

- 42 Lipscombe, T. *et al.* (1994) *J. Wildl. Dis.* 30, 567–571
 43 Lipscombe, T. *et al.* (1994) *J. Wildl. Dis.* 30, 572–576
 44 Rima, B.K. *et al.* (1987) *J. Gen. Virol.* 68, 1723–1735
 45 Rima, B.K. *et al.* (1990) *Res. Vet. Sci.* 49, 114–116
 46 Curran, M.D. *et al.* (1990) *Vet. Rec.* 127, 430–431
 47 Visser, I.K.G. *et al.* (1990) *Arch. Virol.* 111, 149–164
 48 Carter, S.D. *et al.* (1990) *J. Zool.* 222, 391–398
 49 Visser, I.K.G. *et al.* (1989) *Vaccine* 7, 521–526
 50 Duncan, R.J.S. (1988) in *ELISA and other Solid Phase Immunoassays* (Kemeny, D.M. and Challacombe, S.J., eds), pp. 30–54, John Wiley & Sons
 51 Romero, C.H. *et al.* *Virology* (in press)
 52 van Binnendijk, R.S. *et al.* (1993) *J. Virol.* 67, 2276–2284
 53 Rossiter, P.B. *et al.* (1987) *Vet. Rec.* 120, 459–461
 54 Appel, M.J. (1987) in *Infections of Carnivores* (Appel, M.J., ed.), p. 133, Elsevier
 55 Hammond, P.S., McConnell, B.J. and Fedak, M.A. (1993) *Symp. Zool. Soc. London* 66, 211–224
 56 Thompson, P.M. *et al.* (1989) *J. Appl. Ecol.* 26, 521–525
 57 Plowright, W. (1982) *Symp. Zool. Soc. London* 50, 1–29
 58 Zwanenburg, K.C.T. and Bowen, W.D. (1990) in *Population Biology of Sealworm (Pseudoterranova decipiens) in Relation to its Intermediate and Seal Hosts (Canadian Bulletin of Fisheries and Aquatic Sciences, no. 222)* (Bowen, W.D., ed.), pp. 185–197, Dept of Fisheries and Oceans, Canada
 59 Grenfell, B. and Dobson, A., eds *The Ecology of Infectious Disease in Natural Populations*, Cambridge University Press (in press)

Having a blast: exploring the pathogenicity of *Magnaporthe grisea*

Nicholas J. Talbot

One of the great challenges faced by plant pathologists is to determine, at the molecular level, what allows a fungus to cause disease in its host plant. This is an ambitious objective because pathogenicity is such a complicated phenotype. Plant-pathogenic fungi generally live their whole lives on, or within, their host plants. 'Pathogenicity' therefore encompasses processes that are intrinsic to all stages of the life cycles of these fungi, including infection-related morphogenesis, host colonization, the derangement of host metabolism and the dissemination of the fungus to fresh hosts. Hence, when molecular-genetic studies identify single genes that are required for the pathogenicity of a fungus, they may well encode products that are totally unrelated in function, fulfilling roles in very diverse areas of cellular metabolism or development. This is an exciting prospect, but one that demands careful examination of the specialized features of plant-pathogenic fungi. It is clear, for example, that saprophytic fungi have many of the same plant-cell-wall-degrading enzymes as plant pathogens do, yet they cannot colonize living plants¹. Therefore, in defining what constitutes pathogenicity, it is important to identify what separates plant pathogens from saprophytes, both in terms of the diversity and the regulation of gene expression. This will be more informative than merely

The rice blast fungus *Magnaporthe grisea* has an exquisite level of pathogenic specialization, allowing it to infect and colonize rice, subvert the metabolism of the host and spread to new hosts. Through a combination of cytology and molecular-genetic analysis, a picture is gradually emerging of the many interlinked processes that are required for successful infection of the plant.

N.J. Talbot is in the Dept of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, UK EX4 4QG.
 tel: +44 1392 264673, fax: +44 1392 264668,
 e-mail: N.J.Talbot@cen.exeter.ac.uk

identifying genes that are essential for the completion of the life cycle of the organism, and may become increasingly necessary to place newly identified pathogenicity genes into the wider context of fungal parasitism.

With this thought very much in mind, this review examines the life cycle of a model plant-pathogenic fungus, *Magnaporthe grisea*². I describe what is known, and what is not known, about the cell and molecular biology of each

stage of the life cycle of the fungus.

The disease

Magnaporthe grisea (anamorph: *Pyricularia grisea*) is a filamentous ascomycete fungus that parasitizes over 50 grasses, including economically important crops, such as barley, wheat, rice and millet³. These hosts each support pathogenic forms of the fungus that are normally restricted to one or a small number of very closely related species⁴, but the organism is best known as the causal agent of rice blast disease.

Blast is the most serious disease of cultivated rice and is a serious problem in most rice-growing regions of the world. The *M. grisea*-rice interaction is a major model for understanding plant disease, largely because of its great economic importance, but also because of the genetic and molecular-genetic tractability of the fungus (for a review, see Ref. 5). However, it is the

degree of pathogenic specialization shown by the fungus, both morphogenetically and physiologically, that is the real lure to rice blast researchers.

Rice blast is a leaf spot disease that is characterized by large ellipsoid lesions on the surface of rice leaves. In older rice plants, the fungus spreads into the panicle (the inflorescence that holds the rice grain) causing neck blast symptoms that can result in complete loss of the rice crop. *Magnaporthe grisea* is an ascomycete, and it is the possession of a sexual stage that allows genetic analysis of the pathogen in the laboratory. In the field, however, the fungus reproduces asexually by producing conidia. These three-celled spores are produced from lesions during periods of high humidity and are carried to new hosts by wind or splash dispersal. Once the conidium lands on a rice leaf, the remarkable life cycle of *M. grisea* begins.

