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The appressorium at a glance

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ABSTRACT

Many plant pathogenic fungi have the capacity to infect their plant hosts using specialised cells called appressoria. These structures act as a gateway between the fungus and host, allowing entry to internal tissues. Appressoria apply enormous physical force to rupture the plant surface, or use a battery of enzymes to digest the cuticle and plant cell wall. Appressoria also facilitate focal secretion of effectors at the point of plant infection to suppress plant immunity. These infection cells develop in response to the physical characteristics of the leaf surface, starvation stress and signals from the plant. Appressorium morphogenesis has been linked to septin-mediated reorganisation of F-actin and microtubule networks of the cytoskeleton, and remodelling of the fungal cell wall. In this Cell Science at a Glance and accompanying poster, we highlight recent advances in our understanding of the mechanisms of appressorium-mediated infection, and compare development on the leaf surface to the biology of invasive growth by pathogenic fungi. Finally, we outline key gaps in our current knowledge of appressorium cell biology.

KEY WORDS: Appressorium, Plant pathogen, Autophagy, Cell cycle control, Effector, Host–pathogen interface, Melanin biosynthesis, Septin

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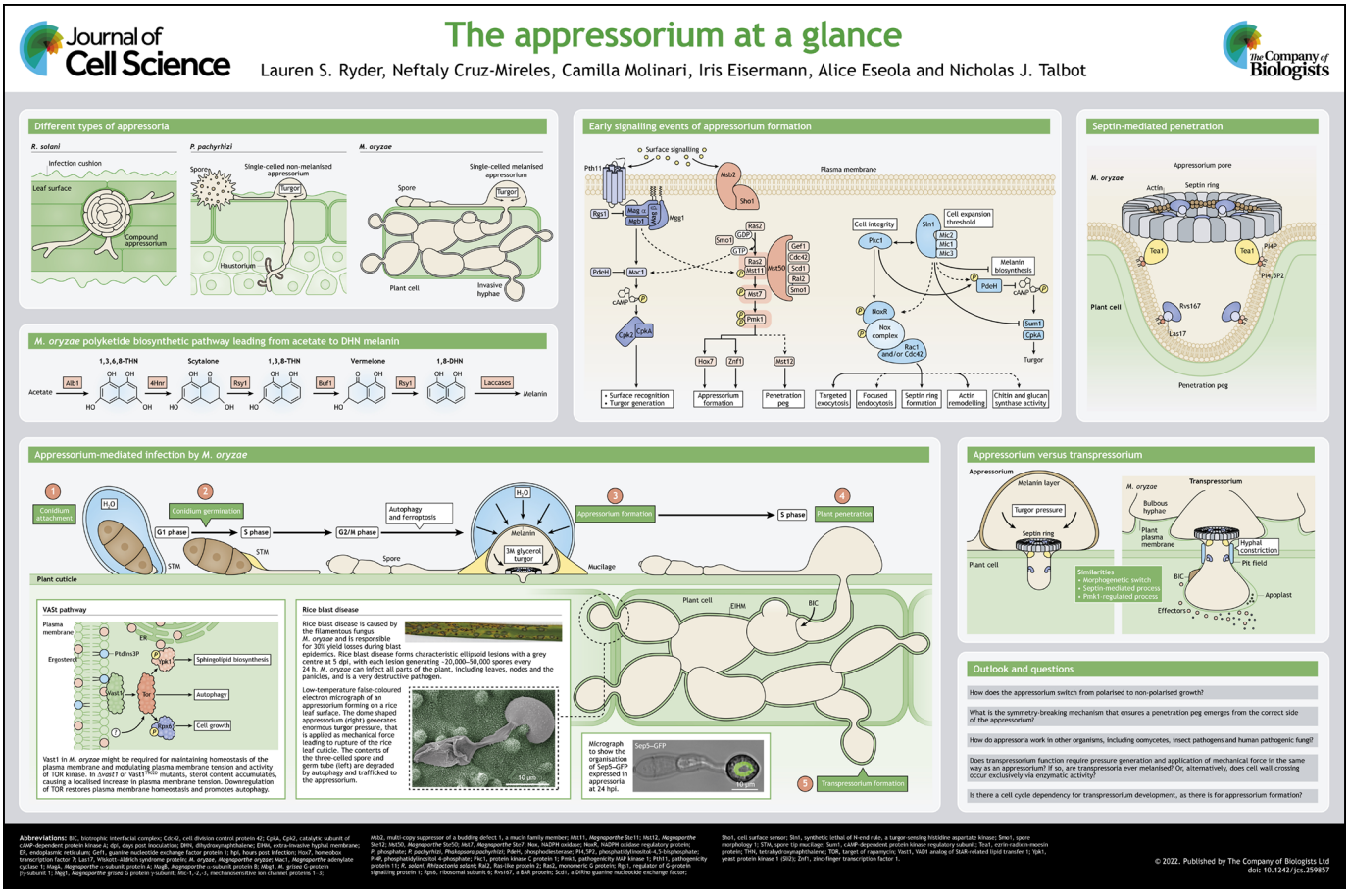
Introduction

To gain entry to host tissue, many fungal pathogens develop infection cells to breach the tough outer surface of a plant or animal (Mendgen et al., 1996; Talbot, 2019). These include hyphopodia, infection cushions and appressoria (Bozkurt and Kamoun, 2020; Choquer et al., 2021; Goos and Gessner, 1975; Ryder and Talbot, 2015; Talbot, 2019). Here, we focus on appressoria, which are the



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most-studied fungal infection cells (Chethana et al., 2021a,b), and are vital to many of the most destructive plant diseases (Dean et al., 2012).

The name appressorium was first coined in the 19th century by Albert Bernhard Frank who defined them as "a swelling on a germ tube or hypha, especially for attachment in an early stage of infection" (Frank, 1883). However, the function of appressoria was later found to be not only for attachment, but also to rupture the host cuticle (Deising et al., 2000). Appressoria are classified into two main groups, single-celled appressoria – including proto-appressoria, hyaline appressoria and melanised appressoria – and compound appressoria, such as infection cushions (see poster) (Chethana et al., 2021a). In addition to plant pathogens, appressoria can be formed by endophytes, saprobes and symbionts, including some arbuscular mycorrhizal fungi, as well as lichens and insect pathogenic fungi (Deising et al., 2000; Demoor et al., 2019; Hyde et al., 2020; Lozano-Tovar et al., 2013; Ryder and Talbot, 2015). The devastating rice blast fungus *Magnaporthe oryzae* (Eseola et al., 2021) elaborates appressoria (see poster), which melanise and generate enormous intracellular turgor to facilitate mechanical penetration of the rice leaf (Hyde et al., 2020; Mendgen et al., 1996; Ryder and Talbot, 2015). Non-melanised (or slightly melanised) appressoria are formed by other cereal pathogens, including the powdery mildew pathogen *Blumeria graminis* and corn smut fungus *Ustilago maydis,* as well as the soybean rust fungus *Phakopsora pachyrhizi* (see poster). Multi-cellular infection cushions are mainly found in root pathogens, such as *Rhizoctonia solani* (see poster). In addition to fungi, filamentous oomycete pathogens, such as *Phytophthora* and *Pythium* species, form appressoria that are small compared to fungal appressoria, non-melanised and separated from their germ tubes by false septa. By contrast, in fungi, a septum normally separates the appressorium from the spore (Chethana et al., 2021a). Unlike a fungal appressorium, which enters the underlying plant cell at a 90° angle, penetration hyphae of the oomycete late blight pathogen *Phytophthora infestans* appear to enter at a diagonal angle, using a specific 'naifu' cutting action to breach the host surface. (Bronkhorst et al., 2021).

Relatively little is known about the evolutionary origin of appressoria. It has been proposed that they evolved first in mutualistic fungi (Mendgen et al., 1996); arbuscular mycorrhizal fungi, for example, develop similar hyphopodia to penetrate roots (Bonfante and Genre, 2010). Recent phylogenetic analysis suggests that simple non-pigmented appressoria appeared first, and later diversified into melanised or compound appressoria (Chethana et al., 2021b). However, there are open questions regarding the origin of proto-appressorium structures and their relationship to other differentiated non-hyphal structures, such as conidia, for example, or the terminally differentiated haustoria used by fungal pathogens to colonise plant cells after infection (Bozkurt and Kamoun, 2020), as well as resting bodies, such as sclerotia, which are often melanised (Mendgen et al., 1996).

