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ON THE TRAIL OF A CEREAL KILLER: Exploring the Biology of *Magnaporthe grisea*

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■ **Abstract** The blast fungus *Magnaporthe grisea* causes a serious disease on a wide variety of grasses including rice, wheat, and barley. Rice blast is the most serious disease of cultivated rice and therefore poses a threat to the world's most important food security crop. Here, I review recent progress toward understanding the molecular biology of plant infection by *M. grisea*, which involves development of a specialized cell, the appressorium. This dome-shaped cell generates enormous turgor pressure and physical force, allowing the fungus to breach the host cuticle and invade plant tissue. The review also considers the role of avirulence genes in *M. grisea* and the mechanisms by which resistant rice cultivars are able to perceive the fungus and defend themselves. Finally, the likely mechanisms that promote genetic diversity in *M. grisea* and our current understanding of the population structure of the blast fungus are evaluated.

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INTRODUCTION

Magnaporthe grisea (Hebert) Barr, the causal agent of rice blast disease (93, 102), displays remarkable morphogenetic and biochemical specialization to its pathogenic lifestyle and is an efficient and devastating agent of disease. Each year rice blast causes losses of between 10 and 30% of the rice harvest. The scale of the problem is well illustrated by a disease outbreak in Bhutan in 1995 (98). More than 700 hectares of rice were affected and led to losses of 1090 tonnes of rice. This was in spite of the fact that many diverse cultivars of rice exhibiting varying resistance levels were under cultivation (98) and should theoretically have limited the scale of an epidemic. In addition to rice, *M. grisea* causes disease on a wide variety of alternative hosts including agriculturally significant plants such as finger millet (*Eleusine coracana*)— an important food security crop in India and southern and east African countries—which provide nutrition and essential minerals such as calcium, phosphorus and iron to poor rural communities. Finger millet blast is a devastating disease that causes complete harvest loss when it occurs prior to grain formation (22). Blast disease of wheat is also an increasing problem and serious outbreaks have occurred in the northern Parana state of Brazil (40).

I begin this review by considering the tools developed to study *M. grisea*, without which the fungus would have remained as experimentally intractable as many fungal pathogens. Then, I describe a selection of recent studies that have begun to illuminate the mechanisms of plant infection by *M. grisea* and the biology of invasive growth. From there the review examines how resistant rice varieties perceive *M. grisea*, and actively defend themselves from attack, and the mechanisms by which genetic diversity is generated in the fungus. Finally, I outline future challenges that must be overcome in order to understand the biology of *M. grisea*. Inevitably with a review such as this, only a small selection of studies are referred to and there are gaps in the coverage of certain topics. Where possible I refer to review articles to bridge some of the gaps, but I apologize to readers whose favorite subjects are not covered as comprehensively as others I have chosen. My principal aim has been to give a flavor of the diversity and scope of research carried out on this fascinating organism.

TOOLS FOR STUDYING THE BIOLOGY OF *MAGNAPORTHE GRISEA*

A number of attributes have allowed *M. grisea* to emerge as a model phytopathogen (93). First and foremost has been the ability to culture the fungus away from its host plant in standard growth media (102), closely followed by the ability to carry out classical genetic analysis (52, 102, 103). *M. grisea* is a filamentous ascomycete fungus that is heterothallic. Two mating types of the fungus are present, *MAT1-1* and *MAT1-2*, and when fertile isolates carrying opposite mating types are paired together on an appropriate growth medium such as oatmeal agar at 20°C, they will

form sexual fruiting bodies called perithecia within 21 days (103). Perithecia are flask-shaped bodies that carry asci—bags containing ascospores, the products of meiosis—in abundance. Asci can be dissected to liberate the ascospores, which are arranged as unordered octads (four pairs of spores representing the products of meiosis that have undergone a subsequent mitotic division) or as larger populations of randomly selected ascospores. In either case the segregation patterns of genetic markers can be readily followed and the genetic basis of phenotypic traits determined (103). In nature the different host-limited forms of *M. grisea* show distinct differences in fertility (77, 102). Among isolates of *M. grisea*, rice pathogenic strains are predominantly infertile and only in rare instances have fertile strains been recovered from the field (52). A commonly studied strain of *M. grisea*, Guy11, a *MAT1-2* strain from French Guiana, has proved extremely valuable in this regard in a large number of genetic studies (60). A large amount of work was also carried out to generate laboratory strains of *M. grisea* that can be genetically crossed (23, 103). Fertility was introgressed into rice pathogenic forms of the fungus by successively back-crossing rice pathogenic isolates of *M. grisea* with those pathogenic toward weeping lovegrass (*Eragrostis curvularia*), or finger millet, which show greater fertility (103). As a result of these studies, a series of highly fertile laboratory strains of the fungus have been developed and made available to the international research community (23, 103).

M. grisea can be readily transformed using a number of selectable markers, including complementation of auxotrophic markers such as *argB*, or by introducing resistance to antibiotics such as hygromycin B, bleomycin, bialophos, and sulfonylurea (93, 102). Although transformation is not efficient (typically 40 transformants are generated per microgram of transforming DNA), the procedure is reliable and sufficient for most experiments. Enhanced transformation frequencies can be achieved using *Agrobacterium tumefaciens*–mediated transformation (82). Targeted gene replacement is widely used in *M. grisea* to study gene function, and vectors typically have 1 to 2 kb of flanking DNA on either side of a gene of interest. Homologous recombination replaces a gene of interest at a frequency of around 20% of transformants in *M. grisea*, although the process is highly locus dependent [for review see (93)]. Recent use of in vitro transposon mutagenesis with much larger flanking regions provides a means of carrying out much more efficient gene disruption at a high throughput (15, 33). Cell biological analysis of *M. grisea* is facilitated by the fact that the fungus can be manipulated away from the plant and induced to undergo its entire prepenetration phase of development—which involves production of a specialized infection cell, the appressorium—on plastic surfaces (7, 17, 31).

THE EARLY STAGES OF PLANT INFECTION

Rice blast infections are initiated when an asexual spore lands on the surface of a rice leaf and attaches itself to the cuticle by release of an adhesive found in an apical compartment of the spore (31). Conidiospores are carried from plant to plant

by dewdrops and the presence of free water is required for germination (7, 31). Spore germination is rapid in *M. grisea*, and within two hours of landing on the leaf, a polarized germ tube is formed. The germ tube normally emerges from one of the apical cells of the conidium and extends for only a short distance (15–30 μm) before swelling at its tip and changing direction while becoming flattened against the leaf surface (7). This process, known as hooking, precedes development of the appressorium and is thought to constitute a "recognition phase" of development in which the characteristics of the substratum are monitored before commitment to appressorium morphogenesis (7). Development of the appressorium requires a hard, hydrophobic surface and the absence of exogenous nutrients (17). The presence of soluble cutin monomers such as *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid or lipid monomers like 1,16-hexadecanediol also induces appressorium formation even on normally noninductive surfaces (28). This combination of signals leads to initiation of multiple signal transduction cascades that brings about terminal differentiation of the germ tube apex into an appressorium.

