Investigating the cell biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

Xia Yan and Nicholas J Talbot

CrossMark

Rice blast disease is a major constraint on worldwide rice production and understanding the biology of plant infection is a priority for development of new disease control strategies. Recent advances in live cell imaging, coupled with tractability of both host and pathogen to molecular genetics and genomics, has made the rice blast pathosystem an important model for understanding plant disease. Here we review recent advances in understanding the cell biology of plant infection and, in particular, the remarkable ability of the rice blast fungus to invade plant tissue and manipulate the host plant using a battery of secreted effector proteins. These fungal effectors suppress plant immunity, alter cellular organisation, and facilitate rapid fungal growth.

Address
School of Biosciences, College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, United Kingdom

Introduction

Rice blast disease is one of the most serious problems affecting cultivated rice worldwide. The disease occurs wherever rice is grown and recurrent epidemics occur on a regular basis. In the last 5 years alone, rice blast epidemics have struck many rice-growing regions, including Kenya [1], Italy [2] and, most recently (in 2016) Bangladesh, where wheat blast was also observed for the first time [3]. In the developed world, controlling rice blast is expensive and difficult to achieve. A combination of disease-resistant cultivars, more efficient use of nitrogen fertilisers, and fungicides do, however, achieve some measure of control. Conversely, in the developing world, rice blast outbreaks can cause total harvest loss, meaning financial ruin for farmers, or even starvation for those most acutely affected. The spread of rice blast disease throughout sub-Saharan Africa, where rice consumption has increased

greatly in popularity in recent years, is of particular concern [1]. Currently, there is little identifiable disease resistance in locally adapted rice cultivars, such as the popular New Rice for Africa (NERICA) interspecific hybrid varieties of rice [4], and fungicides are prohibitively expensive. Controlling the disease in these regions is therefore a major challenge.

In this review we look at some of the most significant advances in our understanding of the cell biology of infection by the pathogenic fungus, *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*) which causes rice blast disease [5] (Figure 1). Due mainly to its economic significance, the rice blast fungus has become a major model system for understanding the molecular and cellular basis of fungal pathogenicity. Significant efforts in the last 20 years have established tools for its study, which is greatly aided by the fungus' amenability to classical genetics. Recently, there have been significant advances in some of our basic understanding of the biology of rice blast disease. In this review, we focus first on advances in understanding the biology of plant infection by the fungus and how the fungus uses a specialised cell called an appressorium to gain entry to the rice plant. Secondly, we focus on the biology of invasive growth, where there have been very significant developments in identifying fungal proteins which manipulate the host plant, suppressing immunity and allowing the fungus to rapidly colonise plant tissue. We will not cover signal transduction pathways associated with plant infection by *M. oryzae* in great detail, because these have been reviewed recently [6], or tool development for the study of rice blast disease for which two recent reviews have also appeared [7,8]. We will concentrate instead on how an understanding of the cell biology of both host and pathogen has led to new insight into the establishment of rice blast and provided new opportunities and avenues of future research.

The cell biology of appressorium development

To bring about plant infection, *M. oryzae* must first adhere to the hydrophobic leaf surface, a non-stick surface, composed of a waxy cuticle. Spores of the rice blast fungus carry with them their own adhesive, which is found in an apical compartment of the spore and released upon hydration of the conidium [9]. This glue, called spore tip mucilage, sticks the conidium to the waxy surface, but as soon as germination occurs, the fungus needs a means by which it can tightly adhere to the cuticle. The hydrophobins, Mpg1 and Mph1, have long been implicated in surface perception and attachment, and a recent study

