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SHORT COMMUNICATION

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Cell cycle-dependent regulation of plant infection by the rice blast fungus *Magnaporthe oryzae*

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ABSTRACT

The rice blast fungus *Magnaporthe oryzae* forms a specialized infection structure called appressorium which uses a turgor-driven mechanical process to breach the leaf cuticle and gain entry into plant tissue. Appressorium development and plant infection are regulated by cell cycle progression and critically depend upon two, temporally separated S-phase checkpoints. Following conidial germination on the rice leaf surface, an S-phase checkpoint is essential for appressorium differentiation and operates through the DNA damage response pathway. By contrast, appressorium maturation and penetration peg development require S-progression that depends on turgor control. In this mini-review, we describe cellular mechanisms associated with cell cycle-dependent regulation of appressorium development and the potential operation of morphogenetic checkpoint control of plant infection.

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Rice blast disease is one of the most devastating and economically important crop diseases. Rice blast is caused by the ascomycete fungus *Magnaporthe oryzae* and accounts for destruction of up to 30% of the total annual rice harvest.[1] The global human population is estimated to increase by 2.2 billion people by 2050 and a huge increase in food production will be necessary if this population is to be sustainably fed. One of the world's most important crops is rice (*Oryza sativa*) with more than 700 million tonnes produced per annum each year, providing 23% of the world's calories. As the world population grows, however, it is predicted that rice yields will need to double by 2050 to meet increasing demand.[2] At least 800 million of the world's population live with insufficient nutrition and at least 10% of the world's food crop production is lost due to plant diseases and infection.[3] Controlling rice blast disease could make a critical contribution to global food security. *M. oryzae* is, however, also able to infect a range of other cereals and is the causal agent of wheat blast disease, which was first reported in 1985 in Paraná state of Brazil. In March 2016 wheat blast appeared in Bangladesh and destroyed more than 15000 hectares of wheat. The disease has already re-occurred in 2017 and now threatens wheat production across South Asia.

The infection mechanism of *Magnaporthe oryzae*

Fungal pathogens have developed a variety of strategies to gain entry into the host plants. Access to host cells can occur either through natural openings, such as stomata, or by direct penetration of the leaf cuticle. *M. oryzae* forms a specialized infection structure called an appressorium that operates by applying mechanical force to rupture the leaf cuticle (Fig. 1A). Mechanical force is generated by accumulation of enormous osmotic pressure inside the appressorium as a consequence of water influx as an osmotic response to high internal glycerol concentrations.[4,5] This leads to cytoskeletal re-orientation at the base of the appressorium, which requires the action of a family of small GTPases called septins, which are specifically recruited to the appressorium pore. Septins form hetero-oligomers and form a toroidal ring structure that scaffolds F-actin at the appressorium pore, providing cortical rigidity, and acting as a diffusion barrier for organisation of polarity and secretion proteins.[6-8] Rupture of the leaf occurs at this point by development of a narrow, rigid penetration peg that breaches the cuticle of the leaf to gain entry to the host primary cell (Fig. 1B). The peg differentiates into primary invasive hyphae which form a Biotrophic Interfacial Complex

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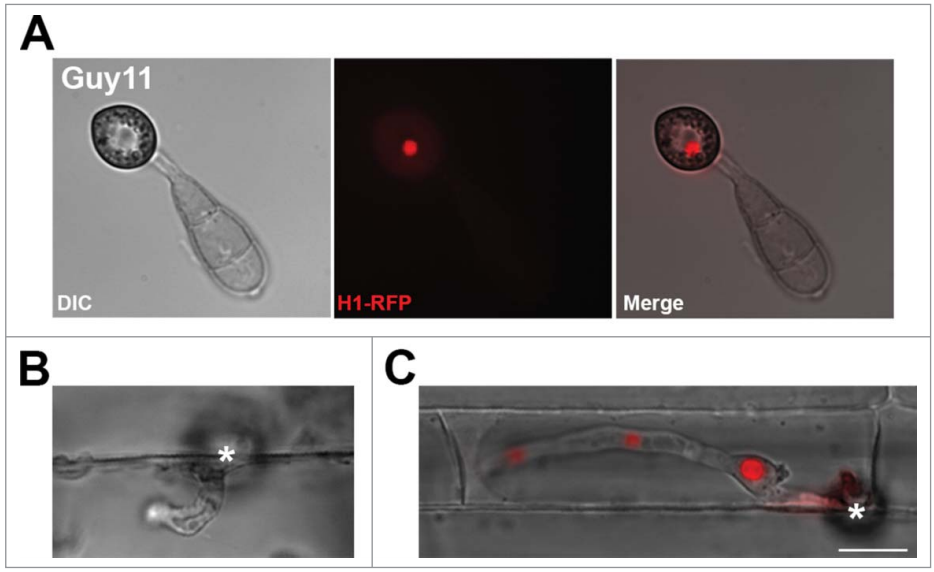