Cyclic AMP Signaling During Appressorium Formation

A cyclic AMP (cAMP) response pathway is believed to be triggered at an early stage of *M. grisea* germ tube elongation because Δ*mac1* mutants, which lack the enzyme adenylate cyclase, required for synthesis of cAMP, are unable to form appressoria and are consequently nonpathogenic (1, 12, 54). Addition of cAMP to Δ*mac1* mutants allows them to complete appressorium development and restores their pathogenicity, demonstrating the importance of this signal for morphogenesis. High concentrations of exogenously applied cAMP also induce appressorium formation in *M. grisea* on normally noninductive (hydrophilic) surfaces, emphasizing the significance of cAMP-mediated processes (12, 59). The cAMP signal may be generated in response to surface hydrophobicity or germ tube contact with a hard surface (17). During germ tube extension the *MPG1* hydrophobin-encoding gene is highly expressed, and secretion of the hydrophobin at this time provides a means by which the fungus secures its attachment to the hydrophobic leaf cuticle (92, 94).

The MPG1 hydrophobin is likely to spontaneously self-assemble on a hydrophobic surface (53, 85), increasing the wettability of the leaf surface and ensuring the effectiveness of hydrophilic mucilage and other adhesives that are secreted at the germ tube–rice leaf interface (109). Absence of the MPG1 hydrophobin results in mutants that are inefficient in appressorium production and poorly pathogenic, indicating that surface attachment is a prerequisite for the signaling pathways that regulate appressorium formation (92). Consistent with this idea, application of cAMP to Δ*mpg1* mutants restores appressorium formation, and the *MPG1* gene appears to be positively regulated by the cAMP–dependent protein kinase A (PKA) pathway (87). At this time *MPG1* is also positively regulated by the product of the *NPR1* gene. *NPR1* encodes a regulator of nitrogen source utilization and is required for appressorium formation and pathogenicity (56, 87).

How cell surface proteins, such as MPG1 and CBP1 (a recently described chitin-binding protein secreted during appressorium formation), bring about appressorium development and generation of the cAMP signal is currently unclear but may involve the product of the *PTH11* gene (18, 48). *PTH11* was identified in an insertional mutagenesis screen for nonpathogenic mutants (90), and *pth11* mutants are severely impaired in appressorium formation on hydrophobic surfaces. *PTH11* encodes a membrane-localized protein with nine membrane-spanning domains and a long cytoplasmic, hydrophilic amino-terminal domain (18). The virulence and appressorium developmental defects associated with *pth11* mutants can also be overcome by addition of cAMP, which demonstrates that *PTH11* operates upstream of the accumulation of cAMP during appressorium morphogenesis. Interestingly, *pth11* mutants also respond to exogenous diacylglycerol, which restores appressorium formation but not pathogenicity, indicating that a signaling pathway involving diacylglycerol generation, and perhaps protein kinase C signaling, is involved in early stages of appressorium formation [maybe in response to plant signals (96)] but is insufficient to bring about subsequent pathogenic development (18).

A heterotrimeric G protein involving the product of the *MAGB* gene is also a component in the early stages of appressorium formation; *magB* mutants are unable to make appressoria and instead make undifferentiated germ tubes that fail to hook or respond to the contact surface (63). Heterotrimeric G proteins are composed of a Gα subunit and G$\beta\gamma$ subunits that interact with a seven-transmembrane (G protein-coupled) receptor at the cell membrane (6). Gα subunits adopt different conformations depending on whether they bind GTP or GDP and dissociate from the $\beta\gamma$ subunit in the GTP-associated form. In this activated form the Gα subunit is diffusible in the cytoplasm and free to interact with effector proteins. Gα subunits also have intrinsic GTPase activity so that they can be quickly recycled to the inactive GDP-associated form (6). *MAGB* encodes a Gα subunit with a number of features associated with the Gi family of G proteins, including a conserved myristoylation motif at the N terminus and a pertussis toxin-responsive ADP-ribosylation site at the C terminus (63). The protein is thus likely to be an inactivator of downstream effector proteins. Because *magB* mutants were unable to form appressoria, it seemed likely that the protein operates upstream of the cAMP response pathway. However, deletion of an inhibitory Gα subunit might be predicted to result in increased cAMP levels. This is not the case for MAGB, however, because exogenous cAMP can restore appressorium development to wild-type levels, suggesting that *MAGB* is required to bring about generation of the cAMP signal. Site-directed mutagenesis of *MAGB* has offered insight into the likely biological function of the Gα subunit (24). A mutation that abolishes GTPase activity, and should result in constitutive activation of the Gα signaling pathway, produced mutants ($magB^{G42R}$) that made appressoria normally, although they displayed a large number of pleiotropic effects such as autolysis of older hyphae, impairment of conidiation, perithecial development, and reduced virulence. In contrast, a $magB^{G203R}$ mutation, which should prevent dissociation of the Gα and G$\beta\gamma$ subunits and thus prevent any G protein signaling, had little effect on *M. grisea*, and the mutant allele was able

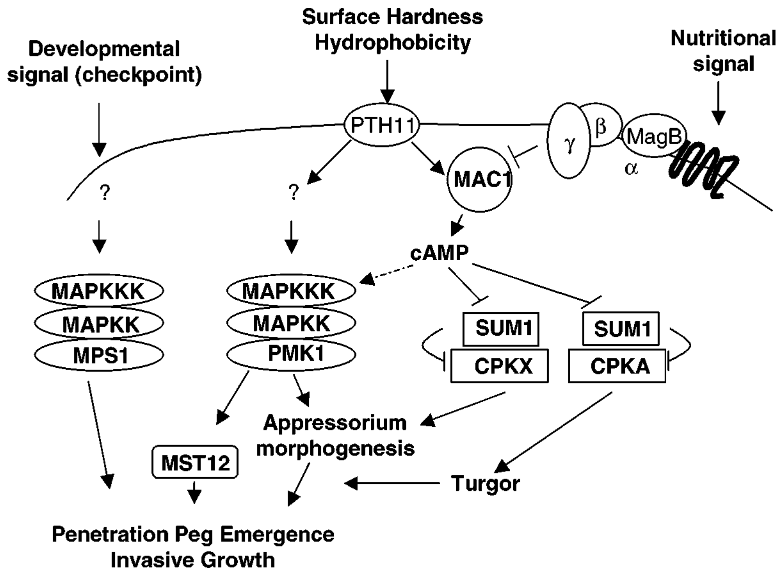

Figure 1 Model for signal transduction pathways that operate to regulate appressorium morphogenesis. In this model appressorium development is positively regulated by physical surface signals that are perceived by the PTH11 receptor protein and activate MAC1 adenylate cyclase. Negative regulation of appressorium development, here in response to exogenous nutrients, occurs via dissociation of the MAGB-containing heterotrimeric G protein, releasing the $\beta\gamma$ subunit, which acts as a repressor of MAC1. The PMK1 MAPK signaling pathway regulates appressorium morphogenesis and the later stages of invasive growth, acting via the MST12 transcription factor. The cAMP response pathway is also responsible for regulating carbohydrate and lipid metabolism during turgor generation. In this model the MPS1 regulatory pathway for penetration peg emergence is triggered by a developmental checkpoint, perhaps following completion of appressorium morphogenesis.