Although our knowledge regarding the origin of appressoria is limited, recent breakthroughs have revealed key cellular mechanisms associated with their development. Here, we summarise these new insights with a special focus on the rice blast fungus *M. oryzae*, where appressoria have been intensively studied (Wilson and Talbot, 2009; Eseola et al., 2021).

Early appressorium development

Appressoria in the rice blast fungus are unicellular and differentiate from a polarised germ tube shortly after germination of an asexual spore called a conidium. In *M. oryzae*, conidia are three-celled tear-drop-shaped (pyriform) spores that stick to the hydrophobic waxy cuticle of rice leaves by secreting a glue called spore tip mucilage (Hamer et al., 1988). Once attached, the conidium germinates to produce a polarised germ tube that ceases apical growth within 4 h (Wilson and Talbot, 2009), flattening and hooking at its tip, which tightly adheres to the leaf surface. The fungus then secretes morphogenetic proteins called hydrophobins, such as Mpg1 and Mhp1 (Kim et al., 2005; Talbot et al., 1993), which polymerise, undergoing interfacial self-assembly, to form an amphipathic monolayer between germ tube and leaf surface, thereby enabling cutinases and mucilage to glue the developing cell to the cuticle (Kim et al., 2005; Pham et al., 2016; Talbot et al., 1993; Whiteford and Spanu, 2002). Hydrophobin self-assembly therefore acts as a primer for attachment factors – a prerequisite to efficient formation of an appressorium (Pham et al., 2016). Recent work has also highlighted the important role of spermine synthase (Ssp1) in acting as an antioxidant in the endoplasmic reticulum (ER), enabling production and secretion of spore tip mucilage necessary to appressorium adhesion (Rocha et al., 2020).

To develop an appressorium, the blast fungus perceives physical cues, such as surface hardness and hydrophobicity, and chemical signals, including leaf waxes and cutin monomers. G-protein coupled receptors (GPCRs) mediate recognition of these signals (Liu and Dean, 1997). Pth11, for example, is a GPCR involved in surface perception, which, when activated, leads to dissociation of the heterotrimeric Gα proteins MagA and MagB. When surface hydrophobicity is detected, the Gβγ subunit Mgb1 separates from the complex and binds to membrane-bound adenylate cyclase (Mac1), which catalyses generation of cyclic AMP (cAMP) (Adachi and Hamer, 1998). Rgs1 meanwhile regulates G-protein signalling and is required for correct perception of inductive surfaces (Liu et al., 2007; Ramanujam et al., 2012). cAMP-dependent protein kinase A signalling then regulates appressorium morphogenesis and turgor generation. In parallel, the sensor proteins Msb2 and Sho1 activate the Pmk1 mitogen-activated protein kinase (MAPK) pathway in response to surface hydrophobicity, cutin monomers and leaf waxes (Liu et al., 2011). Pmk1 pathway activation also involves Mgb1, as well as Ras2 and Cdc42 (Wilson and Talbot, 2009) (see poster). Mgb1 therefore plays important roles in both the cAMP and Pmk1 MAPK cascades, and likely regulates multiple steps in the morphogenetic transition required for infection (Nishimura et al., 2003). Additionally, Mgb1 interacts directly with Mst50, a putative scaffold protein that integrates multiple upstream signals to activate Pmk1 (Park et al., 2006), through its interaction with Gef1, Cdc42, Scd1, Ral2 and the Ras GAP protein Smo1 (Li et al., 2017; Qu et al., 2021). Activation of Pmk1 also requires the Mst7 MAPK kinase (MAPKK) and Mst11 MAPK kinase kinase (MAPKKK) (Zhao et al., 2005), which both bind to Mst50 (Park et al., 2006). The importance of Pmk1 to appressorium development is illustrated by the fact that Δ*pmk1* null-mutants, or those in which the kinase can be chemically inactivated, such as analogue-sensitive *pmk1* mutants (pmk1AS) (Sakulkoo et al., 2018), fail to form appressoria or to infect rice plants, even when the surface cuticle is removed (Sakulkoo et al., 2018; Xu and Hamer, 1996). Furthermore, homologues of Pmk1 are necessary for plant infection in more than 20 fungal pathogens, including both appressorium- and non-appressorium-forming species (Jiang et al., 2018; Turrà et al., 2014). Pmk1 therefore likely serves a conserved regulatory function governing invasive fungal growth (Jiang et al., 2018; Turrà et al., 2014). Phosphorylation targets of Pmk1, including the transcription factors Mst12 and Hox7, have

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recently been identified (Osés-Ruiz et al., 2021). Hox7 is essential for appressorium formation, whereas Mst12 is required for penetration and invasive growth (Cao et al., 2016; Kim et al., 2009; Osés-Ruiz et al., 2021; Park et al., 2002; Yue et al., 2016). Pmk1 ultimately controls expression of more than 6500 genes that are differentially regulated during appressorium development (Osés-Ruiz et al., 2021), highlighting the morphogenetic complexity of appressorium formation.

Appressorium development is also tightly coordinated with cell cycle progression (see poster). Inhibition of S-phase impairs hooking and initiation of appressorium formation, while the G2-M transition is necessary for appressorium maturation (Osés-Ruiz et al., 2017; Saunders et al., 2010; Veneault-Fourrey et al., 2006). In *B. graminis*, inhibition of S-phase prevents maturation of appressoria (Hansjakob et al., 2012), while in *U. maydis*, arrest of filaments at the G2-phase checkpoint is necessary for appressorium formation (Castanheira et al., 2014).

Appressorium turgor generation

Appressorium turgor in *M. oryzae* is generated by accumulation of glycerol and other polyols to high concentrations, which draws water into the cell by osmosis, creating turgor of up to 8.0 MPa (de Jong et al., 1997). The melanin-rich cell wall is impermeable to glycerol, but freely permeable to water which rapidly enters the cell. Compatible solutes used by other appressorium-forming pathogens have yet to be identified, but glycerol and mannitol are abundant in many fungi, and proline may be important in oomycete appressoria (Talbot, 2019). In *M. oryz*ae, trehalose and glycogen are rapidly degraded during spore germination, and trehalose-6-phosphate synthesis is also required for fungal virulence (Foster et al., 2003), regulating an NADPH-dependent genetic switch that controls glucose-6-phosphate metabolism and nitrogen source utilisation (Wilson et al., 2010). This is vital for the metabolic changes necessary for invasion of plant tissue (Fernandez and Wilson, 2012; Wilson et al., 2010). During appressorium maturation, lipid bodies move to the developing appressorium in a Pmk1-dependent manner, and their degradation, and subsequent fatty acid metabolism and glycerol synthesis, requires the cAMP/PKA pathway (Wang et al., 2007). Mutants unable to synthesise melanin cannot retain glycerol, and do not generate turgor pressure or cause disease (Chumley and Valent, 1990). Most fungal melanins are derived from the precursor molecule 1,8-dihydroxynaphthalene (DHN), ultimately derived from acetate, which enters a series of sequential enzymatic steps via the enzymes Alb1, 4Hnr, Rsy1 and Buf1 (see poster). DHN monomers polymerise to yield melanin through the oxidative activity of laccases, and it is deposited in a layer within the appressorium cell wall (see poster). Melanin is critical for appressorium function in many species (Lin et al., 2012), but there are exceptions, such as the soybean rust fungus *P. pachyrhizi*, which produces hyaline non-melanised appressoria that can generate 5.13 MPa of turgor (Chang et al., 2014; Loehrer et al., 2014). Fungal appressoria have therefore evolved distinct means of pressure generation (Chang et al., 2014; Loehrer et al., 2014; Ludwig et al., 2014).

The final prerequisite for appressorium formation in *M. oryzae* is autophagic degradation of the contents of the three-cell conidium (see Box 1 and poster). Autophagy is normally considered a recycling, cell survival strategy (Klionsky, 2007), but it can also regulate a form of programmed cell death (Tsukada and Ohsumi, 1993). In *M. oryzae*, autophagy in the conidium is necessary for appressorium function (Kershaw and Talbot, 2009; Liu et al., 2016; Lv et al., 2017; Ying et al., 2016; Zhang et al., 2013; Zhou et al., 2020). Death of the conidium might, however, require an iron-dependent process called ferroptosis induced by accumulating lipid peroxides in the cell (Dixon et al., 2012; Kagan et al., 2017; Shen et al., 2020). Ferroptosis can be subverted by suppressing lipid peroxide levels within the cell using iron chelators to impair appressorium maturation (Liang et al., 2021; Shen et al., 2020; Shen and Naqvi, 2021).