The first stages of plant infection

The conidia of *M. grisea* do not remain viable for long periods and so have to attach to a rice leaf as quickly as possible to infect. Rice leaves are coated with a waxy cuticle⁶ making them extremely hydrophobic and therefore a potentially hostile environment for a microorganism to colonize. Rice has one of the most hydrophobic surfaces of all plants, having contact angles of up to 170° with a 10 µl drop of water (N.J. Talbot and H. Wösten, unpublished). In comparison, the contact angles of broad-bean leaves are 29–60° (Ref. 7), while artificial surfaces, such as Teflon, have contact angles of 90–130°. *Magnaporthe grisea* conidia stick to the repellent rice surface by releasing an adhesive from a compartment in the spore apex⁸. The release of this mucilage is triggered by wetting of the conidium (Table 1 shows a time course of events).

Once attached, the spore quickly germinates and sends out a short germ tube. This process is not contact-mediated, as conidia will also germinate in suspension⁹. Conidial germination requires the breakdown of the energy source of the spore. *Magnaporthe grisea* conidia are thought to accumulate the polyol mannitol as a storage product, which can form up to 10% of the dry weight of a conidium. Mannitol is not metabolized directly, but is first oxidized to fructose by a mannitol-phosphate dehydrogenase¹⁰. The germ tube normally emerges from one of the terminal cells of the three-celled spore and, like all fungal hyphae, grows by apical extension. It appears, however, to be modified for surface perception, including the positioning of apical vesicles close to the substrate ('nose down') and the flattening of the germ tube while it is in contact with the leaf¹¹. Within 4 h, the germ tube stops growing at the tip and a terminal 'hooking' of the hypha appears, which marks the beginning of the cellular differentiation that is required for infection of the plant.

Appressorial development

Appressorial development (see Box 1) proceeds by a complex morphogenetic sequence. Most knowledge of this process is based on cytological observations, but molecular-genetic studies have been initiated that are beginning to identify genes involved in the devel-

opment of these cells^{11–13}. The initial steps in appressorial formation are the swelling of the tip of the germ tube and a linked mitotic division, resulting in two daughter nuclei. One of these nuclei migrates into the incipient appressorium, while the other returns to the cell of the conidium from which the mother nucleus originated¹¹. A septum then forms to complete the cell division. The cell wall of the appressorium then begins to thicken by forming an outer fibrillar layer over the existing wall and by the addition of an inner-wall layer of melanin. This layer increases in thickness up to the time of penetration, but is completely absent at the point where the cell contacts the rice leaf. The appressorium sticks to the rice leaf using a thick layer of adhesive material that partially infiltrates the underlying rice cuticle, and then inflates to its full potential (Fig. 1). This coincides with the disappearance of a large number of glycogen rosettes that are visible within the cytoplasm of the cell¹¹. The process is completed with the emergence of a penetration peg at the interface that ruptures the cuticle and enters the epidermis of the rice leaf.

Multiple external signals appear to regulate appressorial morphogenesis¹⁴, although it is clear that surface hardness⁹ and hydrophobicity¹⁵ are important factors in inducing appressorial formation. Appressoria can be induced readily, for example, on surfaces such as Teflon or wax-coated plastics^{8,12} (see Fig. 1d). How the inductive signals are perceived is not yet clear, although there is some evidence that a cyclic-AMP-dependent (cAMP-dependent) signal transduction pathway may be involved because exogenous application of cAMP can induce appressorial formation even on poorly inductive surfaces, such as glass¹⁶.

Two independent lines of research suggest that *M. grisea* appressoria mechanically break the surface of the rice leaf. First, penetration pegs from *M. grisea* appressoria can puncture biologically inert plastic membranes¹⁷. Second, gene-disruption experiments have shown that the *CUT1* gene encoding cutinase, an enzyme that degrades the major component of plant cuticles, is dispensable for full pathogenicity¹⁸.

The appressorium produces the force to break the rice cuticle by generating an enormous internal turgor pressure (see Box 1). This has been measured indirectly and estimated to be as high as 8.0 MPa (Ref. 17). These high appressorial turgor pressures are necessary for the penetration of artificial membranes, such as Mylar [poly(ethylene terephthalate) fibre]¹⁷. Reducing the turgor pressure, which can be done by increasing the external osmotic pressure, prevents penetration of these membranes. Rupture of the surface of the rice leaf can be inhibited similarly.

The internal turgor pressure is believed to be generated by an influx of water caused by the osmotic gradient produced in the cell. The appressorial wall is freely permeable to water, but the efflux of any larger molecules from the cell is blocked by the presence of the melanin layer, which significantly reduces the porosity of the appressoria^{11,17}. The importance of this layer in appressorial function has also been shown genetically. Melanin is a polyketide and its biosynthesis

Table 1. Time course of the major events during pathogenesis by *Magnaporthe grisea*, with speculations about the underlying gene activities required