Figure 1
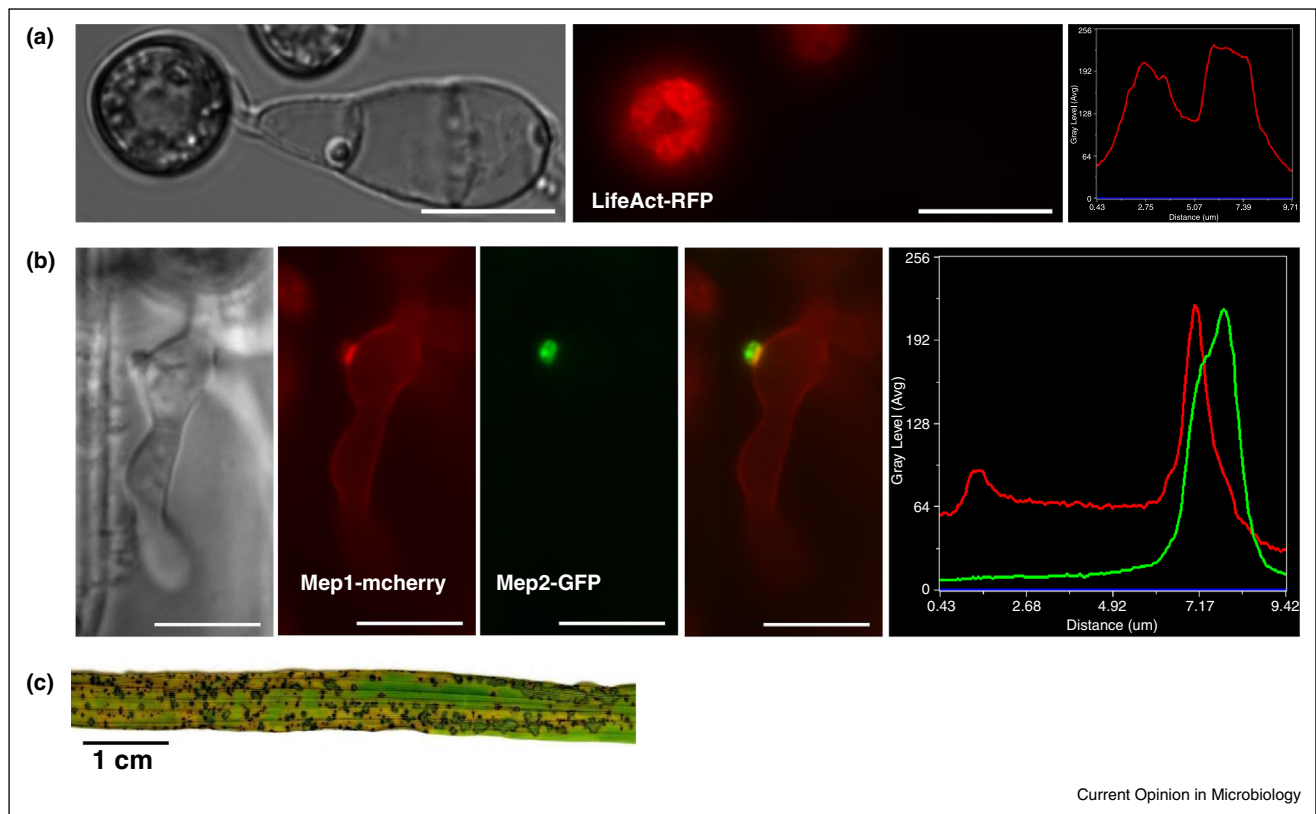
Developmental stages of rice blast disease and live cell imaging of infection.
(a) F-actin network organisation at the appressorium pore prior to re-polarisation and plant infection. Micrographs of LifeAct-RFP in wild type strain Guy11. Conidia were inoculated on glass coverslips and images were taken at 16 h post-inoculation. Line scan analysis of the fluorescence signal confirmed the toroidal shape of the F-actin network at the appressorium pore. **(b)** Visualisation of the expression and localisation of apoplastic effectors and cytoplasmic effectors during invasive growth. Images were taken with strains expressing and apoplastic effector Mep1-mCherry and cytoplasmic Mep2-GFP in Guy11 28 h post-inoculation. Mep2 accumulates predominantly at the biotrophic interfacial complex (BIC), arrowed, while Mep1 accumulates in the apoplast with only a minor component at the BIC, as shown by line scan analysis of the fluorescence signal (Xia Yan & N. J. Talbot, unpublished). Scale bars in panels a and b = 10 μm. Rice blast disease symptoms observed at 5 days after inoculation.

has crystallised these proteins and characterised them in unparalleled detail [10••]. This has revealed that the Mpg1 hydrophobin spontaneously self-assembles at the fungus-host interface into an amyloid like, rodlet structure that can be revealed by atomic force microscopy. This is reminiscent of the rodlet layers observed on the spore surface of *M. oryzae*, which are absent in *mpg1* mutants [11]. Pham and colleagues have provided the molecular validation of a model for appressorium attachment, that was first formulated more than 20 years ago [11]. At that time Mpg1 was shown to be necessary for efficient plant infection by *M. oryzae* [12] and to encode a hydrophobin that self-assembles at the rice interface [11]. The authors have now demonstrated how this occurs at the level of protein-protein interactions to build the polymeric rodlet structure and how this forms an unordered amyloid-like structure. The Mhp1 hydrophobin, meanwhile forms a fibrillar layer. Interestingly, the authors also demonstrated a hydrophobin interaction with cutinase, the methyl

esterase secreted by *M. oryzae* on the leaf surface, validating an idea proposed earlier [13] that cutinases may interact with hydrophobins to provide a means for very tight adhesion to the cuticle, which is necessary for appressorium development. Hydrophobins therefore play vital roles in the early events of plant infection, conditioning the sensing processes by which the fungus recognises the hydrophobic leaf interface as an inductive surface for appressorium development. Recent evidence has also revealed that regulation of hydrophobin gene expression during appressorium development requires the *MoYAK1*-encoded protein kinase which is necessary for aerial hyphal development, appressorium formation and virulence of *M. oryzae* [14].

Two major signalling pathways have long been implicated in appressorium morphogenesis by *M. oryzae* [6] and recent studies have identified new components and provided insight into their activation. The Pmk1 MAPK

kinase pathway is necessary for appressorium formation and widely conserved in pathogenic fungi [15]. Recently, two thioredoxin genes, *TRX1* and *TRX2*, have been characterised that modulate the Pmk1 pathway. Thioredoxins play important roles in intra-cellular ROS signalling and pathogenesis. Interestingly, *TRX2* may regulate the activation of Pmk1 MAPK via the upstream MAPK kinase Mst7 and is required for appressorium development [16]. Upstream of Mst7, the molecular mechanisms involved in activation of the Mst11 MAPKK kinase have been studied revealing that the regulatory region of Mst11 exhibits self-inhibitory binding and that an interaction with the essential Ras2 GTPase is necessary for activation of the Pmk1 pathway [17•].