The regulation of infection-associated autophagy also involves the target of rapamycin (TOR) signalling pathway (Beck and Hall, 1999; Conrad et al., 2014; Shashkova et al., 2015). In fungal pathogens, TOR influences the cAMP response and cell wall integrity pathway, as well as autophagy (López-Berges et al., 2010; Marroquin-Guzman and Wilson, 2015; Oh et al., 2008; Qian et al., 2018; Yu et al., 2014). In *M. oryzae*, the TOR pathway can be

Box 1. Appressorium-associated autophagy

Autophagy is an evolutionarily conserved cellular pathway in all eukaryotes, and plays an important role in appressorium-mediated plant infection (Zhu et al., 2021). In *M. oryzae*, functional characterisation of autophagy-related proteins Atg1, Atg10, Atg12, Atg14–Atg16 and Atg18 showed they are necessary for appressorium function (Kershaw and Talbot, 2009), and functional studies have since been carried out on the Atg1–Atg13–Atg17 complex, an Atg9 trafficking system at the interface between endocytosis and autophagy (Zhu et al., 2018). Environmental stress, including starvation, ROS accumulation and treatment with rapamycin, for instance, are known to lead to a reduction in the activity of TOR kinase in yeast and a range of eukaryotes (Noda and Ohsumi, 1998; Yorimitsu et al., 2007). Subsequently, in yeast, this results in dephosphorylated Atg13, binding of Atg17 and activation of the Atg1 kinase. Consequently, the formation of a single membrane structure, the phagophore, which surrounds and engulfs cytoplasm, organelles and other cellular components, develops into spherical autophagosomes, which then expand and fuse with a vacuole, where cargo is degraded by hydrolases in autophagic bodies (Klionsky, 2007; Mizushima and Komatsu, 2011). TOR kinase is a critical cell growth regulator involved in cellular metabolism, growth, and suppressing catabolic processes. In animals, the mTORC1 complex phosphorylates and inhibits the activity of ULK1, the autophagy-initiating kinase, homologous to yeast Atg1 (Kim and Guan, 2011; Kim et al., 2011), and is itself regulated by the upstream targets phosphoinositide 3-kinase (PI3K) and AKT proteins (Heras-Sandoval et al., 2014). In *Saccharomyces cerevisiae*, two complexes are formed – TORC1, which is sensitive to rapamycin and regulates cell growth and autophagy, and TORC2, which regulates plasma membrane homeostasis, responding to osmotic stress, for example, and modulating plasma membrane tension (Berchtold et al., 2012; Ebner and Haucke, 2018; Niles and Powers, 2014; Riggi et al., 2018). In *M. oryzae,* TOR is implicated in regulating appressorium infection-associated autophagy in response to starvation stress, cell cycle control and modulation of the cAMP/PKA pathway (Fernandez et al., 2014; Franceschetti et al., 2011; Marroquin-Guzman et al., 2017; Marroquin-Guzman and Wilson, 2015). Recently, a novel VASt-domain-containing protein was identified and named Vast1 in *M. oryzae* (Zhu et al., 2021). VASt-domain-containing proteins are not well understood, but have been suggested to have lipid-binding functions (Khafif et al., 2014) In *M. oryzae*, Vast1 is anchored to the plasma membrane and responds to external stress by regulating sterol content in the plasma membrane (Zhu et al., 2021) (see poster). In the absence of Vast1, the sterol content of the membrane increases, elevating membrane tension. Phosphorylation of the putative TOR1 downstream targets Rps6 and Ypk1 was significantly reduced in a Vast1-null mutant when treated with rapamycin (see poster). Furthermore, it has been shown by site-targeted mutagenesis that a threonine site (T902) is essential for localisation and function of Vast1. Therefore, Vast1 has been proposed to contribute to fungal development and pathogenicity through regulation of lipid homeostasis in a phosphorylation signalling mechanism mediated by TOR (Zhu et al., 2021).

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activated under high levels of intracellular glutamine and glucose, which inhibit appressorium formation. Inactive TOR is, therefore, thought to be necessary for cell cycle arrest and autophagy (Fernandez and Orth, 2018; Marroquin-Guzman et al., 2017; Marroquin-Guzman and Wilson, 2015). Furthermore, the serine/threonine phosphatase Tip41 mediates crosstalk between the TOR and the cell integrity pathway (Qian et al., 2018), mirroring a similar regulatory process in yeast (Helliwell et al., 1998; Kamada et al., 2005). Interestingly, in *M. oryzae* a VAD1 analogue of StAR-related lipid transfer (VASt-domain)-containing protein Vast1, monitors plasma membrane tension and sterol content of the plasma membrane, modulating the activity of TOR and thereby regulating autophagy (Zhu et al., 2021).

Maturation and repolarisation

For successful penetration, it is necessary for the appressorium to switch from isotropic expansion to polarised growth. This requires reorganisation of the F-actin cytoskeleton at the appressorium pore, from which the penetration peg emerges (Bourett and Howard, 1992; Dagdas et al., 2012). F-actin forms a higher-order ring structure at the pore that requires septin guanosine triphosphatases (GTPases) (see poster). Septins are crucial for recruiting and organising F-actin, forming a hetero-oligomeric ring at the pore (Dagdas et al., 2012). Septins rigidify the cortex of the appressorium, scaffold actin and act as a lateral diffusion barrier for polarity determinants, such as Las17 (Dagdas et al., 2012; Dulal et al., 2020; Van Ngo and Mostowy, 2019). Likewise, the localisation of Rvs167, a BAR-domain-containing protein, is septin-dependent (Dagdas et al., 2012) and might enable the generation of membrane curvature that is associated with polymerisation of cortical F-actin (Mattila et al., 2007). Interestingly, organisation of the septin ring also relies on an intact microtubule cytoskeleton (Dulal et al., 2021). Septin interactions with phosphatidylinositol (PtdIns) 4-phosphate (PI4P) or phosphatidylinositol-4,5-bisphosphate (PI4,5P2) and the ezrin, radixin and moesin-like protein Tea1 at the plasma membrane also occur at the appressorium pore (see poster). Septin organisation requires the very-long-chain fatty-acid-rich (VLCFA) domains of PIPs, and inhibition of VLCFA biosynthesis prevents septin ring formation and plant infection, which has been used as a means of selecting broad-spectrum fungicides (He et al., 2020). This highlights the conserved nature of septin-dependent processes in the appressorium, because these fungicides control diverse plant and insect-pathogenic fungi (He et al., 2020).

Determining how the appressorium perceives a signal to repolarise is a long-standing question. In *M. oryzae*, the histidine-aspartate kinase Sln1 enables the appressorium to sense when a threshold of turgor has been reached so it can switch to polarised growth (Ryder et al., 2019). Sln1 was first identified as an osmosensor in yeast and regulates hyperosmotic adaptation through the Hog1 MAPK signalling pathway. The equivalent MAPK in *M. oryzae*, Osm1 is, however, not involved in appressorium turgor generation (Dixon et al., 1999) and plays a very different role during plant infection (Liu et al., 2020), so *M. oryzae* Sln1 must operate in a distinct manner to control appressorium turgor. Sln1 negatively regulates melanin biosynthesis and the cAMP-response pathway, and works in parallel with the protein kinase C1 (Pkc1)-dependent cell integrity pathway by phosphorylating the phosphodiesterase enzyme PdeH to modulate cAMP levels, which in turn controls lipolysis and glycerol production (Ryder et al., 2019). Pkc1 might also target the regulatory subunit of NADPH oxidase, a homologue of mammalian $p67^{phox}$ (also known as NCF2), which is necessary for regulated synthesis of reactive oxygen species (ROS) required for septin-mediated cytoskeletal reorganisation (Ryder et al., 2019, 2013) (see poster). This observation is consistent with studies in humans, where it was demonstrated that PKC is required for phosphorylation of $gp91^{phox}$ (also known as CYBB) and its binding to Rac2, $p67^{phox}$ (NoxR in fungi) and $p47^{phox}$ (also known as NCF1; Bem1 in fungi) (Raad et al., 2008).

