukaryot Cell* 2007, **6**:1701-1714.

3. Kronstad JW, Hu G, Choi J: **The cAMP/protein kinase A pathway and virulence in *Cryptococcus neoformans***. *Mycobiology* 2011, **39**:143-150.

4. Zhou X, Zhang H, Li G, Shaw B, Xu JR: **The cyclase-associated protein Cap1 is important for proper regulation of infection-related morphogenesis in *Magnaporthe oryzae***. *PLoS Pathog* 2012, **8**:e1002911.

5. Liang S, Wang ZY, Liu PJ, Li DB: **A G gamma subunit promoter T-DNA insertion mutant — A1-412 of *Magnaporthe grisea* is defective in appressorium formation, penetration and pathogenicity**. *Chin Sci Bull* 2006, **51**:2214-2218.

6. Liu H, Suresh A, Willard FS, Siderovski DP, Lu S, Naqvi NI: **Rgs1 regulates multiple Galpha subunits in *Magnaporthe* pathogenesis, asexual growth and thigmotropism**. *EMBO J* 2007, **26**:690-700.

7. Ramanujam R, Naqvi NI: **PdeH, a high-affinity cAMP phosphodiesterase, is a key regulator of asexual and pathogenic differentiation in *Magnaporthe oryzae***. *PLoS Pathog* 2010, **6**:e1000897.

8. Zhang H, Tang W, Liu K, Huang Q, Zhang X, Yan X, Chen Y, Wang J, Qi Z, Wang Z *et al.*: **Eight RGS and RGS-like proteins orchestrate growth, differentiation, and pathogenicity of *Magnaporthe oryzae***. *PLoS Pathog* 2011, **7**:e1002450.

9. DeZwaan TM, Carroll AM, Valent B, Sweigard JA: ***Magnaporthe grisea* Pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues**. *Plant Cell* 1999, **11**:2013-2030.

10. Nishimura M, Fukada J, Moriwaki A, Fujikawa T, Ohashi M, Hibi T, Hayashi N: **Mstu1, an APSES transcription factor, is required for appressorium-mediated infection in *Magnaporthe grisea***. *Biosci Biotechnol Biochem* 2009, **73**:1779-1786.

11. • Yan X, Li Y, Yue X, Wang C, Que Y, Kong D, Ma Z, Talbot NJ, Wang Z: **Two novel transcriptional regulators are essential for infection-related morphogenesis and pathogenicity of the rice blast fungus *Magnaporthe oryzae***. *PLoS Pathog* 2011, **7**:e1002385.
Two putative downstream transcription factors of cAMP–PKA pathway were identified by insertional mutagenesis

12. Kim S, Park SY, Kim KS, Rho HS, Chi MH, Choi J, Park J, Kong S, Goh J, Lee YH: **Homeobox transcription factors are required for conidiation and appressorium development in the rice blast fungus *Magnaporthe oryzae***. *PLoS Genet* 2009, **5**:e1000757.

13. Hamel LP, Nicole MC, Duplessis S, Ellis BE: **Mitogen-activated protein kinase signaling in plant-interacting fungi: distinct messages from conserved messengers**. *Plant Cell* 2012, **24**:1327-1351.

14. • Liu W, Zhou X, Li G, Li L, Kong L, Wang C, Zhang H, Xu JR: **Multiple plant surface signals are sensed by different mechanisms in the rice blast fungus for appressorium formation**. *PLoS Pathog* 2011, **7**:e1001261.
Studies in *U. maydis*, *M. oryzae*, and *F. oxysporum* showed that Msb2 orthologs function upstream of a conserved MAPK pathway for plant infection. Sho1 only plays a minor role in these fungal pathogens

15. • Lanver D, Mendoza-Mendoza A, Brachmann A, Kahmann R: **Sho1 and Msb2-related proteins regulate appressorium development in the smut fungus *Ustilago maydis***. *Plant Cell* 2010, **22**:2085-2101.
Studies in *U. maydis*, *M. oryzae*, and *F. oxysporum* showed that Msb2 orthologs function upstream of a conserved MAPK pathway for plant infection. Sho1 only plays a minor role in these fungal pathogens

16. • Perez-Nadales E, Di Pietro A: **The membrane mucin Msb2 regulates invasive growth and plant infection in *Fusarium oxysporum***. *Plant Cell* 2011, **23**:1171-1185.
Studies in *U. maydis*, *M. oryzae*, and *F. oxysporum* showed that Msb2 orthologs function upstream of a conserved MAPK pathway for plant infection. Sho1 only plays a minor role in these fungal pathogens

17. Park G, Bruno KS, Staiger CJ, Talbot NJ, Xu JR: **Independent genetic mechanisms mediate turgor generation and penetration peg formation during plant infection in the rice blast fungus**. *Mol Microbiol* 2004, **53**:1695-1707.