to complement the phenotypic defects of Δ*magB* mutants (24). Taken together, these experiments suggest that the G$\beta\gamma$ subunit may be a repressor of adenylate cyclase activity under certain conditions and that deletion of the Gα subunit causes the dissociated G$\beta\gamma$ subunit to constitutively repress adenylate cyclase and prevent appressorium formation. In such a model the MAGB heterotrimeric G protein would operate as a negative regulator of appressorium morphogenesis (Figure 1). It is not clear, however, how the heterotrimeric protein interacts with a receptor or to which extracellular signal it responds. Interestingly, *M. grisea* possesses two other Gα subunits encoded by *MAGA* and *MAGC*, which affect sexual development of the fungus.

Activation of adenylate cyclase results in synthesis of intracellular cAMP and triggering of a pathway for appressorium morphogenesis. cAMP signaling in eukaryotes normally involves activation of cAMP-dependent PKA. cAMP binds to

the regulatory subunit of PKA, which inactivates the protein and releases it from the catalytic subunit, which is then free to phosphorylate downstream target proteins (54). The regulatory subunit of PKA in *M. grisea* was identified by selection of a bypass suppressor mutant, which restored appressorium formation to a Δ*mac1* mutant (1). This was due to a mutation in a cAMP-binding pocket of the regulatory subunit, leading to constitutive cAMP-independent PKA signaling. The mutant, Δ*mac1 sum1-99*, displayed accelerated conidial germination, germ tube extension, and appressorium development but was still impaired in disease symptom formation (1). Deletion of the *CPKA* gene, which encodes a catalytic subunit of PKA, affects appressorium morphogenesis, which leads to a delay in appressorium formation and subsequent production of small, nonfunctional appressoria (70, 113). The Δ*cpkA* mutants are therefore completely nonpathogenic, although they retain the capacity to cause disease if inoculated into plants through wounds, removing the need to complete appressorium-mediated infection. Clearly, the *CPKA*-encoded PKA is significant for appressorium development, but the fact that appressoria can still form in Δ*cpkA* mutants is surprising given that CPKA encodes the major PKA activity detected in developing germ tubes (1). Furthermore, Δ*cpkA* mutants still respond to exogenously applied cAMP undergoing hook formation and accelerated appressorium development, indicating that another PKA catalytic subunit may be involved in surface sensing and the early stages of appressorium development.

The PMK1 MAPK Signaling Pathway for Appressorium Morphogenesis

The role of mitogen-activated protein kinases (MAPKs) in the development of dimorphic and filamentous fungi has recently been reviewed (110). MAPKs operate in association with upstream kinases in order to transmit an environmental or developmental signal from the cell periphery to the nucleus to bring about gene expression. MAPKs are regulated by a MAPK kinase or MEK (for MAPK/ERK kinase), which in turn is activated by a third kinase termed MAPKKK or MEKK (for MEK kinase). These proteins are sometimes held together as a single complex by a scaffold protein, for example, the STE5 protein in the pheromone signaling pathway in *Saccharomyces cerevisiae* (29). Three distinct MAPK genes in *M. grisea* (*PMK1*, *MPS1*, and *OSM1*) have so far been identified and play diverse roles in pathogenesis-related development (20, 111, 112). The PMK1 MAPK is a functional homolog of *FUS3/KSS1* in yeast, which play roles in the pheromone signaling pathway and the regulation of pseudohyphal growth (29). *PMK1* can substitute for either kinase gene in yeast and can complement the mating defect of a *fus3/kss1* double mutant. PMK1 is involved in appressorium formation, and Δ*pmk1* mutants fail to make appressoria on any surface or in response to cAMP or 1,16-hexadecanediol. Δ*pmk1* mutants do, however, respond to exogenous cAMP, undergoing pronounced hooking and terminal swelling of the germ tube tip. This has been taken as evidence that PMK1 operates in a signaling pathway downstream of the initial cAMP-mediated signal for appressorium morphogenesis, although no direct genetic evidence has yet been presented to verify this idea.

In addition to appressorium development, Δ*pmk1* mutants fail to grow invasively in plants and are not pathogenic even when spores are applied to wounds or inoculated into healthy leaves. The capacity of *M. grisea* to undergo infection-related development and subsequent disease establishment therefore requires the *PMK1*-encoded MAPK. Significantly, PMK1-related MAPKs have been identified in a number of other phytopathogenic fungi that cause diverse diseases (84). Where tested, all these MAPK genes appear to be required for pathogenicity, providing evidence that elements of a MAPK signaling pathway for pathogenic development may be widely conserved (100). Because *PMK1* is functionally related to *FUS3* and *KSS1* in yeast that regulate the transcription factor encoded by *STE12*, a homolog of this gene in *M. grisea*, *MST12*, has been characterized. Gene replacement mutants of *MST12* were nonpathogenic, but interestingly, they could still form appressoria (79). The defect in pathogenesis was instead associated with appressorium function because penetration hyphae do not develop from mature Δ*mst12* appressoria. Furthermore, Δ*mst12* mutants failed to produce spreading disease lesions when inoculated into wounded plants, and showed defects in infectious growth. These observations imply that *PMK1* regulates a diverse set of targets playing roles in both the initiation of appressorium development and the subsequent stages of invasive growth. The latter stages of appressorium maturation obviously require signaling through the MST12 transcription factor. Among other downstream targets of *PMK1* are the products of the *GAS1* and *GAS2* genes (115). These novel proteins are only found in related fungal species such as the barley powdery mildew fungus, *Blumeria graminis*, and in abundance during appressorium formation, where they obviously fulfill a vital function in penetration peg emergence (115).