Figure 1. Cell cycle control of appressorium-mediated plant infection in *M. oryzae*. (A) Micrographs showing appressorium formation of Guy11 expressing H1-RFP on hydrophobic coverslips at 24 h. (B) Micrograph to show penetration peg emergence in a rice epidermal cell at 24 h. (C) Micrograph of Guy11 expressing H1-RFP to show nuclear divisions in a primary invasive hypha in a rice epidermal cell at 30 h. (Scale bar, 10 μm).

(BIC) within each invaded rice cell, through which a battery of cytoplasmic effectors are secreted to manipulate and suppress plant immunity (Fig. 1C) [9]. The primary invasive hypha differentiates into bulbous hyphae that occupy and roliferate within the first rice cell and then move to neighbouring cells through primary pit field sites.[9] After 5 days, the fungus sporulate from disease lesions and the infection life cycle starts again.[1]

Cell cycle control in the rice blast fungus

The ability of *M. oryzae* to develop appressoria and infect plants is tightly linked to cell cycle-mediated regulation.[10-12] When the three-cell spore germinates, the apical cell of the conidium undergoes a single round of mitosis. One of the daughter nuclei moves towards the developing appressorium, while the other nucleus returns to the conidium. As the appressorium matures, the conidium undergoes an autophagy-dependent process, leading to conidial cell death and recycling of the spore contents into the incipient appressorium.[13] Autophagy is a prerequisite for plant infection because null mutants of any of the non-selective autophagy-related genes exhibit loss of pathogenicity.[13] At the time of penetration only one nucleus is left in the appressorium dome, and it is only after penetration peg emergence that another round of mitosis occurs.[10,14] It is now known that both initial appressorium development and penetration peg emergence depend on completion of DNA replication or the synthesis phase (S-phase) of the cell cycle.[10,11] When germinating conidia are treated with the DNA replication inhibitor hydroxyurea (HU), the formation of incipient appressoria is blocked.[10,11] Moreover, when mature appressoria are treated with HU, penetration peg emergence and plant infection are also inhibited.[10] These results suggest that an S-phase checkpoint operates to control appressorium initiation and plant penetration but both are regulated in a distinct manner.

In all eukaryotes, surveillance mechanisms monitor the condition of DNA and are mediated through the DNA damage response (DDR) pathway to prevent catastrophic inheritance of abnormal nuclear material.[15-17] When a problem occurs during DNA replication, sensor kinases transduce a signal via a group of serine threonine kinases to inhibit entry into mitosis. They do so by promoting inhibitory phosphorylation of the cyclin-dependent kinase CDK1 (ScCdc28).[17,18] In *M. oryzae*, a homologue of the serine threonine fork head domain (FHA) kinase ScRad53, called Cds1, regulates the S-phase checkpoint during appressorium development.[10] Null mutants of Cds1 are able to override cell cycle arrest in the presence of HU, and undergo a round of mitosis and therefore develop appressoria.[10] Moreover, in the presence of HU the wild type *M. oryzae* strain Guy11 is unable to undergo conidial cell death, suggesting that programmed cell death in the conidium is coupled with mitosis and appressorium formation. This is consistent with treatment of *Δcds1* null mutants with HU after which they are unable to carry out conidial cell death, indicating that the DDR is not involved in this process (Fig. 2).