Penetration and formation of the transpressorium

Plant infection proceeds by emergence of a rigid penetration peg from the base of the appressorium (Bourett and Howard, 1990). Protrusive force generated by appressoria from *Colletotrichum* has been measured at 17 µN using an optical waveguide (Bechinger et al., 1999; Talbot, 2003), which is (easily) sufficient to rupture the cuticle and plant cell wall. During *M. oryzae* infection, the resulting primary invasive hypha does not, however, rupture the plasma membrane of the invaded epidermal cell, but instead the plant membrane is invaginated around the fungal hypha, forming a special compartment called the extra-invasive hyphal membrane (EIHM) (Kankanala et al., 2007; Yi and Valent, 2013). This is analogous to the extrahaustorial membrane (Bozkurt and Kamoun, 2020) and, in the same way, might provide a means of sequestering nutrients from plant cells while also evading recognition. A membrane-rich cap is visible at the tip of the penetration hypha and maintained throughout cell colonisation. This structure is termed the biotrophic interfacial complex (BIC) and appears to be a site for delivery of fungal effectors (Valent and Khang, 2010) (see poster). Effector proteins are secreted fungal proteins that target components of the plant immune system to suppress plant defence (Kamoun, 2006; Valent and Khang, 2010). *M. oryzae* effectors perturb a range of molecular functions, including suppressing chitin-triggered immunity (Mentlak et al., 2012) and reprogramming host transcription (Kim et al., 2020; Mentlak et al., 2011, 2012; Mosquera et al., 2009; Valent and Khang, 2010). Effectors destined for delivery into rice cells accumulate at the BIC, whereas those targeting extracellular immune responses are secreted from the hyphal tip into the apoplastic space, between the fungal cell wall and EIHM (Valent and Khang, 2010; Martin-Urdiroz et al., 2016). In contrast to apoplastic effectors, BIC-localised cytoplasmic effectors are secreted using an unusual Golgi-independent secretion system (Giraldo et al., 2013). Focal secretion of effectors directly from the base of appressoria has also been observed in *Colletotrichum higginsianum* (Kleemann et al., 2012) and *C. orbiculare*, where a membrane-rich structure similar to the BIC has been reported (Irieda et al., 2014; Khang et al., 2010; Valent and Khang, 2010).

When the fungus encounters the junction with a neighbouring cell, the hyphal tip undergoes cortical scanning until it locates a pit field, where plasmodesmata are located. The hyphal tip swells into a structure resembling an appressorium, called a transpressorium (Cruz-Mireles et al., 2021). The transpressorium undergoes hyphal constriction and forms a narrow infection peg that enters the adjacent cell. Remarkably, plant plasma membrane integrity is maintained during this process, with the EIHM surrounding the fungus in the newly invaded cell, where a BIC rapidly forms. There are clear parallels between appressoria and transpressoria, because Pmk1 regulates morphogenesis of both cell types, and transpressorium function is also mediated by septin proteins (Sakulkoo et al., 2018) (see poster).

Conclusions and future directions

Although the regulatory framework governing appressorium development in *M. oryzae* is known, there are very significant gaps in our understanding (listed on the poster). The precise

mechanisms by which surface signals are perceived, for example, are far from clear. There may be a role for thigmotropic sensors and receptors of plant-derived compounds in addition to Pth11, for instance, and the ligand recognised by the Pth11 GPCR is so far unknown. How the upstream signalling pathways activate the Pmk1 pathway is also not at all clear and will require high-resolution proteomic methods, coupled with functional gene studies, to be fully deciphered. Similarly, downstream of Pmk1, we have little understanding of the transcriptional regulatory hierarchy that controls the major changes in gene expression required for appressorium morphogenesis. The immediate targets of Pmk1 are now being defined (Osés-Ruiz et al., 2021), but iterative rounds of phosphoproteomics and chromatin immunoprecipitation (ChIP)-seq analysis will be necessary to identify how the regulatory pathway actually operates. The precise role of TOR signalling also requires further attention, as it might impact on the control of plasma membrane dynamics and turgor control, as well as autophagy, during appressorium development. Understanding Sln1-dependent turgor sensing is also a priority, because it is pivotal to the control of cellular reorganisation and appressorium repolarisation. The direct targets of the Sln1 kinase have not yet been identified, nor its interplay with TOR, protein kinase C, Pmk1 and cell cycle regulation. Finally, the identification of the transpressorium generates many questions (see poster) regarding the parallels between both infection cells. *M. oryzae* is a valuable model for investigating the cell biology of appressoria, but its utility will only be fully realised if the conservation of many of the processes identified is confirmed in other appressorium-forming fungi. This has proceeded in some cases – Pmk1 being a clear example – but there is a need to investigate some of the experimentally recalcitrant appressorium-forming fungi, such as rusts (which are unculturable), to determine the extent to which the discoveries in rice blast can be more generally applied.

Competing interests

The authors declare no competing or financial interests.

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References

Adachi, K. and Hamer, J. E. (1998). Divergent cAMP signaling pathways regulate growth and pathogenesis in the rice blast fungus Magnaporthe grisea. *Plant Cell* **10**, 1361-1373. doi:10.1105/tpc.10.8.1361

Bechinger, C., Giebel, K.-F., Schnell, M., Leiderer, P., Deising, H. B. and Bastmeyer, M. (1999). Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. *Science* **285**, 1896-1899. doi:10.1126/science.285.5435.1896

Beck, T. and Hall, M. N. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* **402**, 689-692. doi:10.1038/45287

Berchtold, D., Piccolis, M., Chiaruttini, N., Riezman, I., Riezman, H., Roux, A., Walther, T. C. and Loewith, R. (2012). Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat. Cell Biol.* **14**, 542-547. doi:10.1038/ncb2480

Bonfante, P. and Genre, A. (2010). Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nat. Commun.* **1**, 48. doi:10.1038/ncomms1046

Bourett, T. M. and Howard, R. J. (1990). In vitro development of penetration structures in the rice blast fungus Magnaporthe grisea. *Can. J. Bot.* **68**, 329-342. doi:10.1139/b90-044

Bourett, T. M. and Howard, R. J. (1992). Actin in penetration pegs of the fungal rice blast pathogen, Magnaporthe grisea. *Protoplasma* **168**, 20-26. doi:10.1007/BF01332647

Bozkurt, T. O. and Kamoun, S. (2020). The plant-pathogen haustorial interface at a glance. *J. Cell Sci.* **133**, jcs237958. doi:10.1242/jcs.237958

Bronkhorst, J., Kasteel, M., van Veen, S., Clough, J. M., Kots, K., Buijs, J., van der Gucht, J., Ketelaar, T., Govers, F. and Sprakel, J. (2021). A slicing mechanism facilitates host entry by plant-pathogenic Phytophthora. *Nat. Microbiol.* **6**, 1000-1006. doi:10.1038/s41564-021-00919-7

Cao, H., Huang, P., Zhang, L., Shi, Y., Sun, D., Yan, Y., Liu, X., Dong, B., Chen, G., Snyder, J. H. et al. (2016). Characterization of 47 Cys_2 -His_2 zinc finger proteins required for the development and pathogenicity of the rice blast fungus Magnaporthe oryzae. *New Phytol.* **211**, 1035-1051. doi:10.1111/nph.13948

Castanheira, S., Mielnichuk, N. and Perez-Martin, J. (2014). Programmed cell cycle arrest is required for infection of corn plants by the fungus Ustilago maydis. *Development* **141**, 4817-4826. doi:10.1242/dev.113415

Chang, H. X., Miller, L. A. and Hartman, G. L. (2014). Melanin-independent accumulation of turgor pressure in Appressoria of Phakopsora pachyrhizi. *Phytopathology* **104**, 977-984. doi:10.1094/PHYTO-12-13-0335-R

Chethana, K. W. T., Jayawardena, R. S., Chen, Y. J., Konta, S., Tibpromma, S., Abeywickrama, P. D., Gomdola, D., Balasuriya, A., Xu, J. P., Lumyong, S. et al. (2021a). Diversity and function of Appressoria. *Pathogens* **10**, 746. doi:10.3390/pathogens10060746

Chethana, K. W. T., Jayawardena, R. S., Chen, Y. J., Konta, S., Tibpromma, S., Phukhamsakda, C., Abeywickrama, P. D., Samarakoon, M. C., Senwanna, C., Mapook, A. et al. (2021b). Appressorial interactions with host and their evolution. *Fungal Divers.* **110**, 75-107. doi:10.1007/s13225-021-00487-5