Time (h)	Event ^a	Metabolic activity/ gene activity recorded ^b	Potential gene action
0	Conidia land on rice surface Spore-tip mucilage released – spore adhesion		Dormancy-release signal
0.5–1.5	Conidia germinate Elongation of the germ tube	Mannitol-phosphate- dehydrogenase activity	Establishment of polarity
2–4	Tip growth ceases Hook formation/apical expansion Mitosis, nucleus migrates into appressorium Septum forms at base of appressorium Bottom cell wall of appressorium thins New cell-wall layers form Membrane cisternae and vesicles abundant		Surface perception Depolarization signal (<i>SMO1?</i> <i>CON1?</i>) Mitotic-induction signal (linked to cell cycle?) Septation signal (linked to mitotic signal?) Cell-wall synthesis Cyclic-AMP-dependent-kinase activity?
4–8	Melanization begins Membrane cisternae and vesicles absent Appressorium pore becomes well defined	<i>ALB1</i> , <i>RSY1</i> and <i>BUF1</i> required for melanization	Reorientation of growth plane to delimit appressorial pore (<i>SMO1?</i>)
16–24	Granular substance (possibly an adhesive) accumulates at interface with rice leaf Glycogen rosettes abundant Pore ring is clearly seen under rim of appressorium	<i>MPG1</i> transcript detected (18 h)	Tight adhesion of appressorium to rice leaf (<i>MPG1?</i> , hydrophobins?, mucilages?) Osmoregulatory signal transduction cascade
24–31	Formation of pore-wall overlay Glycogen rosettes nearly absent Greatest recorded appressorial turgor pressure (26–46 h) Emergence of penetration peg	Actin present at penetration peg	Osmolyte accumulation (a metabolite of glycogen: glycerol?, mannitol?) Re-establishment of polarity Cell-wall-degrading enzymes?
31	Infection hyphae form		
48	Bulbous secondary hyphae form Spread to adjacent epidermal cells		Dimorphism to determinate growth pattern Assimilative phase begins Sugar transport? Extracellular enzymes?
72	Spread into mesophyll cells Some browning and autofluorescence of mesophyll cells often seen 10% of total biomass is fungal		Cell-wall-degrading enzymes? Detoxification of rice secondary metabolites?
96	Lesions are visible to naked eye on rice leaves Longer hyphae visible at lesion margins Conidiation occurs under conditions of very high humidity	Tenuazonic-acid activity detected in leaves <i>MPG1</i> transcript abundant	Phytotoxin-biosynthetic activity? Idiophasic gene expression? Conidiation regulatory genes
144	Lesions begin to coalesce Hyphae fill leaf tissue Conidiation occurs under conditions of very high humidity	Mannitol, arabinol and trehalose found in leaves Acid-invertase activity detected; glucose and fructose levels increase	

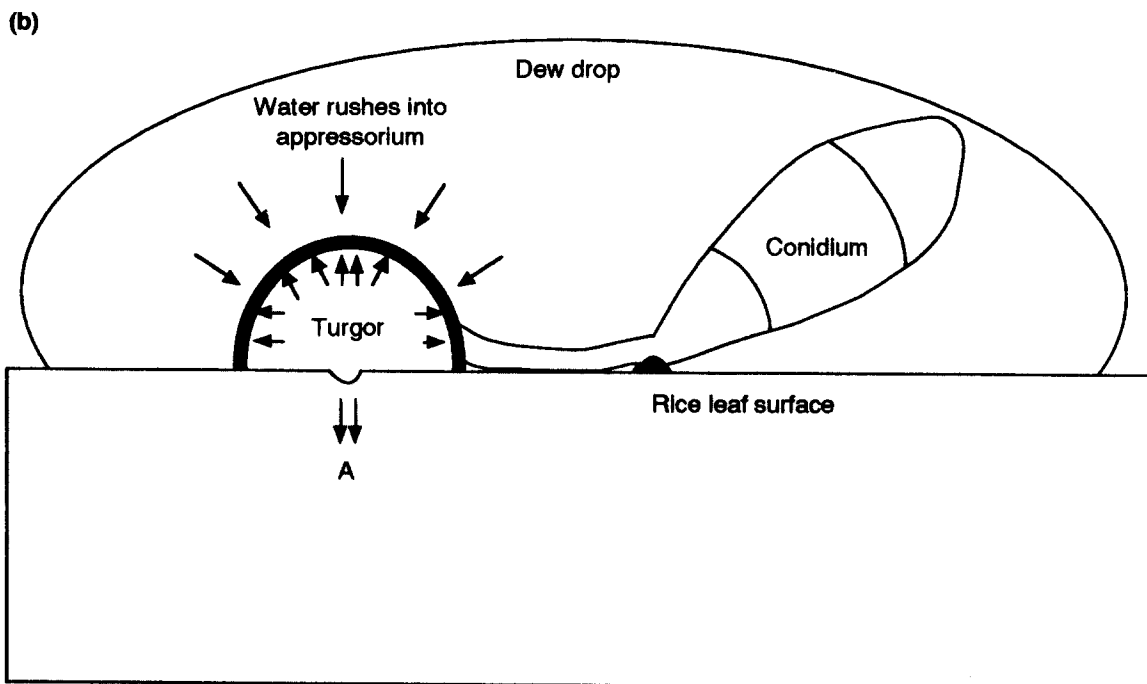
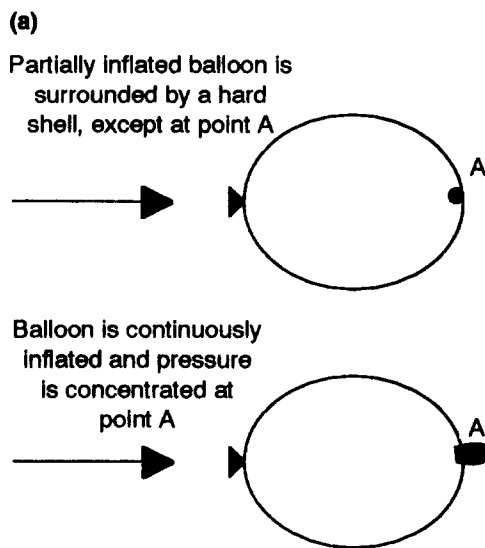
^aFrom Refs 10,30,31,32.

