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

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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.

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Introduction

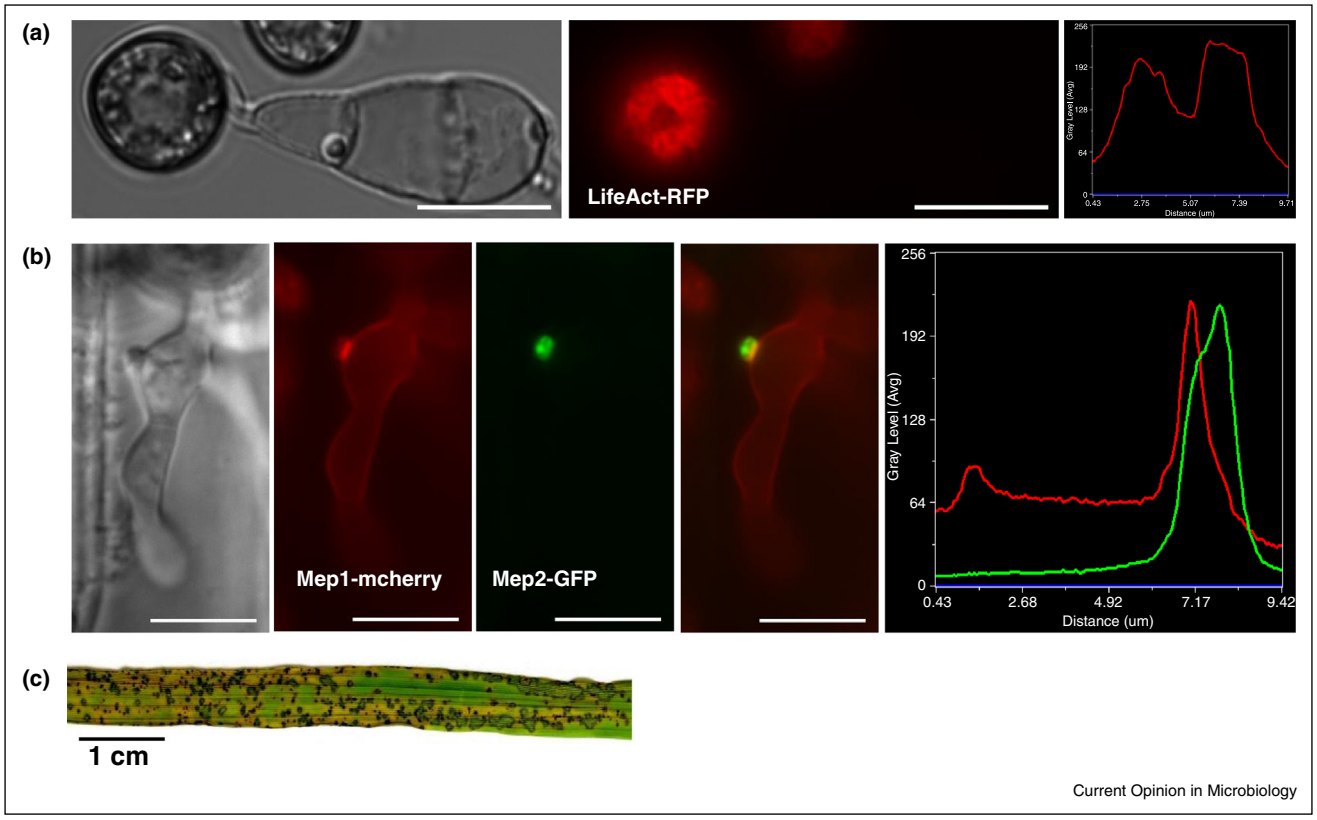
Rice blast disease is one of the most serious problems affecting cultivated rice worldwide. The disease occurs wherever rice is grown and recurrent epidemics occur on a regular basis. In the last 5 years alone, rice blast epidemics have struck many rice-growing regions, including Kenya [1], Italy [2] 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