Choquer, M., Rascle, C., Goncalves, I. R., de Vallée, A., Ribot, C., Loisel, E., Smilevski, P., Ferria, J., Savadogo, M., Souibgui, E. et al. (2021). The infection cushion of Botrytis cinerea: a fungal 'weapon' of plant-biomass destruction. *Environ. Microbiol.* **23**, 2293-2314. doi:10.1111/1462-2920.15416

Chumley, F. G. and Valent, B. (1990). Genetic-analysis of melanin-deficient, nonpathogenic mutants of Magnaporthe-grisea. *Mol. Plant Microbe Interact.* **3**, 135-143. doi:10.1094/MPMI-3-135

Conrad, M., Schothorst, J., Kankipati, H. N., Van Zeebroeck, G., Rubio-Texeira, M. and Thevelein, J. M. (2014). Nutrient sensing and signaling in the yeast Saccharomyces cerevisiae. *FEMS Microbiol. Rev.* **38**, 254-299. doi:10.1111/1574-6976.12065

Cruz-Mireles, N., Eseola, A. B., Osés-Ruiz, M., Ryder, L. S. and Talbot, N. J. (2021). From appressorium to transpressorium—defining the morphogenetic basis of host cell invasion by the rice blast fungus. *PLoS Pathog.* **17**, e1009779. doi:10.1371/journal.ppat.1009779

Dagdas, Y. F., Yoshino, K., Dagdas, G., Ryder, L. S., Bielska, E., Steinberg, G. and Talbot, N. J. (2012). Septin-mediated plant cell invasion by the rice blast fungus, Magnaporthe oryzae. *Science* **336**, 1590-1595. doi:10.1126/science.1222934

de Jong, J. C., McCormack, B. J., Smirnoff, N. and Talbot, N. J. (1997). Glycerol generates turgor in rice blast. *Nature* **389**, 244-244. doi:10.1038/38418

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J. et al. (2012). The top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **13**, 414-430. doi:10.1111/j.1364-3703.2011.00783.x

Deising, H. B., Werner, S. and Wernitz, M. (2000). The role of fungal appressoria in plant infection. *Microbes Infect.* **2**, 1631-1641. doi:10.1016/S1286-4579(00)01319-8

Demoor, A., Silar, P. and Brun, S. (2019). Appressorium: the breakthrough in Dikarya. *J. Fungi.* **5**, 72. doi:10.3390/jof5030072

Dixon, K. P., Xu, J. R., Smirnoff, N. and Talbot, N. J. (1999). Independent signaling pathways regulate cellular turgor during hyperosmotic stress and appressorium-mediated plant infection by Magnaporthe grisea. *Plant Cell* **11**, 2045-2058. doi:10.1105/tpc.11.10.2045

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S. et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **149**, 1060-1072. doi:10.1016/j.cell.2012.03.042

Dulal, N., Rogers, A., Wang, Y. and Egan, M. (2020). Dynamic assembly of a higher-order septin structure during appressorium morphogenesis by the rice blast fungus. *Fungal Genet. Biol.* **140**, 103385. doi:10.1016/j.fgb.2020.103385

Dulal, N., Rogers, A. M., Proko, R., Bieger, B. D., Liyanage, R., Krishnamurthi, V. R., Wang, Y. and Egan, M. J. (2021). Turgor-dependent and coronin-mediated F-actin dynamics drive septin disc-to-ring remodeling in the blast fungus Magnaporthe oryzae. *J. Cell Sci.* **134**, jcs251298. doi:10.1242/jcs.251298

Ebner, M. and Haucke, V. (2018). Mechanical signals regulate TORC2 activity. *Nat. Cell Biol.* **20**, 994-995. doi:10.1038/s41556-018-0181-5

Eseola, A. B., Ryder, L. S., Osés-Ruiz, M., Findlay, K., Yan, X., Cruz-Mireles, N., Molinari, C., Garduño-Rosales, M. and Talbot, N. J. (2021). Investigating the cell and developmental biology of plant infection by the rice blast fungus Magnaporthe oryzae. *Fungal Genet. Biol.* **154**, 103562. doi:10.1016/j.fgb.2021.103562

Fernandez, J. and Orth, K. (2018). Rise of a cereal killer: the biology of Magnaporthe oryzae biotrophic growth. *Trends Microbiol.* **26**, 582-597. doi:10.1016/j.tim.2017.12.007

Fernandez, J. and Wilson, R. A. (2012). Why no feeding Frenzy? Mechanisms of nutrient acquisition and utilization during infection by the rice blast fungus Magnaporthe oryzae. *Mol. Plant-Microbe Interact.* **25**, 1286-1293. doi:10.1094/MPMI-12-11-0326

Fernandez, J., Marroquin-Guzman, M. and Wilson, R. A. (2014). Evidence for a transketolase-mediated metabolic checkpoint governing biotrophic growth in rice cells by the blast fungus Magnaporthe oryzae. *PLoS Pathog.* **10**, e1004354. doi:10.1371/journal.ppat.1004354

Foster, A. J., Jenkinson, J. M. and Talbot, N. J. (2003). Trehalose synthesis and metabolism are required at different stages of plant infection by Magnaporthe grisea. *EMBO J.* **22**, 225-235. doi:10.1093/emboj/cdg018

Franceschetti, M., Bueno, E., Wilson, R. A., Tucker, S. L., Gómez-Mena, C., Calder, G. and Sesma, A. (2011). Fungal virulence and development is regulated by alternative pre-mRNA 3′end processing in Magnaporthe oryzae. *PLoS Pathog.* **7**, e1002441. doi:10.1371/journal.ppat.1002441

Frank, B. (1883). Ueber einige neue und weniger bekannte Pflanzenkrankheiten. *Berichte der Deutschen Botanischen Gesellschaft* **1**, 29-34.

Giraldo, M. C., Dagdas, Y. F., Gupta, Y. K., Mentlak, T. A., Yi, M., Martinez-Rocha, A. L., Saitoh, H., Terauchi, R., Talbot, N. J. and Valent, B. (2013). Two distinct secretion systems facilitate tissue invasion by the rice blast fungus Magnaporthe oryzae. *Nat. Commun.* **4**, 1996. doi:10.1038/ncomms2996

Goos, R. D. and Gessner, R. V. (1975). Hyphal modifications of sphaerulina-pedicellata - appressoria or hyphopodia? *Mycologia* **67**, 1035-1038. doi:10.1080/00275514.1975.12019838

Hamer, J. E., Howard, R. J., Chumley, F. G. and Valent, B. (1988). A mechanism for surface attachment in spores of a plant pathogenic fungus. *Science* **239**, 288. doi:10.1126/science.239.4837.288

Hansjakob, A., Riederer, M. and Hildebrandt, U. (2012). Appressorium morphogenesis and cell cycle progression are linked in the grass powdery mildew fungus Blumeria graminis. *Fungal Biol.* **116**, 890-901. doi:10.1016/j.funbio.2012.05.006

He, M., Su, J., Xu, Y., Chen, J., Chern, M., Lei, M., Qi, T., Wang, Z., Ryder, L. S., Tang, B. et al. (2020). Discovery of broad-spectrum fungicides that block septin-dependent infection processes of pathogenic fungi. *Nat. Microbiol.* **5**, 1565-1575. doi:10.1038/s41564-020-00790-y

Helliwell, S. B., Schmidt, A., Ohya, Y. and Hall, M. N. (1998). The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. *Curr. Biol.* **8**, 1211-1214. doi:10.1016/S0960-9822(07)00511-8

Heras-Sandoval, D., Pérez-Rojas, J. M., Hernández-Damián, J. and Pedraza-Chaverri, J. (2014). The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. *Cell. Signal.* **26**, 2694-2701. doi:10.1016/j.cellsig.2014.08.019

Hyde, K. D., Dong, Y., Phookamsak, R., Jeewon, R., Bhat, D. J., Jones, E. B. G., Liu, N.-G., Abeywickrama, P. D., Mapook, A., Wei, D. et al. (2020). Fungal diversity notes 1151–1276: taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Divers.* **100**, 5-277. doi:10.1007/s13225-020-00439-5