18. • Li G, Zhou X, Kong L, Wang Y, Zhang H, Zhu H, Mitchell TK, Dean RA, Xu JR: **MoSfl1 is important for virulence and heat tolerance in *Magnaporthe oryzae***. *PLoS One* 2011, **6**:e19951.
The first published protein chip experiment in *M. oryzae*. This protein chip contains most of the predicted transcription factors

19. Zhou X, Liu W, Wang C, Xu Q, Wang Y, Ding S, Xu JR: **A MADS-box transcription factor MoMcm1 is required for male fertility, microconidium production and virulence in *Magnaporthe oryzae***. *Mol Microbiol* 2011, **80**:33-53.

20. Xue C, Park G, Choi W, Zheng L, Dean RA, Xu JR: **Two novel fungal virulence genes specifically expressed in appressoria of the rice blast fungus**. *Plant Cell* 2002, **14**:2107-2119.

21. Soanes DM, Chakrabarti A, Paszkiewicz KH, Dawe AL, Talbot NJ: **Genome-wide transcriptional profiling of appressorium development by the rice blast fungus *Magnaporthe oryzae***. *PLoS Pathog* 2012, **8**:e1002514.

22. Zhang H, Xue C, Kong L, Li G, Xu JR: **A Pmk1-interacting gene is involved in appressorium differentiation and plant infection in *Magnaporthe oryzae***. *Eukaryot Cell* 2011, **10**:1062-1070.

23. Xu JR, Staiger CJ, Hamer JE: **Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses**. *Proc Natl Acad Sci U S A* 1998, **95**:12713-12718.

24. Jeon J, Goh J, Yoo S, Chi MH, Choi J, Rho HS, Park J, Han SS, Kim BR, Park SY *et al.*: **A putative MAP kinase kinase kinase, MCK1, is required for cell wall integrity and pathogenicity of the rice blast fungus, *Magnaporthe oryzae***. *Mol Plant Microbe Interact* 2008, **21**:525-534.

25. Fujikawa T, Kuga Y, Yano S, Yoshimi A, Tachiki T, Abe K, Nishimura M: **Dynamics of cell wall components of *Magnaporthe grisea* during infectious structure development**. *Mol Microbiol* 2009, **73**:553-570.

26. Mehrabi R, Ding S, Xu JR: **MADS-box transcription factor Mig1 is required for infectious growth in *Magnaporthe grisea***. *Eukaryot Cell* 2008, **7**:791-799.

27. Qi Z, Wang Q, Dou X, Wang W, Zhao Q, Lv R, Zhang H, Zheng X, Wang P, Zhang Z: **MoSwi6, an APSES family transcription factor, interacts with MoMps1 and is required for hyphal and conidial morphogenesis, appressorial function and pathogenicity of *Magnaporthe oryzae***. *Mol Plant Pathol* 2012, **13**:677-689.

28. Nguyen QB, Kadotani N, Kasahara S, Tosa Y, Mayama S, Nakayashiki H: **Systematic functional analysis of calcium-signalling proteins in the genome of the rice-blast fungus, *Magnaporthe oryzae*, using a high-throughput RNA-silencing system**. *Mol Microbiol* 2008, **68**:1348-1365.

29. Choi J, Kim KS, Rho HS, Lee YH: **Differential roles of the phospholipase C genes in fungal development and pathogenicity of *Magnaporthe oryzae***. *Fungal Genet Biol* 2011, **48**:445-455.

30. Viaud MC, Balhadere PV, Talbot NJ: **A *Magnaporthe grisea* cyclophilin acts as a virulence determinant during plant infection**. *Plant Cell* 2002, **14**:917-930.

31. Choi J, Kim Y, Kim S, Park J, Lee YH: **MoCRZ1, a gene encoding a calcineurin-responsive transcription factor, regulates fungal**

growth and pathogenicity of *Magnaporthe oryzae*. *Fungal Genet Biol* 2009, **46**:243-254.

32. • Kim S, Hu J, Oh Y, Park J, Choi J, Lee YH, Dean RA, Mitchell TK: **Combining ChIP–chip and expression profiling to model the MoCRZ1 mediated circuit for Ca/calcineurin signaling in the rice blast fungus**. *PLoS Pathog* 2010, **6**:e1000909.
The first published ChIP–chip experiment in *M. oryzae*. Several important pathogenicity factors were found to be regulated by MoCrz1

33. Dixon KP, Xu JR, Smirnoff N, Talbot NJ: **Independent signaling pathways regulate cellular turgor during hyperosmotic stress and appressorium-mediated plant infection by *Magnaporthe grisea***. *Plant Cell* 1999, **11**:2045-2058.

34. Motoyama T, Kadokura K, Ohira T, Ichiishi A, Fujimura M, Yamaguchi I, Kudo T: **A two-component histidine kinase of the rice blast fungus is involved in osmotic stress response and fungicide action**. *Fungal Genet Biol* 2005, **42**:200-212.

35. Motoyama T, Ochiai N, Morita M, Iida Y, Usami R, Kudo T: **Involvement of putative response regulator genes of the rice blast fungus *Magnaporthe oryzae* in osmotic stress response, fungicide action, and pathogenicity**. *Curr Genet* 2008, **54**:185-195.

