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Magnaporthe oryzae and Rice Blast Disease

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THE IMPACT OF BLAST DISEASE: PAST, PRESENT, AND FUTURE

Rice blast, caused by the haploid, ascomycetous fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) (Couch and Kohn, 2002), remains the most explosive and potentially damaging disease of the world's rice crop (Wang and Valent, 2009). Estimates are that the crops lost to the blast fungus would feed 60 million people annually, and strategies for durable, sustainable disease control remain elusive. The fungus infects all above-ground parts of the plant, with leaf blast (Fig. 1), neck rot, and panicle blast being responsible for significant losses in the field. In addition, wheat blast, caused by related *M. oryzae* strains, was identified in 1985 in Paraná State of Brazil (Igarashi et al., 1986), and it rapidly spread to the important wheat-producing regions of Brazil and Bolivia (Prabhu et al., 1992). Since then, blast has become a major wheat disease in this region (Urashima et al., 2004). The wheat blast fungus infects mainly wheat heads in the field, and fungicides that control rice blast are not effective in controlling wheat blast. Although wheat blast has not yet spread from South America, close relatives to the wheat isolates emerged in the early to mid-1990s to cause gray leaf spot (GLS), a severe turf grass disease, infecting perennial ryegrass and tall fescue in the United States and in Japan (Viji et al., 2001; Farman, 2002a; Tosa et al., 2004; Tredway et al., 2005). *M. oryzae* represents a growing agricultural threat.

The blast fungus executes diverse developmental processes, including the best-studied development associated with its asexual disease cycle (Fig. 2A through C). At least in laboratory studies, the fungus can also infect roots, executing a different developmental process characteristic of root pathogens such as the closely related wheat take-all fungus, *Gaeumannomyces graminis* (Sesma and Osbourn, 2004). The fungus produces hyaline to pale gray asexual conidia, each with three cells containing a single nucleus. Since the three nuclei in a conidium are identical, the fungus is routinely purified by isolation of a single conidium. The fungus is a member of the Pyrenomycetes, producing ascospores in unordered asci within perithecia (Fig. 2D). Fully fertile strains are self-sterile hermaphrodites, with compatibility for mating governed by alternate alleles of the mating-type locus *MAT1*. Small, crescent-shaped microconidia, 6 μm in length and 0.7 μm in width, are produced from phialides by some sexually fertile isolates (Kato et al., 1994). These microconidia have not been observed to germinate, and their role in nature is unknown.

For rice blast, differences in rice cultivar specificity were first distinguished by Sasaki in Japan in 1922, and he reported the first genetic analysis of resistance in rice (Ou, 1985). Since that time hundreds of races (or pathotypes) have been defined by their virulence spectrums on differential rice cultivars (Wang and Valent, 2009). Major effort has been directed toward identifying dominant resistance (*R*) genes in rice or wild relatives and toward developing blast-resistant cultivars. This has resulted in identification of >80 *R* genes that prevent disease by some pathogen races, as well as 350 quantitative trait loci (Ballini et al., 2008). Despite this wealth of *R* genes, genetic control has not been effective, due to the variability of the fungus and its ability to "defeat" introduced *R* genes within 1 to 2 years of their deployment in the field. Expectations are that blast disease will become even more serious under the conditions of agricultural intensification required to feed the growing world population (Wang and Valent, 2009). For example, both increased fertilizer use and decreasing water supply favor development of rice blast disease. Food security in the coming century depends on eliminating the potential of rice blast disease to cause catastrophic losses to rice production.

M. GRISEA SPECIES COMPLEX

The *Magnaporthe grisea* species complex includes pathogens of more than 50 grass species (Talbot, 2003). *Magnaporthe oryzae* was recently segregated as a distinct species from *M. grisea* based on a multilocus phylogenetic analysis and on mating properties of the strains (Couch and Kohn, 2002). *M. grisea* isolates are pathogenic on crabgrass, *Digitaria sanguinalis*, and related grasses, and *M. oryzae* is associated with pathogens of diverse grasses with agricultural significance. For example, *M. oryzae* includes pathogens of rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*),