^bFrom Refs 10,33,35.

Box 1. Appressoria

Fungi are unique among the plant pathogens in producing specialized cells to infect their hosts. The production of these cells, appressoria, is common among ascomycete and basidiomycete plant pathogens^{43,44}, but also occurs in distantly related groups, such as the oomycetes⁴⁵ and the entomopathogenic fungi⁴⁶. Appressoria can have various shapes, but, in general, they are swollen, round or dome-shaped structures that form at the ends of fungal germ tubes. They are used to penetrate the cuticles of leaves, stems or roots, or natural plant openings, such as stomata, and their formation can be triggered by precise thigmotropic responses that allow them to form in the correct position^{47,48}.

Infection by appressoria is brought about either by enzymatic degradation of the cuticle⁴⁹ or by mechanical rupturing of the plant surface. As the name appressorium suggests, the cells often have high internal pressures that generate mechanical force. A useful analogy to describe the potential mechanism of infection by the appressorium is a balloon surrounded by a rigid papier-mâché covering, apart from one small round opening (a). If the balloon is inflated continuously, then its only means of expansion is through the small opening. This is an effective means of concentrating a very high pressure in a tiny area (point A), and may be similar to how appressoria break the tough cuticles of plant cells. But how is this pressure built up in a biological system? The mechanism is still largely unclear (see text), but water is thought to rush into the appressorium against an osmotic gradient. This gradient is probably generated by the appressorium accumulating a solute that cannot escape from the cell because of the low porosity of the appressorial wall. This concentrates the internal pressure at the infection point (b), until a penetration peg is forced into the plant.



in *M. grisea* proceeds via the polymerization of 1,8-dihydroxynaphthalene. Three genes within this biosynthetic pathway have been identified so far by selecting colour mutants that lack the grey melanin pigment^{19,20}. These mutants were albino, rosy or buff coloured and so the corresponding genes were named *ALB1*, *RSY1* and *BUF1*. These genes are essential for appressorial function because strains carrying mutations at any one of

these loci make completely nonfunctional appressoria²¹, and the mutants are consequently nonpathogenic.

Three other genes have been implicated in appressorial development. The first, *SMO1*, was identified as a mutation affecting conidial shape²² (Fig. 2). It appears to play diverse roles in conditioning the shape of conidia, asci and appressoria. Because all these structures develop from hyphal tips, it has been suggested that