The cyclic AMP-dependent protein kinase A signalling pathway is also known to play an important role in regulating plant infection, controlling appressorium maturation and turgor-driven infection [18]. Further components of this signalling cascade have recently been identified, including Som1 and Cdtf1 which function downstream of CPKA and which regulate cellular differentiation during plant infection [19]. Moreover, a C2H2 zinc-finger transcription factor Znf1 was recently characterised that is essential for pathogenicity and appressorium differentiation of *M. oryzae* [20]. While both the Pmk1 MAP kinase pathway and cAMP-dependent protein kinase A pathway positively regulate appressorium morphogenesis, little is known about how infection cell development is inhibited from being incorrectly activated. A clue to one of the likely mechanisms has come from analysis of the target of rapamycin (TOR) kinase signalling pathway, which appears to repress the cAMP PKA pathway in response to high glutamine levels. The transcription factor Asd4, which is necessary for appressorium development, normally modulates intracellular glutamine, thereby bypassing TOR-mediated repression. If this process is disrupted, for example, by mutation of *ASD4*, this prevents appressorium formation due, at least in part, to TOR kinase activation [21•].

During maturation of the appressorium, the cell integrity pathway, involving the Mps1 MAPK, has been shown to be necessary for appressorium re-polarisation [22]. Recently, it has been demonstrated that Protein Kinase C, a central component of the cell integrity pathway, is essential for viability of *M. oryzae*. Conditional inactivation of the kinase using a chemical genetic approach rendered the fungus unable to grow, consistent with the kinase being essential for growth. This was verified by RNAi and by failure to generate a viable gene deletion mutant [23]. The *MoGTI1* transcription factor-encoding gene affects penetration and invasive growth and appears to be controlled by the Mps1 MAP kinase [24], although nuclear localisation of MoGti1 was found to be subject to regulation by Pmk1 [25].

Understanding the cell biology of appressorium function

Once formed, *M. oryzae* appressoria develop enormous cellular turgor due to accumulation of glycerol as a compatible solute within the infection cell [26]. Due to the presence of melanin in the appressorium cell wall, the appressorium is impermeable to glycerol efflux, but fully permeable to water, therefore ensuring the rapid and continual influx of water into the cell to develop hydrostatic turgor [9,26]. Once a critical turgor threshold is reached, the fungus reorganises the actin cytoskeleton in a process mediated by septin GTPases that scaffold, organising F-actin at the base of the appressorium in a specialised zone known as the appressorium pore [27,28]. Septins form a ring-shaped heteromeric complex at the appressorium pore, which acts as a lateral diffusion barrier, holding in place polarity determinants, endocytic proteins, and factors such as Las17, part of the Arp2/3 complex involved in actin polymerisation. Assembly of this re-polarisation machinery at the appressorium pore is regulated by the Nox2 NADPH oxidase complex, suggesting that synthesis of reactive oxygen species plays a role in regulating F-actin dynamics, required for penetration peg development [28] (Figure 1). The octameric exocyst complex, necessary for polarised protein secretion, is also organised in a ring conformation at the appressorium pore and its organisation is septin-dependent [29•]. The appressorium pore is therefore critical for early secretion at the fungal-host interface, allowing delivery of cargo enzymes and structural proteins essential for penetration peg growth, as well as the secretion of effector proteins at the point of plant infection. Consistent with this idea, syntaxin 8 (MoSyn8), a Qc-SNARE protein homolog, has been shown to play an important role in rice blast disease. A Δ*Mosyn8* mutant exhibits defects in endocytosis and F-actin organisation, appressorium turgor pressure generation, and host penetration [30]. Δ*Mosyn8* mutants are also impaired in secretion of the effectors Avr-Pia and Avr-Piz-t, consistent with their secretion occurring at the point of host entry. Appressorium maturation is also associated with autophagy, consistent with the identified role for TOR regulation [21•], and a recent study showed how three components, MoVps35, MoVps26 and MoVps29, of the retromer complex are essential for appressorium-mediated host penetration, acting in an autophagy-dependent manner [31].