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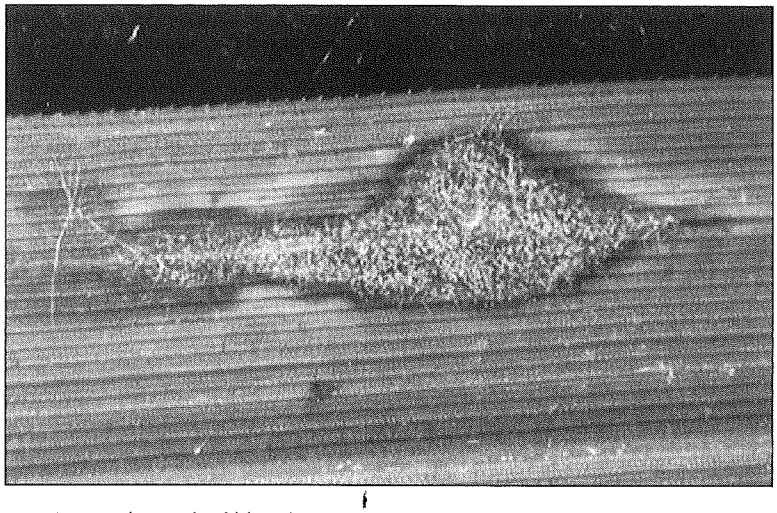

FIGURE 1 A sporulating leaf blast lesion on rice. Fully susceptible lesions first become visible ~5 days postinoculation (dpi) and mature ~7 dpi. Such lesions range from 0.5 to >1 cm in length depending on the rice variety and plant maturity in the field, and they produce several thousand conidia a day for about 2 weeks (Ou, 1985). (Photo courtesy of J. M. Bonman.)

oats (*Avena sativa*), finger millet (*Eleusine coracana*), Italian (foxtail) millet (*Setaria italica*), weeping lovegrass (*Eragrostis curvula*), and perennial ryegrass (*Lolium perenne*). This designation supported earlier conclusions of Kato and colleagues (Kato et al., 2000). They examined pathogenicity, mating compatibility, and restriction fragment length polymorphisms of *M. grisea* isolates from various hosts and found that isolates from *Oryza*, *Setaria*, *Panicum*, and *Eleusine* host species formed a genetically close, interfertile group (the crop isolate or CC group) that was distinct from *Pyricularia grisea* (Kato et al., 2000), the name first assigned to isolates from *Digitaria* host species. The *Triticum* and *Lolium* pathotypes were subsequently shown to belong to the *M. oryzae* (CC) group (Tosa et al., 2004).

Although in general, fungal isolates collected in nature are specialized for that host species, some isolates can infect other species as well (Urashima et al., 1993; Viji et al., 2001). Indeed, Heath and colleagues (Heath et al., 1990) presented comprehensive cytological analyses of a rice isolate and a finger millet isolate infecting rice, finger millet, and weeping lovegrass. They suggested that different plant-by-strain combinations represent a continuum of plant-fungus interactions. That is, isolates that were not adapted for a particular host could colonize that host at some infection sites, resulting in occasional small susceptible lesions or dark brown resistance spots (Valent et al., 1991). Studies of fully susceptible interactions, in which the fungus succeeds at most infection sites, require fungal isolates that are adapted for that host species.

Population Structure and Dynamics

The degree of sexual fertility exhibited by fungal isolates in different host-specific populations within the *M. grisea* species complex ranges from highly fertile hermaphrodites, to female-sterile strains that mate only with hermaphrodites, to totally infertile strains. Host range is generally predictive of fertility (Orbach et al., 1996). Most rice pathogens isolated from the field have low levels of fertility, being either female-sterile or totally infertile. In contrast, most isolates from finger millet or wheat are highly fertile hermaphrodites. Breeding of rice pathogens for improved fertility, as well as identification of rare hermaphroditic rice isolates, have made genetic analysis possible with rice pathogens (Wang and Valent, 2009).