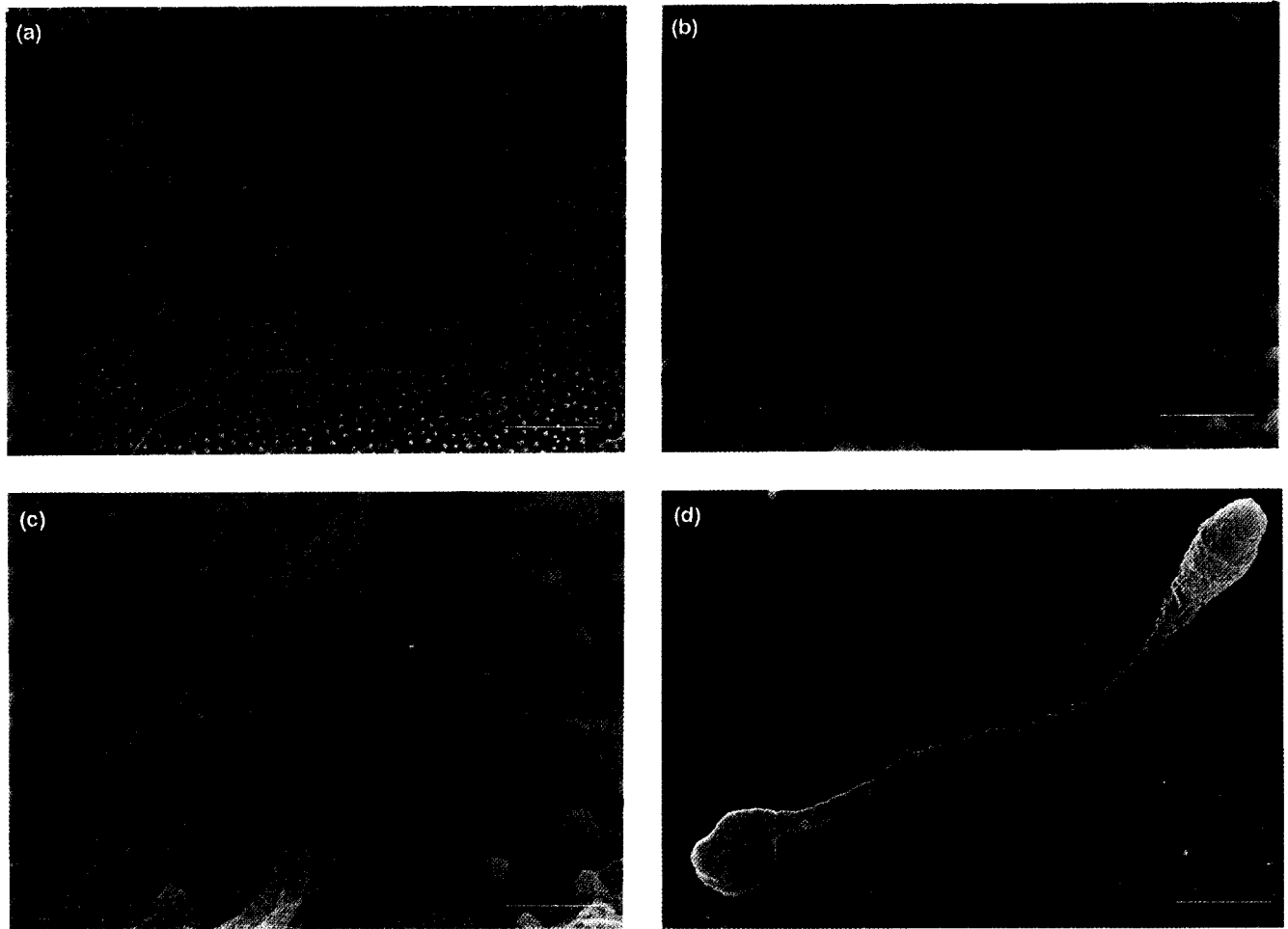


Fig. 1. Infection-related morphogenesis in *Magnaporthe grisea*. **(a)** Scanning electron micrograph of the surface of a rice leaf 14 h after inoculation with conidia from a compatible strain of *M. grisea*. The conidia have germinated and elaborated appressoria. Scale bar = 50 μm . **(b)** Detail of an appressorium on the surface of a rice leaf. The waxy uneven cuticle is clearly visible. An appressorium is present in the foreground and a collapsed conidium in the background. Scale bar = 10 μm . **(c)** Detail of an appressorium showing the tight adhesion of the germ tube to the rice surface. A conidium has attached to a leaf hair and a flattened germ tube has formed before elaboration into the appressorium, which sticks to the surface. A layer of adhesive material can be seen at the base of the appressorium. Scale bar = 10 μm . **(d)** Induction of appressorial formation on an artificial surface. The figure shows a detail of an appressorium formed on a hydrophobic Teflon membrane inoculated with a conidial suspension and incubated for 14 h. Scale bar = 10 μm .

the product of the *SMO1* gene may be involved in the depolarization signal that is required for cellular differentiation from the indeterminate hyphal growth form²². *Smo* mutants produce misshapen appressoria from very long germ tubes, which can also form on non-inductive surfaces, such as glass. Interestingly, *smo1*⁻ mutants have significantly reduced pathogenicity on rice²³, showing that the misshapen appressoria are less effective at infecting the plant. A similar gene affecting conidial shape, *CON1*, also affects the ability of strains to make appressoria. In this case, however, the *con1*⁻ mutants produce long hyphal-like indeterminate conidia, and the emerging germ tubes have no appressorial differentiation, even on inductive surfaces²⁴. This mutation could also be involved in the depolarization signals that are a prerequisite to cell differentiation from hyphae.

The third gene, *MPG1*, is expressed at high levels during appressorial formation, and *mpg1*⁻ null mutants

have a greatly reduced ability to make appressoria¹³. Instead, conidia produce very long germ tubes that undergo periodic swelling and hooking, but do not elaborate fully (Fig. 3). Where appressoria do form, they are misshapen. The product is predicted from the sequence to be a fungal hydrophobin. Hydrophobins are a recently discovered class of proteins that are involved in the development of a variety of fungal structures, including mushrooms and conidia²⁵⁻²⁸. The characteristics of these proteins give clues to the function of the product of *MPG1*: hydrophobins are small, secreted hydrophobic proteins. The only one that has been completely purified so far is the product of the *Sc3* gene from *Schizophyllum commune* (*Sc3p*)²⁹; this led to the remarkable discovery that the hydrophobin assembles into an amphipathic protein monolayer structure at a gas-water interface²⁹. This structure self-assembles on the surface of aerial hyphae and is responsible for their hydrophobicity³⁰. Appressorial

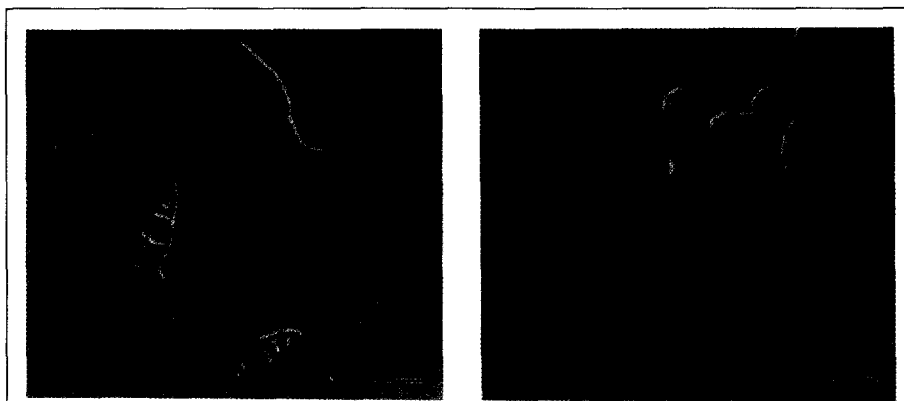


Fig. 2. The *Smo* mutant phenotype in *Magnaporthe grisea*. Differential interference light micrographs of conidia from (a) wild-type *M. grisea* strain (Guy-11) and (b) a *smo1*⁻ mutant (4395-1-2). *Smo* mutants have misshapen conidia with reduced polarity and different planes of cell division. The mutation also affects ascus and appressorial development. Scale bar = 10 μ m.

morphogenesis in *M. grisea* occurs in the aqueous environment of dew-soaked rice leaves, which suggests that, if the product of the *MPG1* gene is secreted and acts in the same way as *Sc3p*, the *MPG1*-encoded protein may self-assemble at the surface of the rice leaf and act as an adhesion protein. This tight adhesion may therefore be an important signal for appressorial morphogenesis.