The cell biology of invasive growth by *M. oryzae*

Once the fungus has entered rice tissue, it develops bulbous, branched hyphae that invaginate the plant plasma membrane and expand within the first occupied epidermal rice cell. The extra invasive hyphal membrane (EIHM) that surrounds the fungus becomes a highly specialised compartment, separate from the remaining plant plasma membrane [32]. Using laser confocal microscopy of rice sheath cells, it has become possible to

visualise how *M. oryzae* maintains membrane integrity of rice cells that it invades, while cells that the fungus exits, often lose membrane integrity and viability [33•]. Use of vital stains such as fluorescein diacetate (FDA) and propidium iodide (PI) enable dynamics of host cell death to be investigated [33•], revealing the extent of biotrophic growth–when the fungus occupies living rice cells–and onset of the necrotrophic switch, when rice cells lose viability and disease lesions form, from which the fungus then subsequently sporulates. Similarly, the major changes that occur within host cells can also be visualised using rice transgenic lines that express fluorescent fusion proteins targeted to specific organelles or cellular domains. In this way, a recent study has demonstrated how *M. oryzae* invasive hyphae can also invaginate vacuolar membranes during infection leading to vacuolar shrinkage. The study also, however, provides evidence that maintenance of host cell vacuolar integrity may be important for tissue invasion [34••].

During tissue invasion *M. oryzae* secretes effector proteins that interfere with host immunity and this process may involve two distinct secretory pathway depending on the ultimate destination of the effector, with effectors destined for delivery into plant cells accumulating in the biotrophic interfacial complex [35] (Figure 1). The fungus possesses a large repertoire of effector proteins that are co-ordinately expressed during infection and appear to be secreted not only into cells occupied by the fungus, but also into adjacent cells to which they translocate via plasmodesmata [32,33•]. Most of these effectors lack homology to known proteins, but recently protein structural analysis has revealed that *M. oryzae* and other fungal pathogens express a family of structurally-conserved MAX-effectors (named after Magnaporthe Avrs and ToxB like) [36••]. Structural similarity was found by NMR spectroscopy of the 3-dimensional structures of AVR1-CO39 and AVR-Pia. Importantly, the MAX effector family accounts for 50% of cloned avirulence effectors in *M. oryzae* [36••]. This suggests that structurally conserved proteins play roles in suppression of plant immunity, but yet are unrelated at the amino acid sequence level, perhaps providing an explanation for how large and seemingly diverse sets of effector proteins evolved in fungal pathogens. A significant number of effectors has now been characterised in *M. oryzae*, such as Avr1-CO39, Avr-Pita, Avr-Pia, Avr-Pii, Avr-Pik, Avr-Piz-t, Pwl1, Pwl2, Slp1, ACE1, Mc69 and Bas1-4 [37,38,39], as well as newly characterised effectors such as AvrPi9 [40], AvrPib [41], Lug6, Lug9, Lug18 [42], Msp1[43], MoHEG13, MoHEG16 [44], MoCDIP1-5[45], SPD effectors [46] and the Mep effectors (X. Yan and N.J. Talbot, unpublished), a temporally co-regulated set of *M. oryzae* secreted proteins that localise to the biotrophic interfacial complex during infection. In addition to their role in suppressing basal PAMP-triggered immunity (PTI), many *M. oryzae* effectors are recognised by NLR immune receptor proteins encoded by resistance genes, leading to effector-triggered immunity (ETI), that results in cultivar-specific resistance to blast disease. New insight has been gained to how such recognition occurs and how rice has evolved sophisticated responses to rice blast infections. The effector AvrPiz-t, for example, plays roles in immune suppression affecting flg22- and chitin-induced generation of reactive oxygen species and other defense responses [47] It is clear now that AvrPiz-t functions to suppress pathogen-associated molecular pattern-triggered immunity (PTI) in rice, using more than one mechanism [47,48•]. AvrPiz-t interacts with two rice RING E3 ligases, APIP6 and APIP10, interfering with E3 ligase activity and promoting protein degradation [47,49]. APIP10 promotes degradation of the rice blast nucleotide-binding leucine-rich repeat (NB-LRR) resistance protein Piz-t [49]. Suppression of rice APIP10 in the Piz-t background leads to cell death and accumulation of Piz-t, indicating that E3 ligase APIP10 negatively regulates turnover of Piz-t [48•]. This points to the effector having multiple roles and interacting partners, by which it manipulates plant immunity, but by which it also has the capacity to be recognised by the NB-LRR, Piz-t. Similarly, the effector Avr-Pii may also have multiple binding partners that play distinct role in host defence that are targeted by the fungus. Two rice Exo70 proteins have been shown to bind Avr-Pii [50•], consistent with a role in suppression of plant defence-associated membrane trafficking, but Avr-Pii has also been shown to interact with rice NADP-malic enzyme2 (Os-NADP-ME2) using yeast two-hybrid (Y2H) analysis [51•]. A rice mutant of *Os-NADP-ME2* showed increased disease susceptibility, suggesting that *M. oryzae* attenuates host defences via Avr-Pii-mediated inhibition of Os-NADP-ME2. Taken together, it seems that effectors such as AvrPiz-t and Avr-Pii target multiple components of the host immunity machinery of rice and have evolved multiple specificities.

A breakthrough in our understanding of the evolution of ETI has recently come from analysis of R-genes that have integrated within them, protein domains associated with effector targets. In *M. oryzae*, two examples are so far known, in which a heavy metal-binding domain is integrated into the NLR. In the case of Avr1-CO39, the protein is recognised by direct binding between Avr1-CO39 and rice RGA5, which also binds to a second effector, Avr-Pia [52]. The RGA4 protein mediates cell death activation, while RGA5 acts as a repressor of RGA4, and as an Avr receptor. RGA4 and RGA5 interact functionally and physically to confer disease resistance [53•]. Plant immune receptors can therefore contain additional domains besides canonical domains, such as NB-ARC and leucine-rich repeat domains. These additional domains act as integrated decoys recognizing effectors from pathogens. The integration of decoy domains in NLR immune receptors has proved to be widespread [54••].