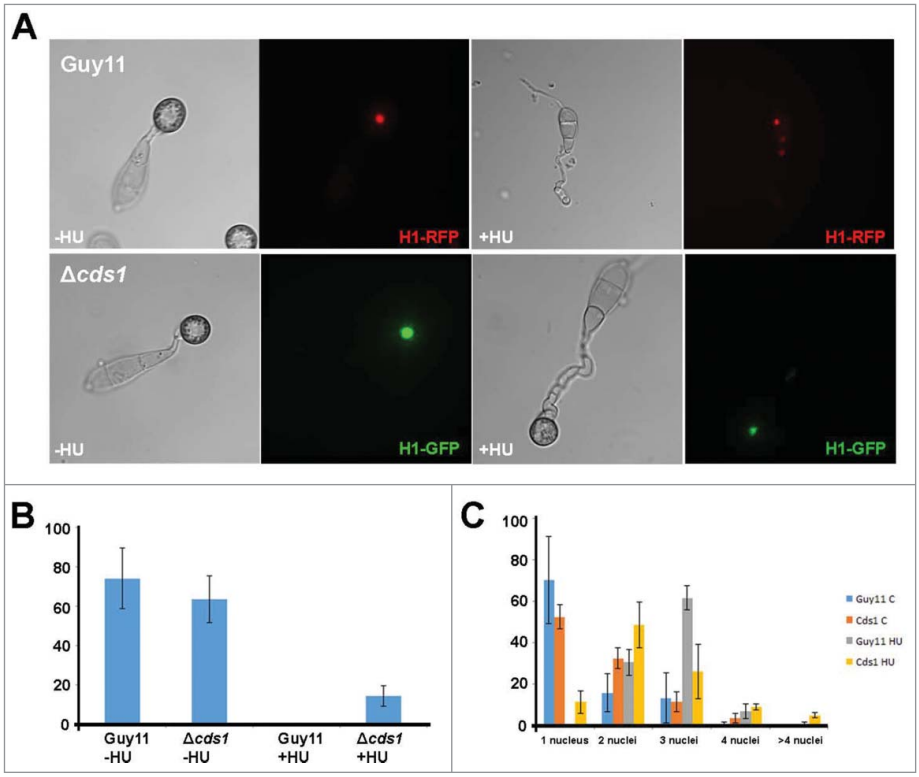

Figure 2. Conidial cell death in *M. oryzae* is independent of the DDR pathway. (A) Micrographs showing appressorium formation of Guy11 expressing H1-RFP and *Δcds1* expressing H1-GFP, after 24 h, following exposure of 200 mM of HU at 1 hpi. (B) Bar chart to show frequency of conidial collapse in Guy11 expressing H1-RFP and *Δcds1* expressing H1-GFP in the presence or absence of HU. (C) Bar chart to show the number of nuclei in Guy11 expressing H1-RFP and *Δcds1* expressing H1-GFP in the presence or absence of HU. (Scale bar, 10 μm).

The mechanism by which the S-phase checkpoint operates during plant penetration is independent of the DDR pathway and, instead, appears to be linked to turgor control. A *Δcds1* null mutant still arrests in S-phase when HU is added to mature appressoria and is unable to cause plant infection.[10] After the first round of mitosis, a minimum turgor threshold must be reached and this is necessary for the appressorium nucleus to pass from G1 to S-phase. Consistent with this, melanin-deficient mutants arrest in G1 and therefore are unable to cause plant infection.[10] Progression through S-phase is important to modulate turgor because appressoria treated with HU show runaway turgor and are unable to repolarize and cause infection. It is only when the nucleus passes into G2/M that the penetration peg elongates and breaches the cuticle of the leaf to start rice infection.

A morphogenetic checkpoint during appressorium-mediated plant infection by *M. oryzae*

The concept of morphogenetic checkpoint was introduced in 1995 by Lew and Reed in their analysis of the control of budding in *Saccharomyces cerevisiae*. Checkpoints are regulatory pathways that ensure correct cell cycle progression in coordination with, in this case, cellular morphogenesis.[19] When perturbation of the cell cycle occurs, morphogenesis stops to provide the cell with time to recover. The opposite also happens and when cells are exposed to any perturbation during development, the cell cycle arrests allowing time for recovery to prevent cellular catastrophe. Therefore, bidirectional signalling communication occurs between morphogenetic components and cell cycle machinery in the cell. For example, cells of *S. cerevisiae* stop the cell cycle and budding in the presence of the actin depolymerizing agent latrunculin.[20] There are now increasing reports showing that the DDR pathway plays a direct role in morphogenesis, because it has been shown, for example, that Rad53 interacts with septins and the inhibitory protein kinase Swe1, thereby connecting morphogenetic components with cell cycle control and the DDR response.[21,22] Swe1 plays an important role because it inhibits CDKs by phosphorylation to cause cell cycle arrest and prevent cellular catastrophe in response to incomplete DNA synthesis, bud formation failure, or other unfavourable conditions [19]. It has also been observed that an S-phase arrest caused by HU, no longer depends on the cell cycle checkpoint, but on extended accumulation of Swe1 as a result of failure in Swe1 degradation.[23] Progression into