Growth within the plant

Research into the nature of fungal growth within the plant has been based solely on cytology and physiological measurements. Clearly, however, a large number of genes are involved. Crosses between pathogens of different compatibility (races) towards specific rice cultivars³¹ identified several polygenic factors that affect both the extent of lesion development and the severity of disease symptoms, suggesting that large combinations of genes are probably required for successful colonization of plant tissues.

Cytological examination shows that penetration pegs rupture the plant cuticle after 28–31 h, breaking

of the fungus in culture, and may represent a unique *in planta* assimilatory phase of development. The fungal hyphae grow quickly throughout the rice leaf, tending to narrow as they traverse from cell to cell and, by as early as 72 h after the original inoculation, up to 10% of the biomass of an infected rice leaf is fungal material¹³. Measurement of sugar fluxes at this time shows that the polyols mannitol and arabitol appear specifically in blast-infected leaves, presumably as fungal storage compounds. A high level of invertase activity and a corresponding flux of glucose and fructose levels also occurs within leaves³⁵. The first obvious disease symptoms now occur.

Disease symptoms

Small oval lesions begin to appear and are accompanied by local chlorosis (chlorophyll deficiency) (Fig. 4b). The lesions grow and become necrotic before coalescing. Clearly, plant cells die during this process, but it is far from obvious how the fungus brings about this cell death. *Magnaporthe grisea* can produce a large number of phytotoxic substances in culture, including pyricularine, pyricularic acid, coumarin, pyrichalasin H, pyricularol, pyriculariol, tyrosol, picolinic acid and tenuazonic acid⁵. *Magnaporthe grisea* culture filtrates also contain a heat-labile phytotoxic activity (perhaps an endoxylanase) that kills rice-suspension-cell cultures by solubilizing ferulated arabinoxylan fragments from rice-cell walls³⁶. The importance of these phytotoxins in pathogenesis, however, has yet to be shown, and only tenuazonic acid is known to be present in diseased leaves at toxic levels³⁷.

Dissemination to new hosts

Conidiation of the large coalescing lesions occurs only when the relative humidity exceeds 93%. During these periods, lesions can produce



Fig. 3. The *Mpg1* mutant phenotype in *Magnaporthe grisea*. Phase-contrast light micrographs of appressorial development in (a) a wild-type *M. grisea* strain (Guy-11) and (b) an *mpg1*⁻ mutant (53-R-39). *Mpg1* mutants have a reduced ability to elaborate appressoria and often produce long, swollen germ tubes on inductive surfaces. Scale bar = 15 μ m.

2000–6000 conidia each night for about 2 weeks. During sporulation, lesions take on a grey mould-like appearance at the centres. Several genes are known to be involved in conidiation of *M. grisea* and, interestingly, these include those genes that have been shown to affect appressorial formation. Both *smo1*⁻ and *con1*⁻ mutants, for example, produce misshapen conidia, while *mpg1*⁻ mutants produce significantly fewer conidia than do wild-type strains¹³.

Pathogenicity functions

What is known

The molecular-genetic analysis of pathogenicity in *M. grisea* is still in its infancy, but has already identified important components of the pathogenic process in the fungus. The principal observation so far is that the genes that have been found to be involved in appressorial formation appear to have pleiotropic effects on conidiation and/or ascus formation. This is due, in part, to the method by which mutants are selected, particularly in the case of *smo1*⁻ and *con1*⁻ mutants, but it also suggests that these developmental processes are all related. This is perhaps not surprising, as all fungal cellular differentiation events require a departure from the hyphal form. The first stage in appressorial development, for example, requires the cessation of polarized growth and radial expansion of the hyphal tip. This is in common with the initial differentiation of conidiophores into phialides (hyphal outgrowths) and then to mature conidia³⁸. Many of the genetic components of the developmental pathway that leads to conidiation in fungi have been identified and characterized³⁸, and it may be interesting to see whether any of these genes is involved in appressorial development.

What is not known

Clearly there is a great deal that is unknown about pathogenesis. The key area of ignorance about infection-related morphogenesis remains the molecular biology of appressorial action. While the development of these cells is similar to that of other types of fungal cell, their mode of action is unique, requiring the generation of an enormous turgor pressure. Current evidence suggests that an osmoregulatory pathway triggering the accumulation of a specific, metabolically compatible solute is central to this process. Osmoregulation in fungi has been studied in most detail in budding yeast, which accumulates glycerol as an osmolyte. The accumulation of glycerol in yeast is triggered by a mitogen-activated-protein-kinase cascade^{39,40} that is controlled by an osmotic sensor that monitors external osmotic potential. This sensor is similar to the two-component regulators from prokaryotic systems⁴¹. It is not yet clear which solute is accumulated by *M. grisea*, although electron microscopy suggests it is a metabolite of glycogen (perhaps mannitol or glycerol)¹¹. The accumulation of the solute may, however, be regulated similarly to in yeast, although with two important differences. First, the osmoregulatory pathway is almost certainly uncoupled from an external osmotic sensor, being triggered instead by a thigmo-