Irieda, H., Maeda, H., Akiyama, K., Hagiwara, A., Saitoh, H., Uemura, A., Terauchi, R. and Takano, Y. (2014). Colletotrichum orbiculare secretes virulence effectors to a biotrophic interface at the primary hyphal neck via exocytosis coupled with SEC22-mediated traffic. *Plant Cell* **26**, 2265-2281. doi:10.1105/tpc.113.120600

Jiang, C., Zhang, X., Liu, H. and Xu, J.-R. (2018). Mitogen-activated protein kinase signaling in plant pathogenic fungi. *PLoS Pathog.* **14**, e1006875-e1006875. doi:10.1371/journal.ppat.1006875

Kagan, V. E., Mao, G. W., Qu, F., Angeli, J. P. F., Doll, S., St Croix, C., Dar, H. H., Liu, B., Tyurin, V. A., Ritov, V. B. et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* **13**, 81-90. doi:10.1038/nchembio.2238

Kamada, Y., Fujioka, Y., Suzuki, N. N., Inagaki, F., Wullschleger, S., Loewith, R., Hall, M. N. and Ohsumi, Y. (2005). Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. *Mol. Cell. Biol.* **25**, 7239-7248. doi:10.1128/MCB.25.16.7239-7248.2005

Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annu. Rev. Phytopathol.* **44**, 41-60. doi:10.1146/annurev.phyto.44.070505.143436

Kankanala, P., Czymmek, K. and Valent, B. (2007). Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell* **19**, 706-724. doi:10.1105/tpc.106.046300

Kershaw, M. J. and Talbot, N. J. (2009). Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. *Proc. Natl. Acad. Sci. USA* **106**, 15967-15972. doi:10.1073/pnas.0901477106

Khafif, M., Cottret, L., Balagué, C. and Raffaele, S. (2014). Identification and phylogenetic analyses of VASt, an uncharacterized protein domain associated with lipid-binding domains in Eukaryotes. *BMC Bioinform.* **15**, 222. doi:10.1186/1471-2105-15-222

Khang, C. H., Berruyer, R., Giraldo, M. C., Kankanala, P., Park, S. Y., Czymmek, K., Kang, S. and Valent, B. (2010). Translocation of Magnaporthe oryzae effectors into rice cells and their subsequent cell-to-cell movement. *Plant Cell* **22**, 1388-1403. doi:10.1105/tpc.109.069666

Kim, J. and Guan, K. L. (2011). Amino acid signaling in TOR activation. *Annu. Rev. Biochem.* **80**, 1001-1032. doi:10.1146/annurev-biochem-062209-094414

Kim, S., Ahn, I. P., Rho, H. S. and Lee, Y. H. (2005). MHP1, a Magnaporthe grisea hydrophobin gene, is required for fungal development and plant colonization. *Mol. Microbiol.* **57**, 1224-1237. doi:10.1111/j.1365-2958.2005.04750.x

Kim, S., Park, S.-Y., Kim, K. S., Rho, H.-S., Chi, M.-H., Choi, J., Park, J., Kong, S., Park, J., Goh, J. et al. (2009). Homeobox transcription factors are required for conidiation and appressorium development in the rice blast fungus Magnaporthe oryzae. *PLoS Genet.* **5**, e1000757-e1000757. doi:10.1371/journal.pgen.1000757

Kim, J., Kundu, M., Viollet, B. and Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132-141. doi:10.1038/ncb2152

Kim, S., Kim, C.-Y., Park, S.-Y., Kim, K.-T., Jeon, J., Chung, H., Choi, G., Kwon, S., Choi, J., Jeon, J. et al. (2020). Two nuclear effectors of the rice blast fungus modulate host immunity via transcriptional reprogramming. *Nat. Commun.* **11**, 5845. doi:10.1038/s41467-020-19624-w

Kleemann, J., Rincon-Rivera, L. J., Takahara, H., Neumann, U., Ver Loren van Themaat, E., van der Does, H. C., Hacquard, S., Stüber, K., Will, I., Schmalenbach, W. et al. (2012). Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen Colletotrichum higginsianum. *PLoS Pathog.* **8**, e1002643. doi:10.1371/journal.ppat.1002643

Klionsky, D. J. (2007). Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat. Rev. Mol. Cell Biol.* **8**, 931-937. doi:10.1038/nrm2245

Li, X., Gao, C., Li, L., Liu, M., Yin, Z., Zhang, H., Zheng, X., Wang, P. and Zhang, Z. (2017). MoEnd3 regulates appressorium formation and virulence through mediating endocytosis in rice blast fungus Magnaporthe oryzae. *PLoS Pathog.* **13**, e1006449. doi:10.1371/journal.ppat.1006449

Liang, M. L., Ye, H. J., Shen, Q., Jiang, X. Y., Cui, G. B., Gu, W. X., Zhang, L. H., Naqvi, N. I. and Deng, Y. Z. (2021). Tangeretin inhibits fungal ferroptosis to suppress rice blast. *J. Integr. Plant Biol.* **63**, 2136-2149. doi:10.1111/jipb.13175

Lin, S. Y., Okuda, S., Ikeda, K., Okuno, T. and Takano, Y. (2012). LAC2 encoding a secreted laccase is involved in appressorial melanization and conidial pigmentation in colletotrichum orbiculare. *Mol. Plant Microbe Interact.* **25**, 1552-1561. doi:10.1094/MPMI-05-12-0131-R

Liu, S. and Dean, R. A. (1997). G protein α subunit genes control growth, development, and pathogenicity of Magnaporthe grisea. *Mol. Plant Microbe Interact.* **10**, 1075-1086. doi:10.1094/MPMI.1997.10.9.1075

Liu, H., Suresh, A., Willard, F. S., Siderovski, D. P., Lu, S. and Naqvi, N. I. (2007). Rgs1 regulates multiple Gα subunits in Magnaporthe pathogenesis, asexual growth and thigmotropism. *EMBO J.* **26**, 690-700. doi:10.1038/sj.emboj.7601536

Liu, W., Zhou, X., Li, G., Li, L., Kong, L., Wang, C., Zhang, H. and Xu, J.-R. (2011). Multiple plant surface signals are sensed by different mechanisms in the rice blast fungus for appressorium formation. *PLoS Pathog.* **7**, e1001261. doi:10.1371/journal.ppat.1001261

Liu, N., Ning, G. A., Liu, X. H., Feng, X. X., Lu, J. P., Mao, L. J., Su, Z. Z., Wang, Y., Zhang, C. L. and Lin, F. C. (2016). An autophagy gene, HoATG5, is involved in sporulation, cell wall integrity and infection of wounded barley leaves. *Microbiol. Res.* **192**, 326-335. doi:10.1016/j.micres.2016.08.008

Liu, X., Zhou, Q., Guo, Z., Liu, P., Shen, L., Chai, N., Qian, B., Cai, Y., Wang, W., Yin, Z. et al. (2020). A self-balancing circuit centered on MoOsm1 kinase governs adaptive responses to host-derived ROS in Magnaporthe oryzae. *Elife* **9**, e61605. doi:10.7554/eLife.61605

Loehrer, M., Botterweck, J., Jahnke, J., Mahlmann, D. M., Gaetgens, J., Oldiges, M., Horbach, R., Deising, H. and Schaffrath, U. (2014). In vivo assessment by Mach–Zehnder double–beam interferometry of the invasive force exerted by the Asian soybean rust fungus (Phakopsora pachyrhizi). *New Phytol.* **203**, 620-631. doi:10.1111/nph.12784

López-Berges, M. S., Rispail, N., Prados-Rosales, R. C. and Di Pietro, A. (2010). A nitrogen response pathway regulates virulence functions in fusarium oxysporum via the protein kinase TOR and the bZIP protein MeaB. *Plant Cell* **22**, 2459-2475. doi:10.1105/tpc.110.075937

Lozano-Tovar, M. D., Ortiz-Urquiza, A., Garrido-Jurado, I., Trapero-Casas, A. and Quesada-Moraga, E. (2013). Assessment of entomopathogenic fungi and their extracts against a soil-dwelling pest and soil-borne pathogens of olive. *Biol. Control* **67**, 409-420. doi:10.1016/j.biocontrol.2013.09.006

Ludwig, N., Löhrer, M., Hempel, M., Mathea, S., Schliebner, I., Menzel, M., Kiesow, A., Schaffrath, U., Deising, H. B. and Horbach, R. (2014). Melanin is not required for turgor generation but enhances cell-wall rigidity in appressoria of the corn pathogen Colletotrichum graminicola. *Mol. Plant Microbe Interact.* **27**, 315-327. doi:10.1094/MPMI-09-13-0267-R