mitosis and division of the nucleus depends on the sequential phosphorylation and degradation of Swe1.[24] A family of Nim1-related kinases promote Swe1 localization to the bud neck for degradation and cause progression of the cell cycle.[25,26] In *S. cerevisiae* there are three Nim1-related kinases Hsl1, Kcc4 and Gin4, and an adaptor protein called Hsl7.[25,26] All Nim1-related kinases localize to the bud neck in a septin-dependent manner, but they play different roles.[27,28] While Gin4 and Kcc4 act in coordination with the PAK kinase Cla4 and are required for septin re-organization, Hsl1 is directly involved in Swe1 degradation.[27] Hsl1 becomes activated when the septin cytoskeleton is properly organized.[29] The septins Cdc11 and Cdc12 activate Hsl1 directly and modify its activity to promote Hsl7 recruitment to the bud neck.[29] Activation of both Hsl1 and Hsl7 recruits Swe1 kinase to the bud neck where it is hyper phosphorylated and inactivated, releasing Clb-Cdc28 from inhibitory phosphorylation and promoting cell cycle progression into mitosis.[26] Therefore, null mutants in *HLS1* and *HSL7* prevent Swe1 localizing to the bud neck causing an arrest at G2/M.[25,30] *M. oryzae* also contains homologues of the morphogenetic checkpoint-associated components and cell cycle regulators and some of them have been already functionally analysed (Table 1). Interestingly, *M. oryzae* contains a Nim1-related kinase homologue, GIN4 (MGG_02810) and a protein arginine N-methlytransferase Hsl7 (MGG_03894) which have been found to be localized to the appressorium pore prior to plant penetration (Oses-Ruiz, M. and Talbot, N. J., unpublished). However, further investigation will be needed to determine the exact involvement of these proteins in appressorium morphogenesis.

By analogy to fungal model organisms, we hypothesize that penetration peg emergence represents an analogous process to the control of budding in *S. cerevisiae*, in which a morphogenesis checkpoint probably plays a key role to coordinate cell cycle progession with penetration peg development (Fig. 3). Interestingly, recent research suggests that accumulation of turgor is not only linked with cell cycle progression but also required for cytoskeleton re-organization at the base of appressorium (Ryder, L.S. and Talbot, N.J., unpublished). Turgor pressure is probably perceived by a sensor acting at the plasma membrane that signals to downstream components leading to cytoskeletal reorganization, cell cycle progression and triggering of morphogenesis checkpoint components. We propose that there is likely to be a triple association between turgor generation, cytoskeletal reorganization and cell cycle progression that is collectively required for appressorium-mediated plant penetration. We hypothesize that if any perturbation occurs to any one of these three factors, then this will generate an effect in the other two, leading to impairment in formation of a penetration peg and prevention of plant infection. The master regulator of these processes is likely to be the Swe1 kinase, which is an essential gene, but to determine its function will require its conditional

Table 1. List of orthologue cell cycle and morphogenetic related genes in *M. oryzae*.

Cell cycle associated proteins	*S. Cerevisae*	*M. oryzae*	accession #	References
Cyclin-dependent kinase				
Mitotic CDK	CDC28	CDC28	MGG_01362	
Cyclins				
G1-type cyclin	CLN3		MGG_03595	
B-type cyclin	CLB2	*CYC1*	MGG_05646	Saunders *et al.,* 2010;
Oses-Ruiz *et al.,* 2017				
B-type cyclin	CLB3	*CYC2*	MGG_07065	Saunders et al., 2010
CDK-activating kinases				
Cdk7-like CDK	KIN28		MGG_13401	
CDK-interacting proteins				
CDK regulatory subunit	CKS1		MGG_00682	Wang et al., 2017
CDK inhibitory phosphorylation				
CDK kinase	SWE1	*WEE1*	MGG_01816	
CDK phosphatase	MIH1		MGG_07734	
Polo kinase	CDC5		MGG_09960	
Mitotic associated kinases				
Serine/threonine protein kinase	KIN3	*NIMA*	MGG_03026	Veneault- Fourrey et al., 2006; Saunders et al., 2010
APC/C complex				
APC/C subunit	APC1	*BIM1*	MGG_03314	Saunders et al, 2010
APC/C subunit	APC2		MGG_04724	
APC/C subunit	CDC27		MGG_17195	
DNA replication				
DNA damage checkpoint regulator	DBF4	*NIM1*	MGG_00597	Saunders et al, 2010
Morphogenetic checkpoint				
Nim1- related kinases	HSL1/KCC4/GIN4	*GIN4*	MGG_02810	
	HSL7	*HSL7*	MGG_03894	
p21-activated kinase (PAK) kinase	CLA4	*CHM1*	MGG_06320	