APPRESSORIUM DEVELOPMENT AND FUNCTION

Once formed, the *M. grisea* apppressorium is a dome-shaped cell with a highly differentiated cell wall structure (7, 100). The cell wall is rich in chitin and contains a layer of melanin on the inner side of the wall. Melanin production is a virulence characteristic of a number of pathogenic fungi and the pigment has diverse functions, acting as an antioxidant, a protective agent from UV exposure, or a secreted toxic metabolite (36). In *M. grisea*, melanin has a different but essential function. Mutants unable to synthesize melanin are easily selected in *M. grisea* because they are distinctively pigmented (102). Three mutants of *M. grisea*, *albino*, *buff*, and *ros*y (corresponding to the *ALB1*, *BUF1*, and *RSY1* loci, respectively), have been studied extensively and are nonpathogenic. This is due to an inability to cross the plant cuticle because of the lack of melanin deposition in the appressorium. *M. grisea* appressoria generate enormous turgor pressure, and an incipient cytorrhysis (cell collapse) assay, in the presence of different concentrations of polyethylene glycol solution, indicated that turgor could rise to as high as 8.0 MPa prior to penetration peg formation. It was also shown that melanin-deficient mutants could not generate turgor of this order, and a hypothesis was formulated suggesting that melanin might

provide an impermeable layer to prevent leakage of an osmotically active metabolite responsible for turgor generation in the fungus (38). Consistent with this idea, the pore size of a mature wild-type appressorium cell wall was calculated to be significantly smaller than that of a melanin-deficient mutant of *M. grisea* (38, 71). Identification of the compatible solute that accumulates in *M. grisea* appressoria allowed this hypothesis to be formally tested. The most abundant solute observed in appressoria is glycerol, which can reach concentrations of up to 3.2 M during turgor generation (16). Melanin-deficient mutants, or a wild-type *M. grisea* strain treated with the melanin biosynthesis inhibitor tricyclazole, generated substantially less appressorial glycerol than a normal, untreated isolate. The role of melanin was, however, most clearly demonstrated when cytorrhysis experiments were repeated using glycerol as the solute. A wild-type strain of *M. grisea* produced appressoria that were readily collapsed by hyperosmotic concentrations of glycerol. Appressoria of isogenic melanin-deficient mutants were similarly collapsed by hyperosmotic glycerol, but they reinflated quickly upon incubation in the solution. In contrast, appressoria of the wild type did not reinflate even after prolonged incubation in glycerol, which shows that the cell wall is impermeable to the polyol (16).

Melanin provides a simple and effective means of preventing solute efflux and allows appressoria of *M. grisea* and related fungi such as *Colletotrichum* species to accumulate substantial turgor. In *M. grisea*, melanin is synthesized through a pentaketide route where acetate units are joined together to form 1,3,6,8-tetrahydroxynaphthalene (4HN), which is then transformed to 1,8-dihydroxynapthalene by two reduction and two dehydration steps, and this product is polymerized by phenol oxidases to the black pigment, melanin. A pentaketide synthase encoded by the *ALB1* gene catalyzes the initial production of the pentaketide substrate for 4HN synthesis. A tetranaphthalene reductase, encoded by the *4HNR* gene, then catalyzes the reduction of 4HN to yield scytalone. Scytalone dehydratase is encoded by the *RSY1* gene and yields trihydroxynapthalene (3HN), which is reduced to vermelone by the product of the *BUF1* gene, a 3HN reductase. Vermelone is then converted to dihydroxynapthalene (2HN) by the *RSY1*-encoded scytalone dehydratase, which is subsequently polymerized to melanin (99).

The Biochemistry of Appressorium Turgor Generation

Glycerol biosynthesis in the appressorium of *M. grisea* is regulated in a way different from that of *S. cerevisiae*, where glycerol accumulates during hyperosmotic stress adaptation. In yeast, glycerol is synthesized predominantly from carbohydrates and regulated by the high osmolarity glycerol response pathway (HOG pathway), a MAPK signaling pathway (29). The *M. grisea* MAPK-encoding gene *OSM1*, which is functionally equivalent to *HOG1* in yeast, however, does not regulate appressorium turgor generation because *Δosm1* mutants are still fully pathogenic and produce turgor (20). The osmoregulatory pathway in *M. grisea* leads instead to arabitol biosynthesis (mannitol, glycerol, and other polyols also accumulate) and requires *OSM1*, but this pathway operates independently of the appressorium turgor generation pathway (20).

Conidia contain substantial amounts of lipid, glycogen, trehalose, mannitol, and other storage products, and because appressoria form in water without exogenous nutrients, glycerol must originate from one, or more, of these sources. Glycogen degradation occurs rapidly during conidial germination and cytology indicates that glycogen is transported and perhaps resynthesized within the appressorium (7, 97). Glycogen degradation occurs during the onset of turgor generation in a process regulated by the cAMP response pathway. Glycogen degradation was retarded in a *ΔcpkA* mutant, whereas in the regulatory PKA mutant *Δmac1sum1-99* the degradation of glycogen occurred quickly, before melanin deposition in the appressorium was complete (97). At present there is no genetic evidence that glycogen metabolism is required for appressorium turgor generation, although genes encoding glycogen synthase, glycogen phosphorylase, and glycogen debranching enzyme are being characterized (L.J. Holcombe & N.J. Talbot, unpublished).

Glycerol production from carbohydrates in yeast involves glycerol-3-phosphate dehydrogenase activity. This enzyme catalyzes reduction of dihydroxyacetone phosphate to glycerol-3-phosphate in a NADH-dependent reaction (2). Glycerol-3-phosphate is then converted to glycerol by two specific glycerol-3-phosphatases encoded by the genes *HOR1* and *HOR2* (37, 76). Glycerol-3-phosphate dehydrogenase (GPD) exists in three forms in *S. cerevisiae*. Two are cytosolic enzymes encoded by *GPD1* and *GPD2* (2). The third GPD is found in the inner mitochondrial membrane and is encoded by the *GUT2* gene. This enzyme carries out flavin adenine dinucleotide (FAD)-dependent oxidation of glycerol-3-phosphate for subsequent metabolism through glycolysis (83). Glycerol can also be produced from dihydroxyacetone by an NADPH-dependent dihydroxyacetone reductase and from glyceraldehyde via an NADPH-dependent glyceraldehyde reductase. In *Aspergillus nidulans* both reactions are catalyzed by a single enzyme, an NADP-dependent glycerol dehydrogenase (GD) (81), and this enzyme may also exist in budding yeast (76). GPD and GD enzyme activities are present in germinating conidia and developing appressoria of *M. grisea* but not induced during appressorium turgor generation (97), so the contribution of glycogen metabolism to turgor generation remains uncertain.

The disaccharide trehalose is also abundant in conidia (an average of 4–5 pg conidium^{-1}) and degrades rapidly as soon as germination occurs (27). Trehalose is synthesized from glucose-6-phosphate and UDP-glucose by trehalose-6-phosphate synthase and an associated phosphatase. In *S. cerevisiae* a multienzyme complex including the products of the *TPS1* and *TPS*2 takes part in trehalose synthesis, which accumulates during stress adaptation (95). In *M. grisea* the trehalose-6-phosphate synthase-encoding gene *TPS1* is required for pathogenicity; *Δtps1* mutants form appressoria that do not generate turgor effectively and are unable to penetrate the host cuticle (27). These mutants, however, retained the capacity to infect wounded rice plants, and therefore the ability to proliferate in plant tissue is not affected by loss of trehalose synthetic activity. Trehalose metabolism (which would be required for trehalose to contribute to glycerol formation for example), however, is not required for appressorium turgor generation. *M. grisea* has two trehalases.