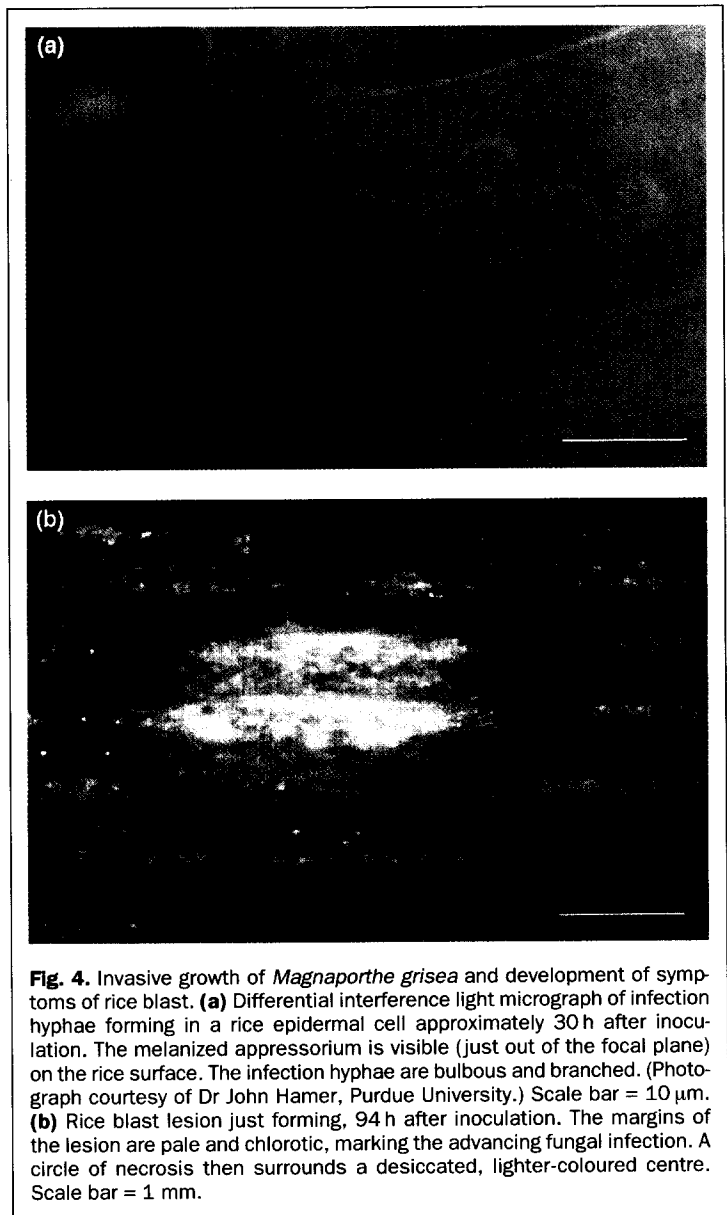


Fig. 4. Invasive growth of *Magnaporthe grisea* and development of symptoms of rice blast. **(a)** Differential interference light micrograph of infection hyphae forming in a rice epidermal cell approximately 30 h after inoculation. The melanized appressorium is visible (just out of the focal plane) on the rice surface. The infection hyphae are bulbous and branched. (Photograph courtesy of Dr John Hamer, Purdue University.) Scale bar = 10 μ m. **(b)** Rice blast lesion just forming, 94 h after inoculation. The margins of the lesion are pale and chlorotic, marking the advancing fungal infection. A circle of necrosis then surrounds a desiccated, lighter-coloured centre. Scale bar = 1 mm.

tropic response to adhesion at a hydrophobic surface. Second, the pathway must be interlinked to the control of cell-wall and polyketide biosynthesis, while perhaps also being negatively regulated during the re-establishment of polarity after infection. Identifying the osmolyte and understanding how its accumulation is regulated are therefore priorities for understanding the action of appressoria.

Very little is known currently about the physiology of invasive growth of the fungus or, indeed, how it brings about the symptoms of the disease. There are two obvious reasons for this. First, most physiological studies of the fungus have been done *in vitro* and, therefore, there is no clear idea of the importance in pathogenesis of the aspects studied. Second, genetic assays for defects in pathogenicity have so far identified genes that are involved in events early in infection. This is not only a consequence of the complexity of infection, but is also because the mutation

must affect appressorial formation to give a non-pathogenic phenotype as, once the fungus enters the plant, it invariably forms a lesion of some sort, even in an incompatible interaction³¹. Therefore, identifying genes that are involved in host colonization will be more difficult because of problems in assaying the phenotype accurately. This is exacerbated by environmental factors, such as temperature, humidity, leaf age and even nitrogen levels, which can all affect lesion size³. This analysis will therefore require a more accurate and reproducible measure of fungal biomass *in planta*^{13,42}, to identify more subtle effects on pathogenesis.

Conclusion

The degree of pathogenic specialization of *M. grisea*, coupled with its amenability to analysis, means that study of this fungus gives an exciting opportunity to dissect the 'pathogenicity' phenotype. Progress is clearly being made by a combination of 'phenotypically driven' studies to recognize genes affecting pathogenicity and differential cDNA screens to survey stage-specific gene expression. The challenge ahead will be not only to identify the large number of likely pathogenicity determinants, but also to ask the more difficult question: is each determinant a unique adaptation of plant pathogens, or merely a fitness characteristic of the fungus? Answering this is necessary to generating a clearer overall concept of what constitutes pathogenesis and what really separates plant-pathogenic fungi from their saprophytic relatives.

Acknowledgements

I am very grateful to John Hamer, Kathy Dobinson, Steve Harris, Verel Shull, Gee Lau (all presently or formerly of Purdue University) and Barbara Valent (DuPont Company) for many stimulating discussions about the nature of pathogenicity in the rice blast fungus. I am also grateful to Jos Wessels and his group (University of Groningen) for enlightening me further about the hydrophobins. I acknowledge gratefully Steve Aves (University of Exeter), Steve Harris (University of Connecticut) and Nick Read (University of Edinburgh) for reading the manuscript critically and Howard Stebbings and Gavin Wakley (University of Exeter) for help with microscopy.