Similarly, AvrPikD is recognised by the intracellular NLR immune receptor Pi-k. Avr-PikD binds a dimer of the Pikp-1 HMA integrated domain with nanomolar affinity [55[••]]. When considered together, it is clear the plant immune receptors have evolved the capacity to integrate commonly targeted protein domains into defence proteins.

Fungal perturbation of plant hormone signalling

In addition to targeting immune function in plants, fungal pathogens can modulate hormonal activity to provide a better environment for their own growth and vitality. Rice produces cytokinin (CK) hormones to control key developmental processes, such as sink/source relations in the plant, cell division or programmed cell-death [56]. In a recent report, a fungal cytokinin synthase, Cks1, has been shown to be essential for CK biosynthesis. Fungal secreted CKs are likely to affect host cytokinin levels during infection because the transcriptional regulation of rice CK-responsive genes is altered in plants infected by the *cks1* mutant. Interestingly, the *cks1* mutant also triggered enhanced induction of plant defences as manifested by an elevated oxidative burst and expression of defence-related markers [56]. A fungal antibiotic biosynthesis monooxygenase (Abm) is also secreted after invasion and most likely converts plant jasmonic acid (JA) into its hydroxylated form (12OH-JA), which does not act in plant defence signalling. In this way the fungus impairs plant immunity signalling by reducing the concentration of a key signalling molecule, to help facilitate fungal tissue colonisation [57[••]]. The rice blast fungus also secretes tenuazonic acid (TeA), a mycotoxin produced by various plant pathogenic fungi. TeA is synthesised from isoleucine and acetoacetyl-coenzyme A by a unique non-ribosomal peptide synthetase and polyketide synthase hybrid enzyme [58[•]]. In addition to deploying their own secondary metabolites, fungal pathogens encounter and metabolise a range of host-derived metabolites while proliferating inside the host. Aminosugar metabolism, for instance, has recently been found to be essential for successful host colonisation [59].

Conclusion

A key challenge for the rice blast fungus is the ability to colonise and invade plant tissue. Appressorium-mediated invasion of the initial epidermal cell is clearly pivotal to establishing rice blast disease and is revealed as a highly orchestrated developmental process, but one which is beginning to be understood at least in outline. Recent evidence has been provided to show how a large repertoire of effectors plays roles in invasion beyond the first colonised cell, suppressing plant immunity and allowing the fungus to grow unimpeded within rice tissue. Many questions, however, remain to be answered. How does the fungus maintain membrane integrity of the plant cells it invades and is its exit from rice cells always accompanied by host cell death and collapse, as recent live cell imaging experiments suggest [33[•],34[••]]. How also does the fungus move from cell-to-cell and in this process is it able to modulate plasmodesmata conductance and suppress immune responses at the cell junctions? How is the modulation of hormone behaviour related to fungal growth and immunity suppression? Finally, from a cell biology perspective, perhaps the most significant questions are those of general importance for all fungal pathogens and, indeed, oomycete pathogens too. How do effectors get secreted by invasive hyphae and make their way across the host plasma membrane and locate specific cellular destinations, including host organelles? Does the fungus, for example, manipulate host endocytic mechanisms to facilitate protein trafficking into plant cells? Alternatively, do exocytic mechanisms involve the generation of exosomes by fungi that have been reported to occur in some plant-fungal interactions? If so, how are they formed and how does their route into host cells interface with plant endocytic mechanisms? These questions will be important to answer in the next few years, as we now have tools to address them, including rapid advances in live cell imaging technologies. Understanding protein trafficking and the communication between host and fungus is arguably the most significant challenge in contemporary plant pathology and the rice blast pathosystem is an ideal one in which to address this challenge.

Acknowledgements

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. Kihoro J, Bosco NJ, Murage H, Ateka E, Makihara D: **Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya**. *Springerplus* 2013, **2**:308.

2. Titone P, Mongiano G, Tamborini L: **Resistance to neck blast caused by *Pyricularia oryzae* in Italian rice cultivars**. *Eur J Plant Pathol* 2015, **142**:49-59.

3. Callaway E: **Devastating wheat fungus appears in Asia for first time**. *Nature* 2016, **532**:421-422.

4. Moseley WG, Carney J, Becker L: **Neoliberal policy, rural livelihoods, and urban food security in West Africa: a comparative study of The Gambia, Cote d'Ivoire, and Mali**. *Proc Natl Acad Sci U S A* 2010, **107**:5774-5779.

5. Zhang N, Luo J, Rossman AY, Aoki T, Chuma I, Crous PW, Dean R, de Vries RP, Donofrio N, Hyde KD *et al.*: **Generic names in Magnaporthales**. *IMA Fungus* 2016, **7**:155-159.