The bifunctional trehalase, encoded by the *TRE1* gene, provides the main intracellular activity during spore germination but is also secreted and required for growth on trehalose as a carbon source. *TRE1* is not required for pathogenicity, but the second trehalase, encoded by the *NTH1* gene, is required for full disease symptom expression by *M. grisea* and was first identified by insertional mutagenesis [as the *pth9* mutant (90)]. *NTH1* is highly expressed during early plant infection and tissue invasion and is important for events after initial entry into the host (27). Trehalose biosynthesis is therefore required for appressorium function but subsequent metabolism of the disaccharide is not. A possible explanation for these observations comes from the fact that *Δtps1* mutants cannot grow on glucose as a sole carbon source. In *S. cerevisiae* the *TPS1* gene is required for regulation of glycolysis (95). Trehalose-6-phosphate synthesis provides a route to limit entry of glucose into glycolysis, and the trehalose-6-phosphate synthesis enzyme also negatively regulates hexokinase activity in vitro. The lack of ability to grow on glucose results from unregulated entry of glucose into glycolysis and rapid accumulation of fructose 1,6 bisphosphate. This depletes the intracellular phosphate pool and leads to a catastrophic decline in ATP levels (95). In *M. grisea* it seems likely that *TPS1* plays a similar role, but there are also important differences such as the fact that *M. grisea Δtps1* mutants cannot grow on lipid or acetate as sole carbon sources (27).

The only glycerol biosynthetic enzyme activity induced during appressorium development in *M. grisea* is intracellular triacylglycerol lipase (97). Lipid bodies are present in abundance in germinating conidia and move to the apex of the germ tube in a process regulated by the PMK1 MAPK pathway. During appressorium morphogenesis, lipid bodies coalesce and are taken up by vacuoles in the appressorium (108). The vacuole appears to be the site of rapid lipolysis, which occurs at the onset of turgor generation. Appressorial lipase activity is substantially reduced in a *ΔcpkA* mutant, indicating that lipid degradation is a cAMP-regulated process. Furthermore, cytological examination of a *ΔcpkA* mutant revealed pronounced retardation of lipid degradation in appressoria. In contrast, a *Δmac1sum1-99* mutant showing cAMP–independent PKA activity exhibited accelerated lipid movement and degradation, completing the process before the onset of appressorium melanization and perhaps explaining the reduced virulence phenotype of *Δmac1sum1-99* mutants (97). The initial release of the *M. grisea* genome sequence has revealed the presence of 7 putative intracellular triacylglycerol lipases and a further 19 extracellular lipases. Determining which of these enzymes contributes to the lipase activity present in appressoria and which are required for pathogenicity will be important challenges in the next few years.

An important consequence of lipid degradation in the appressorium is likely to be the metabolism of fatty acids. An enzyme involved in the process of β-oxidation has already been shown to be required for pathogenicity. The *PTH2* gene, identified by insertional mutagenesis (90), encodes a carnitine acetyl transferase responsible for movement of acetyl CoA across the mitochondrial or peroxisomal membrane. A second important consequence of appressorium lipid metabolism may be a

requirement for glucose generation via the glyoxylate cycle. Glucose may be required for rapid cell wall biosynthesis during infection. A recent study showed that the glyoxylate cycle is important for temporal regulation of pathogenesis in *M. grisea* (107). An isocitrate lyase mutant, *Δicl1*, was retarded in spore germination, in appressorium formation, and in the visible production of disease symptoms. The significance of the glyoxylate cycle in *M. grisea* is consistent with similar findings in a number of pathogenic fungi such as the brassica pathogen *Leptosphaeria maculans* (39) and the human pathogen *Candida albicans* (64) as well as pathogenic bacteria such as *Mycobacterium tuberculosis* (67).

PRODUCTION OF THE APPRESSORIUM PENETRATION PEG AND PLANT INFECTION

Appressorium-mediated plant infection proceeds via generation of a narrow penetration peg at the base of the appressorium. The site of penetration peg emergence is visible initially as the appressorium pore, which is an apparently wall-less layer where the fungal plasmalemma is in direct contact with the plant surface (7). Prior to production of the penetration peg, a bilayered appressorium pore overlay forms and the peg then emerges into the substratum bounded by a single cell wall layer. The peg contains numerous microfilaments, filasomes, and microtubules (7), and cuticle penetration appears to result from a sustained application of physical force, as reported for the related fungus *Colletotrichum graminicola* (5). Insertional mutagenesis has identified a number of genes important for penetration peg emergence in *M. grisea* (3, 90). The *PLS1* gene encodes a novel protein related to the tetraspanin family found in animals (13).

Tetraspanins are membrane proteins containing four membrane-spanning domains and form part of the membrane protein complexes associating with other membrane proteins such as integrins. Animals contain large numbers of paralogous tetraspanin genes (up to 37), but filamentous fungi examined so far appear to possess a single tetraspanin gene belonging to a single family of fungal-specific tetraspannins (30). The *pls1* mutant fails to elaborate a penetration peg and is completely nonpathogenic. Interestingly, *pls1* mutants also fail to infect wounded leaves, showing that penetration peg formation and invasive hyphae formation are completely blocked in the absence of the tetraspanin (13). Potential functions for the PLS1 tetraspanin include focusing mechanical force at the appressorium pore and orchestrating the formation of the actin network at the site of peg emergence. It is also possible, however, that *PLS1* plays a role in integrin-mediated attachment and acts as a signal transduction molecule for peg emergence. A second membrane protein significant at this time is the *PDE1*-encoded P-type ATPase, which was identified in an insertional mutant hunt as a penetration-defective mutant showing reduced disease symptoms (3, 4). *PDE1* encodes a putative aminophospholipid translocase, a class of protein required to generate phospholipid asymmetry in membranes. A fundamental property of most biological membranes is the asymmetric distribution of lipids across the bilayer. Choline phospholipids

(phosphatidylcholine and sphingomyelin) are localized mainly in the outer monolayer of the plasma membrane (or lumenal side of internal organellar membranes), whereas aminophospholipids (phosphatidylserine and phosphatidylethanolamine) are enriched on the inner (cytofacial) side of the plasma membrane. It is possible that PDE1 is important in penetration peg emergence because of the severe membrane stress that may accompany polarity establishment at the appressorium pore, necessitating an enhanced requirement for membrane phospholipid asymmetry. Localization of the PDE1 protein is, however, required to confirm such a role (4).