36. Zhang H, Liu K, Zhang X, Song W, Zhao Q, Dong Y, Guo M, Zheng X, Zhang Z: **A two-component histidine kinase, MoSLN1, is required for cell wall integrity and pathogenicity of the rice blast fungus, *Magnaporthe oryzae***. *Curr Genet* 2010, **56**:517-528.

37. Lara-Rojas F, Sanchez O, Kawasaki L, Aguirre J: ***Aspergillus nidulans* transcription factor AtfA interacts with the MAPK SakA to regulate general stress responses, development and spore functions**. *Mol Microbiol* 2011, **80**:436-454.

38. Guo M, Guo W, Chen Y, Dong S, Zhang X, Zhang H, Song W, Wang W, Wang Q, Lv R *et al.*: **The basic leucine zipper transcription factor Moatf1 mediates oxidative stress responses and is necessary for full virulence of the rice blast fungus *Magnaporthe oryzae***. *Mol Plant Microbe Interact* 2010, **23**:1053-1068.

39. Van Thuat N, Schafer W, Bormann J: **The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum***. *Mol Plant Microbe Interact* 2012, **25**:1142-1156.

40. Valent B, Khang CH: **Recent advances in rice blast effector research**. *Curr Opin Plant Biol* 2010, **13**:434-441.

41. • Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, Terauchi R, Nishizawa Y, Shibuya N, Thomma BP *et al.*: **Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease**. *Plant Cell* 2012, **24**:322-335.
Excellent work demonstrated the suppression of chitin oligomer-triggered plant immunity by a secreted LysM effector protein Slp1

42. Kankanala P, Czymmek K, Valent B: **Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus**. *Plant Cell* 2007, **19**:706-724.

43. • Khang CH, Berruyer R, Giraldo MC, Kankanala P, Park SY, Czymmek K, Kang S, Valent B: **Translocation of *Magnaporthe oryzae* effectors into rice cells and their subsequent cell-to-cell movement**. *Plant Cell* 2010, **22**:1388-1403.
This ground-breaking study showed the importance of BIC in effector delivery

44. Mentlak TA, Talbot NJ, Kroj T: **Effector translocation and delivery by the rice blast fungus *Magnaporthe oryzae***. *Effectors in Plant–Microbe Interactions*. Wiley-Blackwell; 2011: 219–241.

45. Clergeot PH, Gourgues M, Cots J, Laurans F, Latorse MP, Pepin R, Tharreau D, Notteghem JL, Lebrun MH: **PLS1, a gene encoding a tetraspanin-like protein, is required for penetration of rice leaf by the fungal pathogen *Magnaporthe grisea***. *Proc Natl Acad Sci U S A* 2001, **98**:6963-6968.

46. Li L, Xue CY, Bruno K, Nishimura M, Xu JR: **Two PAK kinase genes, *CHM1* and *MST20*, have distinct functions in *Magnaporthe grisea***. *Mol Plant–Microbe Interact* 2004, **17**:547-556.

47. Chen J, Zheng W, Zheng S, Zhang D, Sang W, Chen X, Li G, Lu G, Wang Z: **Rac1 is required for pathogenicity and Chm1-dependent conidiogenesis in rice fungal pathogen *Magnaporthe grisea***. *PLoS Pathog* 2008, **4**:e1000202.

48. Galhano R, Talbot NJ: **The biology of blast: understanding how *Magnaporthe oryzae* invades rice plants**. *Fungal Biol Rev* 2011, **25**:61-67.

49. • Dagdas YF, Yoshino K, Dagdas G, Ryder LS, Bielska E, Steinberg G, Talbot NJ: **Septin-mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae***. *Science* 2012, **336**:1590-1595.
This study beautifully illustrated how septins, F-actin, and other cytoskeletal components are involved in appressorium penetration in *M. oryzae*

50. Egan MJ, Wang ZY, Jones MA, Smirnoff N, Talbot NJ: **Generation of reactive oxygen species by fungal NADPH oxidases is required for rice blast disease**. *Proc Natl Acad Sci U S A* 2007, **104**:11772-11777.

51. Chi MH, Park SY, Kim S, Lee YH: **A novel pathogenicity gene is required in the rice blast fungus to suppress the basal defenses of the host**. *PLoS Pathog* 2009, **5**:e1000401.

52. Huang K, Czymmek KJ, Caplan JL, Sweigard JA, Donofrio NM: ***HYR1*-mediated detoxification of reactive oxygen species is required for full virulence in the rice blast fungus**. *PLoS Pathog* 2011, **7**:e1001335.

53. Guo M, Chen Y, Du Y, Dong Y, Guo W, Zhai S, Zhang H, Dong S, Zhang Z, Wang Y *et al.*: **The bZIP transcription factor MoAP1 mediates the oxidative stress response and is critical for pathogenicity of the rice blast fungus *Magnaporthe oryzae***. *PLoS Pathog* 2011, **7**:e1001302.