Lv, W. Y., Wang, C. Y., Yang, N., Que, Y. W., Talbot, N. J. and Wang, Z. Y. (2017). Genome-wide functional analysis reveals that autophagy is necessary for growth, sporulation, deoxynivalenol production and virulence in Fusarium graminearum. *Sci. Rep.* **7**, 11062. doi:10.1038/s41598-017-11640-z

Marroquin-Guzman, M. and Wilson, R. A. (2015). GATA-dependent glutaminolysis drives appressorium formation in Magnaporthe oryzae by suppressing TOR inhibition of cAMP/PKA signaling. *PLoS Pathog.* **11**, e1004851. doi:10.1371/journal.ppat.1004851

Marroquin-Guzman, M., Sun, G. and Wilson, R. A. (2017). Glucose-ABL1-TOR signaling modulates cell cycle tuning to control terminal appressorial cell differentiation. *PLoS Genet.* **13**, e1006557. doi:10.1371/journal.pgen.1006557

Martin-Urdiroz, M., Oses-Ruiz, M., Ryder, L. S. and Talbot, N. J. (2016). Investigating the biology of plant infection by the rice blast fungus Magnaporthe oryzae. *Fungal Genet. Biol.* **90**, 61-68. doi:10.1016/j.fgb.2015.12.009

Mattila, P. K., Pykäläinen, A., Saarikangas, J., Paavilainen, V. O., Vihinen, H., Jokitalo, E. and Lappalainen, P. (2007). Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism. *J. Cell Biol.* **176**, 953-964. doi:10.1083/jcb.200609176

Mendgen, K., Hahn, M. and Deising, H. (1996). Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annu. Rev. Phytopathol.* **34**, 367-386. doi:10.1146/annurev.phyto.34.1.367

Mentlak, T., Talbot, N. and Kroj, T. (2011). Effector translocation and delivery by the rice blast fungus Magnaporthe oryzae. In *Effectors in Plant-Microbe Interactions* (ed. S. Kamoun and F. Martin), pp. 219-241. Wiley-Blackwell.

Mentlak, T. A., Kombrink, A., Shinya, T., Ryder, L. S., Otomo, I., Saitoh, H., Terauchi, R., Nishizawa, Y., Shibuya, N., Thomma, B. P. et al. (2012). Effector-mediated suppression of chitin-triggered immunity by magnaporthe oryzae is necessary for rice blast disease. *Plant Cell* **24**, 322-335. doi:10.1105/tpc.111.092957

Mizushima, N. and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. *Cell* **147**, 728-741. doi:10.1016/j.cell.2011.10.026

Mosquera, G., Giraldo, M. C., Khang, C. H., Coughlan, S. and Valent, B. (2009). Interaction transcriptome analysis identifies Magnaporthe oryzae BAS1-4 as biotrophy-associated secreted proteins in rice blast disease. *Plant Cell* **21**, 1273-1290. doi:10.1105/tpc.107.055228

Niles, B. J. and Powers, T. (2014). TOR complex 2-Ypk1 signaling regulates actin polarization via reactive oxygen species. *Mol. Biol. Cell* **25**, 3962-3972. doi:10.1091/mbc.e14-06-1122

Nishimura, M., Park, G. and Xu, J.-R. (2003). The G–beta subunit MGB1 is involved in regulating multiple steps of infection–related morphogenesis in Magnaporthe grisea. *Mol. Microbiol.* **50**, 231-243. doi:10.1046/j.1365-2958.2003.03676.x

Noda, T. and Ohsumi, Y. (1998). Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J. Biol. Chem.* **273**, 3963-3966. doi:10.1074/jbc.273.7.3963

Oh, Y., Donofrio, N., Pan, H. Q., Coughlan, S., Brown, D. E., Meng, S. W., Mitchell, T. and Dean, R. A. (2008). Transcriptome analysis reveals new insight into appressorium formation and function in the rice blast fungus Magnaporthe oryzae. *Genome Biol.* **9**, R85. doi:10.1186/gb-2008-9-5-r85

Osés-Ruiz, M., Sakulkoo, W., Littlejohn, G. R., Martin-Urdiroz, M. and Talbot, N. J. (2017). Two independent S-phase checkpoints regulate appressorium-mediated plant infection by the rice blast fungus Magnaporthe oryzae. *Proc. Natl. Acad. Sci. USA* **114**, E237-E244. doi:10.1073/pnas.1611307114

Osés-Ruiz, M., Cruz-Mireles, N., Martin-Urdiroz, M., Soanes, D. M., Eseola, A. B., Tang, B. Z., Derbyshire, P., Nielsen, M., Cheema, J., Were, V. et al. (2021). Appressorium-mediated plant infection by Magnaporthe oryzae is regulated by a Pmk1-dependent hierarchical transcriptional network. *Nat Microbiol* **6**, 1383-1397. doi:10.1038/s41564-021-00978-w

Park, G., Xue, C., Zheng, L., Lam, S. and Xu, J.-R. (2002). MST12 regulates infectious growth but not Appressorium formation in the rice blast fungus Magnaporthe grisea. *Mol. Plant-Microbe Interact.* **15**, 183-192. doi:10.1094/MPMI.2002.15.3.183

Park, G., Xue, C., Zhao, X., Kim, Y., Orbach, M. and Xu, J.-R. (2006). Multiple upstream signals converge on the adaptor protein Mst50 in Magnaporthe grisea. *Plant Cell* **18**, 2822-2835. doi:10.1105/tpc.105.038422

Pham, C. L. L., Rey, A., Lo, V., Soulès, M., Ren, Q., Meisl, G., Knowles, T. P. J., Kwan, A. H. and Sunde, M. (2016). 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.* **6**, 25288. doi:10.1038/srep25288

Qian, B., Liu, X. Y., Jia, J., Cai, Y. C., Chen, C., Zhang, H. F., Zheng, X. B., Wang, P. and Zhang, Z. G. (2018). MoPpe1 partners with MoSap1 to mediate TOR and cell wall integrity signalling in growth and pathogenicity of the rice blast fungus Magnaporthe oryzae. *Environ. Microbiol.* **20**, 3964-3979. doi:10.1111/1462-2920.14421

Qu, Y., Wang, J., Huang, P., Liu, X., Lu, J. and Lin, F.-C. (2021). PoRal2 is involved in Appressorium formation and virulence via Pmk1 MAPK pathways in the rice blast fungus Pyricularia oryzae. *Front. Plant Sci.* **12**, 702368. doi:10.3389/fpls.2021.702368

Raad, H., Paciet, M.-H., Boussetta, T., Kroviarski, Y., Morel, F., Quinn, M. T., Gougerot-Pocidalo, M.-A., Dang, P. M.-C. and El-Benna, J. (2008). Regulation of the phagocyte NADPH oxidase activity: phosphorylation of gp91phox/NOX2 by protein kinase C enhances its diaphorase activity and binding to Rac2, p67phox, and p47phox. *FASEB J.* **23**, 1011-1022. doi:10.1096/fj.08-114553

Ramanujam, R., Yishi, X., Liu, H. and Naqvi, N. I. (2012). Structure-function analysis of Rgs1 in Magnaporthe oryzae: role of DEP domains in subcellular targeting. *PLoS One* **7**, e41084. doi:10.1371/journal.pone.0041084

Riggi, M., Niewola-Staszkowska, K., Chiaruttini, N., Colom, A., Kusmider, B., Mercier, V., Soleimanpour, S., Stahl, M., Matile, S., Roux, A. et al. (2018). Decrease in plasma membrane tension triggers PtdIns(4,5)P2 phase separation to inactivate TORC2. *Nat. Cell Biol.* **20**, 1043-1051. doi:10.1038/s41556-018-0150-z

Rocha, R. O., Elowsky, C., Pham, N. T. and Wilson, R. A. (2020). Spermine-mediated tight sealing of the Magnaporthe oryzae appressorial pore–rice leaf surface interface. *Nat. Microbiol.* **5**, 1472-1480. doi:10.1038/s41564-020-0786-x

Ryder, L. S. and Talbot, N. J. (2015). Regulation of appressorium development in pathogenic fungi. *Curr. Opin. Plant Biol.* **26**, 8-13. doi:10.1016/j.pbi.2015.05.013