References

1 Dean, R.A. and Timberlake, W.E. (1989) *Plant Cell* 1, 265-273
 2 Valent, B. (1990) *Phytopathology* 80, 33-36
 3 Ou, S.H. (1985) in *Rice Diseases* (2nd edn), pp. 109-201, Commonwealth Mycological Institute
 4 Mackill, A.O. and Bonman, J.M. (1986) *Plant Dis.* 70, 125-127
 5 Valent, B. and Chumley, F.G. (1991) *Annu. Rev. Phytopathol.* 29, 443-467
 6 Uchiyama, T. and Okuyama, K. (1990) *Phytochemistry* 29, 91-92
 7 Fogg, G.E. (1947) *Proc. R. Soc. London Ser. B* 135, 503-552
 8 Hamer, J.E. *et al.* (1988) *Science* 239, 288-290
 9 Xiao, J.-Z. *et al.* (1994) *Physiol. Mol. Plant Pathol.* 44, 227-236
 10 Yamada, H. *et al.* (1961) *J. Biochem. (Tokyo)* 49, 404-410
 11 Bourett, T.M. and Howard, R.J. (1990) *Can. J. Bot.* 68, 329-342
 12 Lee, Y-H. and Dean, R.A. (1993) *Exp. Mycol.* 17, 215-222
 13 Talbot, N.J., Ebbole, D.J. and Hamer, J.E. (1993) *Plant Cell* 5, 1575-1590
 14 Jellito, T.C., Page, H.A. and Read, N.D. (1994) *Planta* 194, 471-477
 15 Lee, Y-H. and Dean, R.A. (1994) *FEMS Microbiol. Lett.* 115, 71-75

16 Lee, Y-H. and Dean, R.A. (1993) *Plant Cell* 5, 693-700
 17 Howard, R.J. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 11281-11284
 18 Sweigard, J.A., Chumley, F.G. and Valent, B. (1992) *Mol. Gen. Genet.* 232, 183-190
 19 Woloshuk, C.P. *et al.* (1980) *Pestic. Biochem. Physiol.* 14, 256-264
 20 Chumley, F.G. and Valent, B. (1990) *Mol. Plant-Microbe Interact.* 3, 135-143
 21 Howard, R.J. and Ferrari, M.A. (1989) *Exp. Mycol.* 13, 403-418
 22 Hamer, J.E., Valent, B. and Chumley, F.G. (1989) *Genetics* 122, 351-361
 23 Hamer, J.E. and Givan, S. (1990) *Mol. Gen. Genet.* 223, 487-495
 24 Shi, Z. and Leung, H. (1994) *Mol. Plant-Microbe Interact.* 7, 113-120
 25 Stringer, M.A. *et al.* (1991) *Genes Dev.* 5, 1161-1171
 26 St Leger, R.J., Staples, R.C. and Roberts, D.W. (1992) *Gene* 120, 119-124
 27 Wessels, J.G.H. (1992) *Mycol. Res.* 96, 609-620
 28 Wessels, J.G.H. *et al.* (1991) *Plant Cell* 3, 793-799
 29 Wosten, H.A.B., de Vries, O.M.H. and Wessels, J.G.H. (1993) *Plant Cell* 5, 1567-1574
 30 Wosten, H.A.B. *et al.* (1994) *Eur. J. Cell Biol.* 63, 122-129
 31 Valent, B., Farrall, L. and Chumley, F.G. (1991) *Genetics* 127, 87-101
 32 Bourett, T.M. and Howard, R.J. (1992) *Protoplasma* 168, 20-26
 33 Heath, M.C. *et al.* (1990) *Can. J. Bot.* 68, 1627-1637
 34 Heath, M.C. *et al.* (1992) *Can. J. Bot.* 70, 779-787
 35 Hwang, B.Y., Kim, K.D. and Kim, Y.B. (1989) *J. Phytopathol.* 125, 124-132
 36 Bucheli, P. *et al.* (1990) *Physiol. Mol. Plant Pathol.* 36, 159-173
 37 LeBrun, M-H. *et al.* (1990) *Phytochemistry* 29, 3777-3783
 38 Timberlake, W.E. (1993) *Plant Cell* 5, 1453-1460
 39 Nisida, E. and Gotoh, Y. (1993) *Trends Biochem. Sci.* 18, 128-131
 40 Neiman, A.M. (1993) *Trends Genet.* 9, 390-394
 41 Maeda, T., Wurgler-Murphy, S.M. and Salto, H. (1994) *Nature* 369, 242-245
 42 Oliver, R.P. *et al.* (1993) *Mol. Plant-Microbe Interact.* 6, 521-525
 43 Mendgen, K. and Deising, H. (1993) *New Phytol.* 124, 193-213
 44 Staples, R.C. and Hoch, H.C. (1987) *Exp. Mycol.* 11, 163-169
 45 Hardham, A.R. (1992) *Annu. Rev. Plant Physiol. Mol. Biol.* 43, 491-526
 46 St Leger, R.J. *et al.* (1989) *Exp. Mycol.* 13, 274-288
 47 Hoch, H.C. *et al.* (1987) *Science* 235, 1659-1662
 48 Read, N.D. *et al.* (1992) in *Perspectives in Plant Cell Recognition* (Callow, J.A. and Green, J.R., eds), pp. 137-172, Cambridge University Press
 49 Kollatukudy, P. (1985) *Annu. Rev. Phytopathol.* 23, 223-250

Letters to the Editor

Trends in Microbiology welcomes correspondence. Letters, of not more than 500 words, may relate to topics raised in earlier issues of the journal (in which case they will be sent to the appropriate authors for review and to give them the opportunity to reply), or to other matters of general interest to microbiologists.