Regulation of penetration peg formation requires *MPS1*, which encodes a MAPK (112) functionally related to the Slt2/Mpk1 kinase from *S. cerevisiae*, where it is responsible for regulation of cell wall growth under conditions of membrane stress (14, 29). Gene replacement mutants that lack *MPS1* do not form penetration pegs and are completely nonpathogenic. They also show pleiotropic effects associated with having weakened cell walls, including hypersensitivity to protoplasting enzymes and autolysis of older hyphae. It is likely that *MPS1* regulates cell wall biosynthesis during initial emergence of the penetration peg and its downstream effectors may include a large number of the morphogenetic proteins required to synthesize the functional penetration hypha. Among the virulence factors that contribute to penetration peg emergence and turgor generation is a cyclophilin encoded by the *CYP1* gene (104). Cyclophilins are peptidyl prolyl isomerases that may play roles both in protein folding and in regulation of calcineurin assembly and activity. The latter role is based on the longstanding identification of cyclophilins as cellular targets of the immunosuppressive drug cyclosporin A (104, 105). The role of *CYP1* in regulating virulence-associated activities in *M. grisea* and the observation of a similar role for a cyclophilin in the human pathogen *Cryptococcus neoformans* implicate calcium signaling and calcineurin activity in fungal pathogenesis (105).

COMPATIBLE INTERACTIONS—INVADING THE HOST

The penetration hypha differentiates into a series of bulbous, branched infectious hyphae soon after plant infection. These hyphae resemble pseudohyphal-propagating yeast cells and appear to form buds during their initial appearance (3, 7). After filling the initial epidermal cells, longer, more conventionally cylindrical hyphae ramify out into adjacent tissue and the leaf tissue is rapidly colonized (92, 102) and photosynthesis is severely affected (9). Relatively few genetic determinants of tissue colonization have been identified, largely because the nonpathogenic mutants examined so far have led to identification of proteins involved in appressorium formation and function. Insertional mutagenesis has, however, revealed that synthesis of amino acids such as methionine and histidine are required for disease symptom production by *M. grisea* (3, 90). There is also a requirement for an ATP-driven efflux pump protein encoded by the *ABC1* gene (101). The *ABC1*-encoded protein is similar to yeast ABC transporters involved in multidrug resistance, and mutants lacking *ABC1* are nonpathogenic. *ABC1* is induced by a number of metabolic poisons and antimicrobial agents, including a rice

phytoalexin, and it is possible that the protein provides a means by which such plant defense compounds are tolerated by the invading fungus (101).

M. grisea generates toxins of its own during tissue invasion, including tenuazonic acid, pyricularin, pyrichalasin, and others, although relatively little is known about the significance of any of these compounds to infection (102). The availability of a genome sequence for the fungus provides the means to identify the biosynthetic pathways for these metabolites and the opportunity to test genetically their significance to disease progression. The appearance of necrotic disease lesions is accompanied by the development of aerial conidiophores. Conidia in *M. grisea* are sympodially arrayed at the tips of these aerial hyphae. Mitotic divisions of a single progenitor nucleus occur in the conidiophore, leading to the production of the first three-celled conidium. Thereafter, the hyphal tip moves to the side of the conidium and produces a second spore until three to five conidia are produced in a whorl at the conidiophore tip (57). Mutants affected in conidiation often have pleiotropic effects on appressorium formation and pathogenicity. The *acropetal* mutant, for example, produces chains of misshapen conidia and appears to negatively regulate conidial morphogenesis, allowing sympodial patterning to proceed (57). *Acr1* mutants are reduced in virulence and mature spores do not form appressoria efficiently. A number of other spore morphology and sporulation mutants affect disease progression and appressorium formation including the *smo* and *con* mutants (32, 86), which highlight the developmental parallels between appressorium morphogenesis and conidiation.

INCOMPATIBLE INTERACTIONS—RESISTING INFECTION

Single gene resistance to rice blast operates via a classical gene-for-gene interaction, where the host possesses a single dominant gene conditioning resistance against a race of the pathogen carrying a corresponding dominant avirulence gene (35). There has been considerable study regarding the mechanisms by which resistance to rice blast is inherited, and more than 30 major rice blast resistance gene loci, denoted *Pi* genes, are known (89). Avirulence genes encode protein products that are recognized by plants (a pathogen-associated molecular pattern) possessing the appropriate resistance gene product. Currently, two rice blast resistance genes, *Pi-ta* and *Pib*, have been cloned and characterized and a third locus, *Pi-CO39(t)*, is close to being identified (8, 11, 106). The *Pib* gene encodes a 1251-amino-acid protein that is predicted to be cytoplasmically localized and contains a nucleotide-binding site and a leucine-rich repeat carboxy-terminal domain (106). The protein is therefore typical of the nucleotide-binding site leucine-rich repeat class of resistance gene (35), although with some unusual characteristics including the presence of a duplication within the P-loop in the N terminus of the protein and some clustering of cysteine residues in one of the leucine-repeat sequences (89). The *Pib* gene is expressed in response to challenge with both compatible and incompatible strains of *M. grisea* and in response to certain environmental stresses (106).

The blast resistance gene *Pi-ta* is linked to the centromere of chromosome 12 in rice and encodes a predicted cytoplasmic receptor protein of 928 amino acids with a centrally located nucleotide-binding site and a C-terminal leucine-rich domain (8). The *Pi-ta* gene is constitutively expressed in resistant and susceptible rice varieties. Susceptible rice varieties (which carry the recessive *pi-ta*$^{-}$ allele) encode a protein that has a common single amino acid difference, having a serine instead of alanine at position 918 in the leucine-rich carboxy terminus of the protein. Transient expression experiments in which the *Pi-ta* gene and *AVR-Pita* were coexpressed in rice cells elicited a resistant response, suggesting that the proteins interact with each other inside rice cells to bring about disease resistance (8). Further evidence in support of this idea was obtained with the yeast two-hybrid system, which showed a direct interaction between AVR-$Pita_{176}$ (a processed form of the protein lacking N-terminal secretory and pro-protein sequences) and the leucine-rich domain of the Pi-ta protein (43). No physical interaction was observed between proteins encoded by the *pi-ta* alleles, which carry the single amino acid change in the LRD that brings about susceptibility, and the AVR-$Pita_{176}$ protein. These experiments indicate that the products of the *Pi-ta* resistance gene and *AVR-Pita* avirulence gene physically interact in rice cells to induce resistance. *AVR-Pita* putatively encodes a zinc-dependent metalloprotease that shows greatest similarity (27% identity and 44% similarity) to Npll, a neutral zinc metalloprotease from *Aspergillus oryzae* (78). The *AVR-Pita* gene maps to a position close to one of the telomeres of chromosome 3, and its cloning therefore required considerable effort because the gene was not present in any available genomic libraries. The presence of an *AVR* gene so close to the end of a chromosome has been postulated to provide a mechanism that promotes frequent rearrangements, thereby enhancing the possibility of mutating *AVR* genes and causing a *M. grisea* isolate to become virulent on formerly resistant hosts (8, 78). A number of *AVR* genes appear to be located close to telomeres in *M. grisea* in addition to *AVR-Pita*, such as *AVR-TSUY AVR1-Ku-86* and *AVR1-MedNoi* (19). Other *AVR* genes recombine with telomeric markers such as *AVR-CO3*9 and *AVR1-Irat7*, which are themselves closely linked (19). The full sequence of *M. grisea* will allow more effective testing of this idea, but some *AVR* genes that appear to be unstable, such as the *PWL2* gene (see below), are located away from subtelomeric regions of the genome.