Low levels of sexual fertility and geographic isolation of strains of opposite mating type suggested that field populations infecting rice were predominantly asexual. DNA fingerprinting using multilocus probes confirmed that rice isolates in younger rice-growing regions, such as the Americas, can be divided into relatively low numbers of distinct lineages, with each lineage apparently derived through clonal propagation from a common ancestor (Zeigler, 1998). However, such lineages were not apparent in the ancient Asian populations near the center of origin of rice. The occurrence of hermaphroditic rice isolates in these Asian populations is suggestive of some residual level of sexual recombination, although this has not been demonstrated. Analysis of blast lineages in South America suggested that individuals within lineages may have limited potential for overcoming some *R* genes, leading to the "lineage exclusion" strategy for predicting effective *R* gene combinations to control the disease (Zeigler, 1998; Wang and Valent, 2009).

Evolution of host-specific populations is an important topic that can be addressed within the *M. grisea* species complex. Based on 37 multilocus haplotypes among 497 fungal isolates, Couch and colleagues determined that the rice-infecting population had a single origin host shift from a *Setaria* population around the time of domestication of rice (Couch et al., 2005). That is, the current rice blast population dates from ~7,000 years ago, when cultivated rice (*O. sativa*) was domesticated from *Oryza rufipogon* in the middle Yangtze valley in China. Italian millet, *Setaria italica*, was domesticated in China around the same time. Additional shifts to rice weeds, *Panicum* and *Leersia* species, followed soon after. Additional host shifts might have occurred, especially around the time of the Green Revolution, ~35 years ago, when improved rice varieties spread rapidly over a broad geographic area. However, there is no evidence to support a second host shift event associated with rice. Host shifts probably account for the recent emergences of wheat blast in Brazil and GLS in the United States and Japan. Because rice blast was endemic to the region where wheat blast first occurred, early hypotheses focused on a