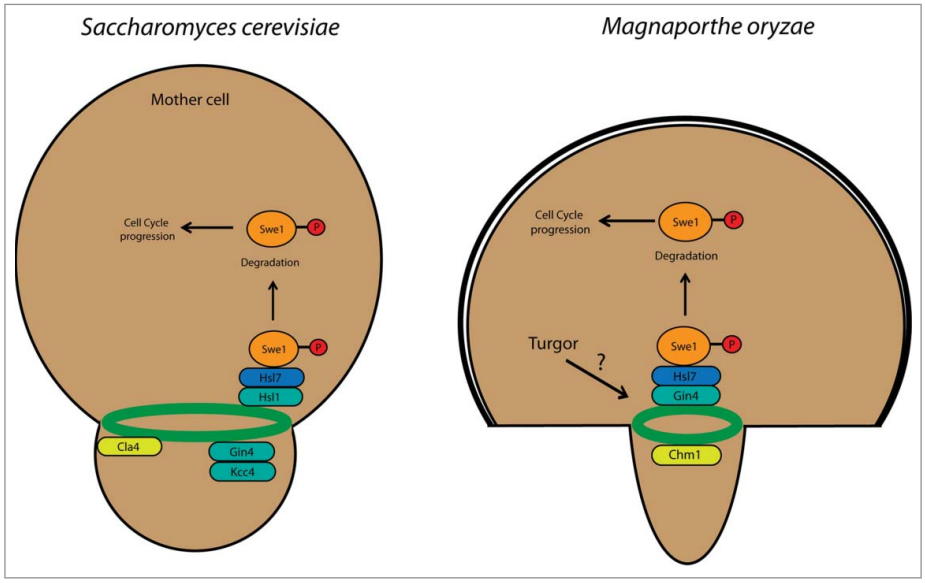

Figure 3. Model to show comparison of the control of budding in *S. cerevisiae* and penetration peg development in *M. oryzae* by operation of a morphogenesis checkpoint. A septin toroidal ring structure is formed both at the bud site in yeast and at the appressorium pore in *M. oryzae*. Septin recruitment depends on the PAK kinase ScCla4 and MoChm1 (yellow). In *S. cerevisiae*, Nim1-related kinases ScKcc4 and ScGin4 are required for septin re-organization at the bud neck. The Nim1-related kinase ScHsl1 (green) is recruited to the septin ring to associate with Hsl7 (blue) and promote ScSwe1 (orange) phosphorylation. Hyperphosphorylated Swe1 is triggered for degradation and allows cell cycle progression. In *M. oryzae* MoGin4 and MoHsl7 localize to the appressorium pore but the association and the mechanism by which cell cycle progression coordinated with turgor has not yet been revealed.

inactivation and/or conditional expression of activated versions of Swe1.

After formation of the appressorium, once the single nucleus has passed into G1, melanin accumulation is triggered and a turgor threshold accumulates in the appressorium. This turgor threshold triggers progression into S-phase and probably also, the recruitment of septins and cytoskeletal components to the appressorium pore. During S-phase, appressorium turgor increases, while septins re-orientate a toroidal F-actin ring structure at the base of the appressorium to recruit polarity and secretory components required for penetration peg development. When the F-actin toroidal ring is formed at the appressorium pore and maximal turgor is reached, morphogenetic checkpoint components are also likely to be recruited to the pore to signal to downstream cell cycle components for progression into G2 at the same time as penetration peg development to breach the rice leaf cuticle. Once the peg has elongated, the appressorium nucleus then progresses into M phase, divides, and this leads to formation of the primary invasive hypha and colonization of host tissue. It is also likely that subsequent differentiation of invasive hyphae, also requires cell cycle-dependent control. Appressorium turgor control, cytoskeletal organisation state and cell cycle progression all serve therefore as the critical input and output signals to facilitate coordinated development and successful plant infection by the rice blast fungus.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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