The *PWL2* gene confers nonpathogenicity (avirulence) on weeping lovegrass and was found to be an unstable locus, where rearrangements often led to loss of the *PWL2* gene and gain of the ability to cause disease on weeping lovegrass (91). *PWL2* encodes a 16-kDa secreted, glycine-rich, hydrophilic protein. *PWL2* was found to be highly polymorphic in strains of *M. grisea* and subsequently a *PWL* gene family was identified by homology, including *PWL1*, *PWL3*, and *PWL4*. Interestingly, *PWL3* and *PWL4* were nonfunctional, although *PWL4* could be made functional if expressed under control of the *PWL2* promoter. This indicates that the genes are expressed quite distinctly and may have diverse potential as avirulence factors (51). It will be interesting to test whether the diverse *M. grisea* hosts will enable definition of further resistance genes capable of recognizing each

member of the *PWL* family in a manner similar to that of the tomato leaf mould fungus *Cladosporium fulvum*, where the pathogenicity factors *ECP1* and *ECP2* act as avirulence gene products (58). The endogenous function of the *PWL* genes, however, remains obscure (51), but it is striking how host specificity in *M. grisea* appears to operate in the same gene-for-gene manner as cultivar specificity.

GENOME STRUCTURE OF *MAGNAPORTHE GRISEA*

In 2002 a draft genome sequence of the 70-15 rice pathogenic isolate of *M. grisea* was released to the international research community (http://www-genome.wi.mit.edu/annotation/fungi/magnaporthe/). Full annotation and analysis of the genome of *M. grisea* is currently underway and will provide an unprecedented opportunity to learn more about the fungus (93, 114). Previous studies have provided a glimpse of some of the features that might be expected. Sequencing of large cloned fragments of the *M. grisea* genome have shown an average gene density of one gene every 4 kb and have indicated the potential presence of considerably more genes than in related saprophytic fungi such as *Neurospora crassa*, but some evidence of conservation of gene order (synteny) in parts of the genome (34). A large-scale EST sequencing effort has already provided the sequences of 7245 unique genes (80, 88, 114), which can be readily accessed and compared with ESTs from related plant pathogenic fungi (http://cogeme.ex.ac.uk/). In addition to single copy genes and gene families, the genome of *M. grisea* contains repeated DNA families that have been proposed to be a source of genetic variability in the fungus (102). Recombination between repeated DNA sequences can lead to translocations, deletions, or inversions, but repeated DNA sequences can also be active transposons that facilitate their own movement in the genome. *M. grisea* strains possess both retrotransposons, which are copied to an RNA intermediate by reverse transcription, and inverted terminal repeat (ITR) transposons, which excise and reinsert within the genome. Among the retrotransposons are long terminal repeat (LTR) transposons such as *Grasshopper* (*grh*) and MAGGY. *grh* is present in a subset of *M. grisea* fingermillet pathogens (21). The transposon has 198-bp LTRs and its transposition generates a 5-bp target site duplication. The diverse but patchy geographic distribution of fingermillet pathogens possessing *Grh* indicates that the transposon has been acquired subsequent to the evolution of this host-limited form of the fungus. The MAGGY retrotransposon is present in high copy number (50–100 copies) in rice pathogenic isolates of *M. grisea* but is also found in variable copy numbers in other host-limited forms including pathogens isolated from *Setaria glauca*, *Paspalum districhum*, and *Panicum* spp. (26). Significantly, the isolates carrying the MAGGY element constitute a single genetic cluster of *M. grisea*, suggesting that MAGGY was originally acquired by a common ancestor to this group of isolates (26, 73). The MAGGY element has LTRs of 253 bp flanked by 6 bp inverted repeat sequences. When it transposes, MAGGY generates a 5-bp target site duplication (26). MAGGY is active in at least two strains of *M. grisea* and can also transpose in heterologous fungal species (72). The presence of degenerate forms of MAGGY in a subset of *M. grisea* isolates

from common millet *Panicum miliaceum*, possessing numerous point mutations, suggests that the element has been effectively "trapped" in certain isolates of *M. grisea* while still active in others (72). Expression of active MAGGY elements is influenced by environmental stresses such as heat shock, oxidative stress, or exposure to copper (42). How such stress responsiveness arose is an interesting problem, but it might be a consequence of the capture of adjacent *cis*-acting stress-responsive motifs during transposition (44) or interelement exchange of such promoter motifs and subsequent selection of actively transposing elements within the host (66). A third LTR-class transposon, MGLR-3, which belongs to the *Gypsy* class of retrotransposons, has 250-bp LTRs that lack ITR sequences. Transposition of the element does not generate a target site duplication, presumably due to generation of blunt ends during cutting and insertion into the genome. MGLR-3 appears ubiquitous among *M. grisea* isolates and is present at relatively high copy number (49).

Phylogenetic analysis of three retrotransposons in *M. grisea* (*Grh*, MAGGY, and MGLR-3) suggests that they may have arisen from a common ancestral retrotransposon, although they have subsequently taken different routes in distribution. MGLR-3 became propagated in the genome of *M. grisea* before the evolution of diverse host-limited forms, whereas MAGGY has become largely limited to rice pathogens and *Grh* to a subset of fingermillet pathogens, perhaps indicating horizontal transfer of a new retrotransposon derivative (or a closely related element from another host) later in the evolutionary history of *M. grisea* (49). A similar picture of diverse distribution can be seen when the ITR transposons and non-LTR retrotransposons are considered. Two different ITR transposons, Pot2 and Pot3, are found in *M. grisea* (25, 46). Both are related to the *TC1/Mariner* class of transposons and contain two ITRs flanking a single open reading frame encoding a transposase. Recently, it was shown that a Pot3 element had integrated in the promoter of the *AVR-Pita* gene, 304 bp upstream of the start codon (50). This insertion led to a gain of virulence on cultivar Yashiro-moshi and showed the potential for transposons to bring about changes in the virulence spectrum of *M. grisea*. The Pot3 element was originally described as part of the MGR586 DNA fingerprinting probe, which has been used extensively in population studies of *M. grisea* (25). Pot3 is distributed among rice pathogenic isolates of *M. grisea* at relatively high copy number but is also present in isolates of the fungus derived from other hosts such as *Pennisetum*, *Panicum*, *Leersia*, and *Triticale*. Pot3 is therefore almost ubiquitous in *M. grisea* isolates, although its presence at high copy number is more common among the rice pathogenic strains of the fungus (25). Three different groups of non-LTR retrotransposons also exist in the *M. grisea* genome, including one long interspersed nuclear element (LINE) called MGL (previously MGR583) that is present at high copy number in rice pathogens (50–80 copies). A short interspersed nuclear element (SINE) called MGSR1 is found in rice pathogens at approximately 40 copies per genome, while grass pathogenic forms have considerably fewer elements. A second SINE called Mg-SINE was found as an insertion element in a Pot2 transposon and is present at high copy number in both rice and non-rice pathogen isolates of *M. grisea* (45, 74, 75).