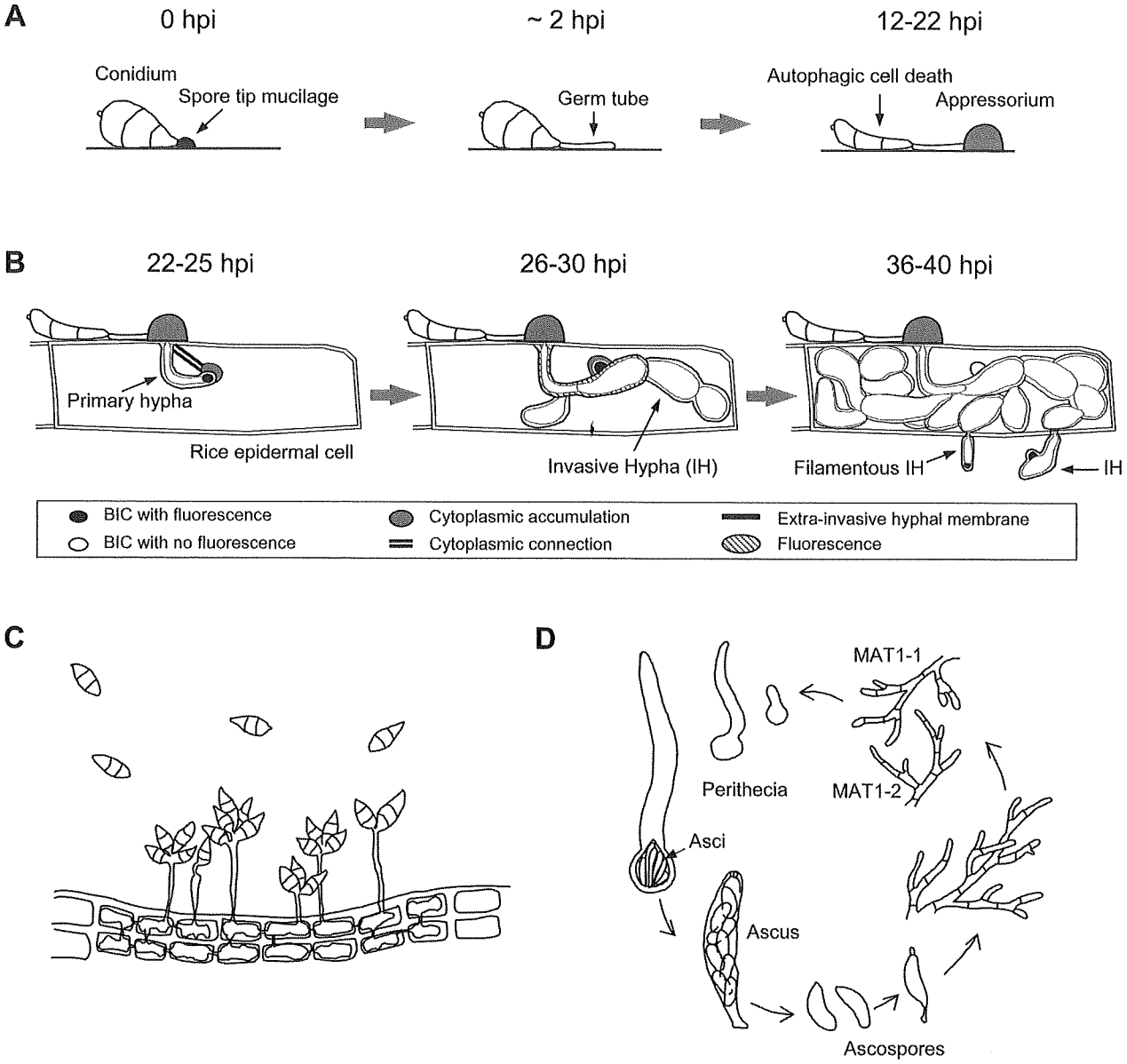

FIGURE 2 Asexual and sexual cycles of the rice blast fungus. (A) A conidium (25 to 30 by 9 to 12 μm) adheres to the leaf surface by using spore tip mucilage, produces a germ tube that senses the inductive surface, and differentiates an appressorium. A mature appressorium uses osmotically generated pressure to force a penetration peg through the plant cuticle and cell wall. (B) Inside the host cell lumen, the penetration peg becomes a filamentous primary hypha, accompanied by migration of cellular contents from the appressorium into the primary hypha. The primary hypha invaginates the host plasma membrane and secretes effectors, which are visualized by translational fusion of effector polypeptides with enhanced green fluorescent protein (GFP), into the membranous cap BIC at its tip at 22 to 25 h postinoculation (hpi). By 26 to 30 hpi, primary hyphae have differentiated into bulbous IH, which are sealed in an EIHM compartment. The BIC has moved beside the first differentiated IH, where it accumulates fluorescent effector proteins as long as IH grow in the cell. By 36 to 40 hpi, IH have undergone extreme constriction to cross the plant cell wall. In neighbor cells, the fungus first grows as filamentous IH secreting effector:GFP fusion proteins into tip BICs and then differentiates into bulbous IH with fluorescent side BICs. Subsequent cell invasions follow the same pattern. (C) Conidiogenesis in *M. oryzae* is holoblastic such that expansion and swelling of the conidiophore apex gives rise to a conidium, followed by a septum being formed to delimit the forming conidium (Howard 1994; Shi and Leung, 1995). The apex then grows to the side to produce the next conidium, resulting in three to five conidia borne sympodially on a conidiophore. (D) Sexual cycle: strains of opposite mating type mate to form pigmented perithecia (500 to 1,200 μm in length) with spherical bases (80 to 260 μm in diameter) and long cylindrical necks. Unordered asci contain eight hyaline, fusiform ascospores (16 to 25 by 4 to 8 μm), each with four cells and a single nucleus per cell.

host shift from rice to wheat. However, pathogenicity assays, the absence of rice pathogen-specific repetitive elements in the wheat isolates, and the extremely high sexual fertility of the wheat isolates compared to that of rice isolates proved that wheat pathogens were distinct from rice pathogens and that they were related to finger millet pathogens (Urashima et al., 1993). Interestingly, the wheat isolates from South America and GLS isolates from the United States and Japan are more closely related to each other than to other studied host-limited populations within the species complex (Viji et al., 2001; Farman, 2002a; Tosa et al., 2004). The growing intensities of wheat blast and GLS highlight the importance of understanding past host shifts and of determining the potential for new shifts to occur on additional crops.