6. Ryder LS, Talbot NJ: **Regulation of appressorium development in pathogenic fungi**. *Curr Opin Plant Biol* 2015, **26**:8-13.

7. Cairns TC, Studholme DJ, Talbot NJ, Haynes K: **New and improved techniques for the study of pathogenic fungi**. *Trends Microbiol* 2016, **24**:35-50.

8. Martin-Urdiroz M, Oses-Ruiz M, Ryder LS, Talbot NJ: **Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*. *Fungal Genet Biol* 2015.

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

10. Pham CL, Rey A, Lo V, Soules M, Ren Q, Meisl G, Knowles TP,
•• Kwan AH, Sunde M: **Self-assembly of MPG1, a hydrophobin protein from the rice blast fungus that forms functional amyloid coatings, occurs by a surface-driven mechanism**. *Sci Rep* 2016, **6**:25288.
Structural definition of the role of a fungal hydrophobin in appressorium-mediated plant infection. Provides molecular characterisation of fungal hydrophobin self-assembly.

11. Talbot NJ, Kershaw MJ, Wakley GE, De Vries O, Wessels J, Hamer JE: **MPG1 encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea*. *Plant Cell* 1996, **8**:985-999.

12. Talbot NJ, Ebbole DJ, Hamer JE: **Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell* 1993, **5**:1575-1590.

13. Skamnioti P, Henderson C, Zhang Z, Robinson Z, Gurr SJ: **A novel role for catalase B in the maintenance of fungal cell-wall integrity during host invasion in the rice blast fungus *Magnaporthe grisea*. *Mol Plant-Microbe Interact* 2007, **20**:568-580.

14. Han JH, Lee HM, Shin JH, Lee YH, Kim KS: **Role of the MoYAK1 protein kinase gene in *Magnaporthe oryzae* development and pathogenicity**. *Environ Microbiol* 2015, **17**:4672-4689.

15. Rispail N, Soanes DM, Ant C, Czajkowski R, Grunler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, Valiante V *et al.*: **Comparative genomics of MAP kinase and calcium-calcineurin signalling components in plant and human pathogenic fungi**. *Fungal Genet Biol* 2009, **46**:287-298.

16. Zhang S, Jiang C, Zhang Q, Qi L, Li C, Xu JR: **Thioredoxins are involved in the activation of the PMK1 MAP kinase pathway during appressorium penetration and invasive growth in *Magnaporthe oryzae*. *Environ Microbiol* 2016.

17. Qi L, Kim Y, Jiang C, Li Y, Peng Y, Xu JR: **Activation of Mst11 and
• feedback inhibition of germ tube growth in *Magnaporthe oryzae*. *Mol Plant Microbe Interact* 2015, **28**:881-891.
Demonstrating the importance of Ras signaling upstream of the Pmk1 MAP kinase pathway.

18. Xu J-R, Urban M, Sweigard JA, Hamer JE: **The CPKA gene of *Magnaporthe grisea* is essential for appressorial penetration**. *Mol Plant-Microbe Interact* 1997, **10**:187-194.

19. 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.

20. Yue X, Que Y, Xu L, Deng S, Peng Y, Talbot NJ, Wang Z: **ZNF1 encodes a putative C2H2 zinc-finger protein essential for appressorium differentiation by the rice blast fungus *Magnaporthe oryzae*. *Mol Plant Microbe Interact* 2016, **29**:22-35.

21. Marroquin-Guzman M, Wilson RA: **GATA-dependent
• glutaminolysis drives appressorium formation in *Magnaporthe oryzae* by suppressing TOR inhibition of cAMP/PKA signaling**. *PLoS Pathog* 2015, **11**:e1004851.
Defining the connection between TOR signaling, nutrient monitoring and regulation of appressorium development.

22. Xu J-R, 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.

23. Penn TJ, Wood ME, Soanes DM, Csukai M, Corran AJ, Talbot NJ: **Protein kinase C is essential for viability of the rice blast fungus *Magnaporthe oryzae*. *Mol Microbiol* 2015, **98**:403-419.

24. Li Y, Wang G, Xu JR, Jiang C: **Penetration Peg formation and invasive hyphae development require stage-specific activation of MoGTI1 in *Magnaporthe oryzae*. *Mol Plant Microbe Interact* 2016, **29**:36-45.

25. Chen Y, Zhai S, Zhang H, Zuo R, Wang J, Guo M, Zheng X, Wang P, Zhang Z: **Shared and distinct functions of two Gti1/Pac2 family proteins in growth, morphogenesis and pathogenicity of *Magnaporthe oryzae*. *Environ Microbiol* 2014, **16**:788-801.

26. de Jong JC, McCormack BJ, Smirnoff N, Talbot NJ: **Glycerol generates turgor in rice blast**. *Nature* 1997, **389** 244-244.

27. 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.

28. Ryder LS, Dagdas YF, Mentlak TA, Kershaw MJ, Thornton CR, Schuster M, Chen J, Wang Z, Talbot NJ: **NADPH oxidases regulate septin-mediated cytoskeletal remodeling during plant infection by the rice blast fungus**. *Proc Natl Acad Sci U S A* 2013, **110**:3179-3184.