Ryder, L. S., Dagdas, Y. F., Mentlak, T. A., Kershaw, M. J., Thornton, C. R., Schuster, M., Chen, J., Wang, Z. and Talbot, N. J. (2013). NADPH oxidases regulate septin-mediated cytoskeletal remodeling during plant infection by the rice blast fungus. *Proc. Natl. Acad. Sci. USA* **110**, 3179-3184. doi:10.1073/pnas.1217470110

Ryder, L. S., Dagdas, Y. F., Kershaw, M. J., Venkataraman, C., Madzvamuse, A., Yan, X., Cruz-Mireles, N., Soanes, D. M., Oses-Ruiz, M., Styles, V. et al. (2019). A sensor kinase controls turgor-driven plant infection by the rice blast fungus. *Nature* **574**, 423-427. doi:10.1038/s41586-019-1637-x

Sakulkoo, W., Osés-Ruiz, M., Oliveira Garcia, E., Soanes, D. M., Littlejohn, G. R., Hacker, C., Correia, A., Valent, B. and Talbot, N. J. (2018). A single fungal MAP kinase controls plant cell-to-cell invasion by the rice blast fungus. *Science* **359**, 1399-1403. doi:10.1126/science.aaq0892

Saunders, D. G. O., Aves, S. J. and Talbot, N. J. (2010). Cell cycle-mediated regulation of plant infection by the rice blast fungus. *Plant Cell* **22**, 497-507. doi:10.1105/tpc.109.072447

Shashkova, S., Welkenhuysen, N. and Hohmann, S. (2015). Molecular communication: crosstalk between the Snf1 and other signaling pathways. *FEMS Yeast Res.* **15**, fov026. doi:10.1093/femsyr/fov026

Shen, Q. and Naqvi, N. I. (2021). Ferroptosis and microbial pathogenesis. *PLoS Pathog.* **17**, e1009298. doi:10.1371/journal.ppat.1009298

Shen, Q., Liang, M. L., Yang, F., Deng, Y. Z. and Naqvi, N. I. (2020). Ferroptosis contributes to developmental cell death in rice blast. *New Phytol.* **227**, 1831-1846. doi:10.1111/nph.16636

Talbot, N. J. (2003). On the trail of a cereal killer: exploring the biology of Magnaporthe grisea. *Annu. Rev. Microbiol.* **57**, 177-202. doi:10.1146/annurev.micro.57.030502.090957

Talbot, N. J. (2019). Appressoria. *Curr. Biol.* **29**, R144-R146. doi:10.1016/j.cub.2018.12.050

Talbot, N. J., Ebbole, D. J. and Hamer, J. E. (1993). Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus Magnaporthe grisea. *Plant Cell* **5**, 1575-1590. doi:10.1105/tpc.5.11.1575

Tsukada, M. and Ohsumi, Y. (1993). Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. *FEBS Lett.* **333**, 169-174. doi:10.1016/0014-5793(93)80398-E

Turrà, D., Segorbe, D. and Pietro, A. D. (2014). Protein kinases in plant-pathogenic fungi: conserved regulators of infection. *Annu. Rev. Phytopathol.* **52**, 267-288. doi:10.1146/annurev-phyto-102313-050143

Valent, B. and Khang, C. H. (2010). Recent advances in rice blast effector research. *Curr. Opin. Plant Biol.* **13**, 434-441. doi:10.1016/j.pbi.2010.04.012

Van Ngo, H. and Mostowy, S. (2019). Role of septins in microbial infection. *J. Cell Sci.* **132**, jcs226266. doi:10.1242/jcs.226266

Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G. and Talbot, N. J. (2006). Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* **312**, 580-583. doi:10.1126/science.1124550

Wang, Z.-Y., Soanes, D. M., Kershaw, M. J. and Talbot, N. J. (2007). Functional analysis of lipid metabolism in Magnaporthe grisea reveals a requirement for peroxisomal fatty acid β-oxidation during appressorium-mediated plant infection. *Mol. Plant Microbe Interact.* **20**, 475-491. doi:10.1094/MPMI-20-5-0475

Whiteford, J. R. and Spanu, P. D. (2002). Hydrophobins and the interactions between fungi and plants. *Mol. Plant Pathol.* **3**, 391-400. doi:10.1046/j.1364-3703.2002.00129.x

Wilson, R. A. and Talbot, N. J. (2009). Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. *Nat. Rev. Microbiol.* **7**, 185-195. doi:10.1038/nrmicro2032

Wilson, R. A., Gibson, R. P., Quispe, C. F., Littlechild, J. A. and Talbot, N. J. (2010). An NADPH-dependent genetic switch regulates plant infection by the rice blast fungus. *Proc. Natl. Acad. Sci. USA* **107**, 21902-21907. doi:10.1073/pnas.1006839107

Xu, J. R. and Hamer, J. E. (1996). MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus Magnaporthe grisea. *Genes Dev.* **10**, 2696-2706. doi:10.1101/gad.10.21.2696

Yi, M. and Valent, B. (2013). Communication Between Filamentous Pathogens and Plants at the Biotrophic Interface. *Annu. Rev. Phytopathol.* **51**, 587-611. doi:10.1146/annurev-phyto-081211-172916

Ying, S. H., Liu, J., Chu, X. L., Xie, X. Q. and Feng, M. G. (2016). The autophagy-related genes BbATG1 and BbATG8 have different functions in differentiation, stress resistance and virulence of mycopathogen Beauveria bassiana. *Sci. Rep.* **6**, 26376. doi:10.1038/srep26376

Yorimitsu, T., Zaman, S., Broach, J. R. and Klionsky, D. J. (2007). Protein kinase A and Sch9 cooperatively regulate induction of autophagy in Saccharomyces cerevisiae. *Mol. Biol. Cell* **18**, 4180-4189. doi:10.1091/mbc.e07-05-0485

Yu, F. W., Gu, Q., Yun, Y. Z., Yin, Y. N., Xu, J.-R., Shim, W.-B. and Ma, Z. H. (2014). The TOR signaling pathway regulates vegetative development and virulence in Fusarium graminearum. *New Phytol.* **203**, 219-232. doi:10.1111/nph.12776

Yue, X., Que, Y., Xu, L., Deng, S., Peng, Y., Talbot, N. J. and Wang, Z. (2016). ZNF1 encodes a putative C2H2 Zinc-finger protein essential for Appressorium differentiation by the rice blast fungus Magnaporthe oryzae. *Mol. Plant Microbe Interact.* **29**, 22-35. doi:10.1094/MPMI-09-15-0201-R

Zhang, L., Wang, J., Xie, X.-Q., Keyhani, N. O., Feng, M.-G. and Ying, S.-H. (2013). The autophagy gene BbATG5, involved in the formation of the autophagosome, contributes to cell differentiation and growth but is dispensable for pathogenesis in the entomopathogenic fungus Beauveria bassiana. *Microbiology* **159**, 243-252. doi:10.1099/mic.0.062646-0

Zhao, X., Kim, Y., Park, G. and Xu, J.-R. (2005). A mitogen-activated protein kinase cascade regulating infection-related morphogenesis in Magnaporthe grisea. *Plant Cell* **17**, 1317-1329. doi:10.1105/tpc.104.029116

Zhou, D. X., Xie, M. H., Bai, N., Yang, L., Zhang, K. Q. and Yang, J. K. (2020). The autophagy-related gene Aolatg4 regulates hyphal growth, sporulation, autophagosome formation, and pathogenicity in Arthrobotrys oligospora. *Front. Microbiol.* **11**, 592524. doi:10.3389/fmicb.2020.592524

Zhu, X.-M., Liang, S., Shi, H.-B., Lu, J.-P., Dong, B., Liao, Q.-S., Lin, F.-C. and Liu, X.-H. (2018). VPS9 domain-containing proteins are essential for autophagy and endocytosis in Pyricularia oryzae. *Environ. Microbiol.* **20**, 1516-1530. doi:10.1111/1462-2920.14076

Zhu, X. M., Li, L., Cai, Y. Y., Wu, X. Y., Shi, H. B., Liang, S., Qu, Y. M., Naqvi, N. I., Del Poeta, M., Dong, B. et al. (2021). A VASt-domain protein regulates autophagy, membrane tension, and sterol homeostasis in rice blast fungus. *Autophagy* **17**, 2939-2961. doi:10.1080/15548627.2020.1848129