Comparative Genomics

To facilitate understanding of *M. oryzae* infection strategies, the genome sequence was determined for strain 70-15, a fertile laboratory strain that is pathogenic on rice (Dean et al., 2005). Like most sexually fertile strains (Orbach et al., 1996), 70-15 has seven chromosomes. The assembled genome reported by Dean et al. (2005) contained 11,109 protein-coding genes, was 38.8 Mb in length, and was comprised of ~90% simple DNA sequence and ~10% repetitive DNA sequence. The currently available assembly 6, which is approaching a finished genome, has 11,074 predicted genes (http://www.broad.mit.edu/annotation/genome/magnaporthe_grisea/MultiHome.html). A community annotation database provides a valuable resource for analysis of *M. oryzae* genes (http://www.mgosdb.org/). The *M. oryzae* genome is enriched for putative secreted proteins, with estimates ranging from 7% (Dean et al., 2005) to 12% (Soanes et al., 2008) of all genes, depending on the prediction program used. Compared to the related pyrenomycete *Neurospora crassa*, expanded gene families in *M. oryzae* include many genes with a potential role in pathogenicity: genes encoding putative enzymes for degrading the plant cuticle and plant cell walls, genes for G-protein-coupled receptors (GPCRs) with potential roles in environmental sensing and response, and genes involved in production of secondary metabolites. The genome has expanded families for genes encoding small, secreted, cysteine-rich proteins. In other systems, these proteins often function as effectors, pathogen proteins secreted into host extracellular spaces or translocated into the cytoplasm of living host cells to control host defenses and cellular processes. From current experience, the subset of these effector genes, the avirulence (*AVR*) effector genes corresponding to rice *R* genes deployed by breeders in the field should exhibit increased polymorphism compared to genes not subject to *R*-gene-mediated selection. Comparing the 70-15 sequence with sequences from diverse rice pathogens should provide clues to identify AVR effectors.

Extensive expression data are available for fungal cell types, such as conidia, appressoria, perithecia, and mycelium from different culture conditions. These data include expressed sequence tags, serial analysis of gene expression, robust-long serial analysis of gene expression, massively parallel signature sequencing, and microarray analyses (Irie et al., 2003; Takano et al., 2003; Ebbole et al., 2004; Soanes and Talbot, 2005; Gowda et al., 2006). Whole *M. oryzae* genome microarray analysis has been performed for mycelium grown in nitrogen-rich and nitrogen-deficient media (Donofrio et al., 2006). In planta expression analyses have also been performed. Studies performed after macroscopic symptoms developed identified both fungal and rice genes expressed in planta (Kim et al., 2001; Rauyaree et al., 2001; Matsumura et al., 2003). Large-scale expressed sequence tag analysis (Jantasuriyarat et al., 2005) and microarray analysis (Vergne et al., 2007) performed at early infection stages before symptom appearance focused on rice gene expression because so little fungus was present in the infected leaf tissue. Mosquera et al. (2009) developed a robust procedure to obtain infected rice sheath tissue RNAs consisting of 20% RNA from biotrophic invasive hyphae (IH) growing in first-invaded cells, and they showed that these morphologically distinct IH specifically express many novel genes that encode putative secreted proteins.

Most repetitive sequences in *M. oryzae* are derived from transposable elements (Dean et al., 2005). Seven types of retrotransposons and three types of DNA transposons are prevalent in the 70-15 genome. These repetitive elements are not uniformly distributed in the genome but form discrete clusters. Analysis of these elements revealed that they have undergone extensive recombination during their evolution. These recombination events could cause deletions or inversions of the intervening sequences or chromosome translocations, which play important roles in genome evolution. Transposon-mediated recombination events may contribute to the *M. oryzae* genome instability that is characteristic of some subpopulations, including rice pathogens and perennial ryegrass pathogens. For example, most rice pathogens have lost sexual fertility and they have undergone extensive chromosome rearrangements (Talbot et al., 1993b; Orbach et al., 1996). Except for rare fertile strains, field isolates from rice often contain different numbers and sizes of chromosomes, even when they have similar molecular fingerprints. In addition, independent karyotype changes were found after prolonged serial transfer of one fungal strain in culture (Talbot et al., 1993b). This rice pathogen karyotype variability is associated with DNA deletions, inversions, and translocations. Genome instability of rice pathogens is also reflected by the rapid spontaneous mutation of certain genes involved in pathogenicity and host specificity, which tend to reside in transposon-rich regions of the genome. For example, rice pathogens undergo frequent spontaneous deletion of the *BUF1* melanin-biosynthesis gene (Valent et al., 1991), which is required for appressorial penetration. A genetic mechanism has been described for instability of certain *BUF1* alleles in sexual crosses (Farman, 2002b).