POPULATION-LEVEL ANALYSIS OF *MAGNAPORTHE GRISEA*

Molecular variability studies of *M. grisea* have proved revealing in defining the pathogen population and gaining insight into the means of blast disease propagation [for review see (116)]. The Pot3 transposable element has been extensively used as a DNA fingerprinting probe, and so far in excess of 2500 *M. grisea* isolates from many different countries have been characterized. It is clear from these studies that *M. grisea* is predominantly a clonally propagating organism, reproducing by conidial production from disease lesions. The influence of agricultural systems is, however, also apparent. In Europe and the Americas, where rice cultivation is relatively new and dominated by modern plant breeding, the introduction of cultivars carrying exotic resistance genes from numerous genetic backgrounds has clearly exerted a selective pressure on the pathogen population such that a few compatible clonal lineages of the fungus predominate. For example, in the United States a study of *M. grisea* that examined 42 isolates of *M. grisea*, representing the eight major pathotypes present in the country, defined eight Pot3 fingerprint groups (sharing at least 80% common Pot3-hybridizing fragments). Six of the eight fingerprint groups corresponded to isolates sharing a given physiological race (pathotype). In the other cases one Pot3 fingerprint group was composed of isolates showing two different pathotypes, and another pathotype could be divided into isolates classified into two fingerprint groups (62). The presence of such easily defined genotypic groups strongly supported a clonal population structure for *M. grisea* in the United States. A much more complex situation, however, exists in Asia, where the long history of rice cultivation and the huge number of traditional cultivars grown has meant that the pathogen population is more diverse, although predominantly spread as successful clonally propagating lineages. In Thailand, for example, a study identified 68 lineages from 527 isolates (68, 69). The isolates were found to represent 175 distinct pathotypes, and thus the relationship of lineage to pathotype was complex. Twenty-one of the pathotypes comprised 53% of the sampled population and were widespread. The remaining 160 pathotypes were all rare, with 117 of them represented by a single isolate of *M. grisea*. Similar complex relationships between pathotype and Pot3 fingerprint groups were observed in studies in India, China, and Korea, while in Colombia a somewhat simpler relationship was found, although rather more complex than the almost complete simple alignment of lineage and pathotype observed in the United States and Europe (61, 116).

At the center of origin of rice (and by inference center of origin of *M. grisea*), there is evidence of sexual recombination influencing the variability of *M. grisea* populations, perhaps as it did before the widespread worldwide cultivation of rice (55). The prevalence of *M. grisea* isolates of both mating types in the Himalayas and the southern Yunnan province of China indicates that sexual reproduction may be occurring or that it certainly has occurred in the recent past. In the Matli region of the Himalayas, for example, 38% of isolates identified were *MAT1-1* and 13% were *MAT1-2*, and isolates showing male fertility or hermaphroditic fertility were

commonly detected (55). The presence of Pot3 fingerprint profiles that could be interpreted as being recombinant forms of other lineages, and the presence of isolates having an intermediate copy number greater than 40 copies of Pot3, is also consistent with a population having been influenced by recombination. Using both Pot3 and a number of single copy molecular markers, Kumar et al. (55) investigated genetic diversity levels and were unable to reject the hypothesis of gametic phase equilibrium—which would be expected for a population undergoing sexual reproduction (55). Gametic phase equilibrium analysis has been used to determine whether recombination has influenced populations of organisms that at the outset appear to be clonal (10, 65). The analysis is based on the probability of random associations of alleles, present in fully recombining populations, as opposed to linkage disequilibrium, which occurs in clonally propagating organisms (65). Taken together, the presence of gametic phase equilibrium and the prevalence of both mating types indicate that sexual recombination has played a significant role in these ancestral populations of *M. grisea*. Further supporting evidence comes from the recent demonstration that repeat-induced point mutation operates in *M. grisea* in the same way as in the related pyrenomycete *Neurospora crassa* and that it occurs during the sexual phase of growth (41).

WHAT IS LEFT TO BE DISCOVERED?

Although our understanding of *M. grisea* has been extended significantly in the past few years, there is clearly much to learn. The development of appressoria by *M. grisea*, for example, requires cAMP and the PMK1 MAP signaling pathway, but the interplay between these two pathways is not at all understood. In the corn smut fungus *Ustilago maydis* it is becoming apparent that the pheromone-regulated MAP signaling pathway and cAMP-dependent signaling process, which collectively regulate production of the filamentous (and infectious) dikaryotic phase of the fungus, cross-talk extensively and essentially act in opposition to one another to regulate yeast-hyphal dimorphism in response to plant, nutritional, and environmental signals (47). How *M. grisea* regulates appressorium formation—a similar departure from hyphal growth—remains a significant challenge and requires more effective ordering of the signaling pathways than that carried out to date. The nature of turgor generation in appressoria of *M. grisea* is also an area about which relatively little is known. How cellular metabolism is altered to accommodate accumulation of high concentrations of a compatible solute and how this process is genetically regulated are significant areas for investigation and differ significantly from any cellular process in *S. cerevisiae*, the standard "pathfinder" organism in eukaryotic biology.

Once within the rice leaf tissue it is not clear how *M. grisea* invades rice cells, and surprisingly, the exact nature of the interface between the invading fungus and its host is not at all well established. The apparent integrity of plant cells invaded by the fungus indicates that invasive hyphae invaginate the host plasmalemma during invasion. However, there is no clear extrahaustorial matrix, or specialized

haustorium visible in *M. grisea* infections, compared with those of other biotrophic fungi, and it remains possible that *M. grisea* hyphae do directly enter plant cells. Understanding the nature of this interface is critical to determining the biological functions of effector proteins such as AVR-Pita. Determining the mechanisms by which genetic diversity are generated in *M. grisea* and the basis for the considerable strain variation [observed in phenotypes of a number of mutants, see (1, 4, 18)] also provides a rich avenue for study.

The final and arguably most difficult challenge is translating the fundamental knowledge gained about the blast fungus into durable disease control mechanisms. Two studies, however, show how fundamental research can impact disease management. First, the application of lineage exclusion breeding, where rice cultivars are bred against prevailing populations of *M. grisea* classified by Pot3 fingerprinting, has been successful in both Colombia and Thailand (68, 116). Second, the optimization of genetic diversity in rice cultivars based on intercropping has shown significant yield increases and disease suppression in the Yunnan province in China (117). These reports show what can be achieved by application of a combination of genetic analysis and field pathology and provide evidence that a thorough understanding of the molecular biology of *M. grisea* will provide durable solutions to thwart this efficient cereal killer.

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