29. Gupta YK, Dagdas YF, Martinez-Rocha AL, Kershaw MJ,
• Littlejohn GR, Ryder LS, Sklenar J, Menke F, Talbot NJ: **Septin-dependent assembly of the exocyst is essential for plant infection by *Magnaporthe oryzae*. *Plant Cell* 2015, **27**:3277-3289.
The eight-membered exocyst complex re-locates to the appressorium pore during plant infection, allowing polarised exocytosis at the point of plant infection.

30. Qi Z, Liu M, Dong Y, Zhu Q, Li L, Li B, Yang J, Li Y, Ru Y, Zhang H *et al.*: **The syntaxin protein (MoSyn8) mediates intracellular trafficking to regulate conidiogenesis and pathogenicity of rice blast fungus**. *New Phytol* 2016, **209**:1655-1667.

31. Zheng W, Zhou J, He Y, Xie Q, Chen A, Zheng H, Shi L, Zhao X, Zhang C, Huang Q *et al.*: **Retromer is essential for autophagy-dependent plant infection by the rice blast fungus**. *PLoS Genet* 2015, **11**:e1005704.

32. 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.

33. Jones K, Kim DW, Park JS, Khang CH: **Live-cell fluorescence
• imaging to investigate the dynamics of plant cell death during infection by the rice blast fungus *Magnaporthe oryzae*. *BMC Plant Biol* 2016, **16**:69.
Live cell imaging study indicating how plant cell death can occur upon entry of the fungus to an adjacent living cell.

34. Mochizuki S, Minami E, Nisizawa Y: **Live-cell imaging of rice
•• cytological changes reveals the importance of host vacuole maintenance for biotrophic invasion by blast fungus, *Magnaporthe oryzae*. *Microbiology Open* 2015, **4**:952-966.
Live cell imaging that reveals host rice vacuolar dynamics are important in progression of rice blast infections.

35. Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B: **Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. *Nat Commun* 2013, **4**:1996.

36. de Guillen K, Ortiz-Vallejo D, Gracy J, Fournier E, Kroj T, Padilla A:
•• **Structure analysis uncovers a highly diverse but structurally conserved effector family in phytopathogenic fungi**. *PLoS Pathog* 2015, **11**:e1005228.
Discovery that a large number of rice blast effectors belong to a conserved structurally related group, the MAX effectors. A potential breakthrough in deciphering fungal effector evolution.

37. Liu W, Liu J, Ning Y, Ding B, Wang X, Wang Z, Wang GL: **Recent progress in understanding PAMP- and effector-triggered immunity against the rice blast fungus *Magnaporthe oryzae*. *Mol Plant* 2013, **6**:605-620.

38. 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.

39. Chen XL, Shi T, Yang J, Shi W, Gao X, Chen D, Xu X, Xu JR, Talbot NJ, Peng YL: **N-glycosylation of effector proteins by an alpha-1,3-mannosyltransferase is required for the rice blast fungus to evade host innate immunity**. *Plant Cell* 2014, **26**:1360-1376.

40. Wu J, Kou Y, Bao J, Li Y, Tang M, Zhu X, Ponaya A, Xiao G, Li J, Li C *et al.*: **Comparative genomics identifies the *Magnaporthe oryzae* avirulence effector AvrPi9 that triggers Pi9-mediated blast resistance in rice**. *New Phytol* 2015, **206**:1463-1475.

41. Zhang S, Wang L, Wu W, He L, Yang X, Pan Q: **Function and evolution of *Magnaporthe oryzae* avirulence gene AvrPib responding to the rice blast resistance gene Pib**. *Sci Rep* 2015, **5**:11642.

42. Dong Y, Li Y, Zhao M, Jing M, Liu X, Liu M, Guo X, Zhang X, Chen Y, Liu Y *et al.*: **Global genome and transcriptome analyses of *Magnaporthe oryzae* epidemic isolate 98-06 uncover novel effectors and pathogenicity-related genes, revealing gene gain and lose dynamics in genome evolution**. *PLoS Pathog* 2015, **11**:e1004801.

43. Wang Y, Wu J, Kim SG, Tsuda K, Gupta R, Park SY, Kim ST, Kang KY: ***Magnaporthe oryzae*-secreted protein MSP1 induces cell death and elicits defense responses in rice**. *Mol Plant Microbe Interact* 2016, **29**:299-312.

44. Mogga V, Delventhal R, Weidenbach D, Langer S, Bertram PM, Andresen K, Thines E, Kroj T, Schaffrath U: ***Magnaporthe oryzae* effectors MoHEG13 and MoHEG16 interfere with host infection and MoHEG13 counteracts cell death caused by Magnaporthe-NLPs in tobacco**. *Plant Cell Rep* 2016, **35**:1169-1185.

45. Chen S, Songkumarn P, Venu RC, Gowda M, Bellizzi M, Hu J, Liu W, Ebbole D, Meyers B, Mitchell T *et al.*: **Identification and characterization of in planta-expressed secreted effector proteins from *Magnaporthe oryzae* that induce cell death in rice**. *Mol Plant Microbe Interact* 2013, **26**:191-202.

46. Sharpee W, Oh Y, Yi M, Franck W, Eyre A, Okagaki L, Valent B, Dean R: **Identification and characterization of suppressors of plant cell death (SPD) genes from *Magnaporthe oryzae***. *Mol Plant Pathol* 2016.

47. Park CH, Chen S, Shirsekar G, Zhou B, Khang CH, Songkumarn P, Afzal AJ, Ning Y, Wang R, Bellizzi M *et al.*: **The *Magnaporthe oryzae* effector AvrPiz-t targets the RING E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice**. *Plant Cell* 2012, **24**:4748-4762.

48. Ning Y, Wang R, Shi X, Zhou X, Wang GL: **A layered defense**
• **strategy mediated by rice E3 ubiquitin ligases against diverse pathogens**. *Mol Plant* 2016.
Demonstrates that E3 ligases exert multiple roles against pathogens such as *M. oryzae* and how effector-mediated activity attempts to counter this.

49. Park CH, Shirsekar G, Bellizzi M, Chen S, Songkumarn P, Xie X, Shi X, Ning Y, Zhou B, Suttiviriya P *et al.*: **The E3 ligase APIP10 connects the effector AvrPiz-t to the NLR receptor Piz-t in rice**. *PLoS Pathog* 2016, **12**:e1005529.

50. Fujisa K, Abe Y, Ito A, Saitoh H, Yoshida K, Kanzaki H, Kanzaki E,
• Utsushi H, Yamashita T, Kamoun S *et al.*: **Rice Exo70 interacts with a fungal effector, AVR-Pii, and is required for AVR-Pii-triggered immunity**. *Plant J* 2015, **83**:875-887.
Study revealing the likely role of a *M. oryzae* effector in perturbing host membrane trafficking during infection, perhaps for immunity suppression or to facilate membrane acquisition around invasive hyphae.

51. Singh R, Dangol S, Chen Y, Choi J, Cho YS, Lee JE, Choi MO,
• Jwa NS: ***Magnaporthe oryzae* effector AVR-Pii helps to establish compatibility by inhibition of the rice NADP-malic enzyme resulting in disruption of oxidative burst and host innate immunity**. *Mol Cells* 2016, **39**:426-438.
Reveals a second potential role for Avr-Pii in suppression of immunity, but in this impairing the role of a metabolic enzyme, in addition to its role in exo70 perturbation reported in 45.

52. Cesari S, Thilliez G, Ribot C, Chalvon V, Michel C, Jauneau A, Rivas S, Alaux L, Kanzaki H, Okuyama Y *et al.*: **The rice resistance protein pair RGA4/RGA5 recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding**. *Plant Cell* 2013, **25**:1463-1481.

53. Cesari S, Kanzaki H, Fujiwara T, Bernoux M, Chalvon V, Kawano Y,
• Shimamoto K, Dodds P, Terauchi R, Kroj T: **The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance**. *EMBO J* 2014, **33**:1941-1959.
Definition of the interaction between a pair of genes required for Co-39 and Pi-a-mediated disease resistance and characterised as having internal decoy domains/

54. Kroj T, Chanclud E, Michel-Romiti C, Grand X, Morel JB:
•• **Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread**. *New Phytol* 2016, **210**:618-626.
A key paper revealing how integrated decoy domains may be a widespread way in which plant resistance proteins have evolved.

55. Maqbool A, Saitoh H, Franceschetti M, Stevenson CE, Uemura A,
•• Kanzaki H, Kamoun S, Terauchi R, Banfield MJ: **Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor**. *Elife* 2015:4.
Beautiful structure-function study that reveals how an integrated decoy domain is necessary for recognition of the rice blast AvrPi-k effector.

56. Chanclud E, Kisiala A, Emery NR, Chalvon V, Ducasse A, Romiti-Michel C, Gravot A, Kroj T, Morel JB: **Cytokinin production by the rice blast fungus is a pivotal requirement for full virulence**. *PLoS Pathog* 2016, **12**:e1005457.

57. Patkar RN, Benke PI, Qu Z, Constance Chen YY, Yang F,
•• Swarup S, Naqvi NI: **A fungal monooxygenase-derived jasmonate attenuates host innate immunity**. *Nat Chem Biol* 2015, **11**:733-740.
An elegant interdisciplinary study showing how *M. oryzae* can convert the phytohormone jasmonate to a hydroxylated form that is unable to act in plant immunity signaling, thereby enhancing fungal invasive growth.

58. Yun CS, Motoyama T, Osada H: **Biosynthesis of the mycotoxin**
• **tenuazonic acid by a fungal NRPS-PKS hybrid enzyme**. *Nat Commun* 2015, **6**:8758.
Comprehensive dissection of tenuazonic acid biosynthesis and its role in blast disease.

59. Kumar A, Ghosh S, Bhatt DN, Narula A, Datta A: ***Magnaporthe oryzae* aminosugar metabolism is essential for successful host colonization**. *Environ Microbiol* 2016, **18**:1063-1077.