The abundance of transposable elements in the rice isolates from the field suggests that *M. oryzae* lacks the repeat-induced point mutation (RIP) mechanism described in the related pyrenomycete *N. crassa*. RIP is involved in detecting and mutating duplicated sequences during the sexual cycle. Dean et al. (2005) reported some low level of sequence signatures of RIP in some transposable elements in the 70-15 genome, but not in others, and concluded that RIP is not operating in *M. oryzae*. In contrast, Ikeda and colleagues (Ikeda et al., 2002) studied highly fertile wheat isolates from Brazil and concluded that a process similar to RIP functions in these strains. They reported that the frequency of transition mutations indicative of RIP correlated with the level of sexual fertility in the strains tested. This leads to the intriguing possibility that some host-specific populations within the *M. oryzae* species complex have different properties with regard to RIP as well as sexual fertility.

Another trait in which host-specific populations differ is telomere instability. Instability of telomere sequences in rice isolates has been documented, and indeed, many of the *AVR* genes identified are located near telomeres (Orbach et al., 2000; Farman, 2007). In contrast, telomeres from

foxtail millet and crabgrass isolates appear to be stable. Recently, it has been shown that ryegrass isolates responsible for GLS show unprecedented levels of telomere instability (Farman, 2007). Mechanisms and biological consequences for telomere instability traits in the host-specific field populations remain interesting subjects for analysis.

THE RICE BLAST DISEASE CYCLE: A NEW PARADIGM FOR HEMIBIOTROPHY

The rice blast pathogen undergoes extensive development to execute its disease cycle (Fig. 2A through C). *M. oryzae* was believed to be a hemibiotroph similar to *Colletotrichum* species, which invade one or a small number of host cells biotrophically and then undergo a distinct switch to necrotrophic killing of host cells before invading them (O'Connell and Panstruga, 2006). To investigate this, Berruyer et al. (2006) transformed a rice pathogen to express cytoplasmic enhanced yellow fluorescent protein (YFP) and used fluorescence stereomicroscopy to follow individual lesions from appressoria to macroscopically visible leaf spots. They found that the fungal growth front always preceded visible symptoms even at later stages, and asymptomatic infected regions were common. From these results and characteristics of sequential cell invasions in rice sheath epidermal cells, Kankanala et al. (2007) concluded that, at least in the highly susceptible interaction, there was not a distinct switch from biotrophy to necrotrophy as occurs for *Colletotrichum* species. Instead, rice blast defines a new paradigm in hemibiotrophy whereby each successive plant cell invasion is biotrophic but invaded plant cells die by the time the fungus moves to living neighbor cells (Kankanala et al., 2007). Many molecular and cellular details of the blast disease cycle have emerged (see also Talbot, 2003; and Ebbole, 2007).

Appressorium Formation and Function

Extensive structural and functional analyses have been reported for appressorium formation, which involves a series of developmental and metabolic processes (Bourett and Howard, 1990; Howard and Valent, 1996; Talbot, 2003; Ebbole, 2007). As shown in Fig. 2A, rice blast disease is initiated by conidia landing on the leaf surface and attaching tightly by virtue of a glue, called spore tip mucilage, that is released from the conidial apex upon hydration (Hamer et al., 1988). Conidia germinate to produce specialized germ tubes that recognize the physicochemical features of the surface they are contacting. Upon recognition of an inductive surface (one that promotes appressorium development), the germ tube ceases apical growth. The germ tube apex swells and hooks, and apical vesicles polarize toward the host surface (Bourett and Howard, 1990). The germ tube apex continues to enlarge and develops a symmetrical dome-shaped appressorium. A mitotic division occurs, and one of the daughter nuclei migrates into the developing appressorium (Bourett and Howard, 1990; Veneault-Fourrey et al., 2006). A septum forms between the appressorium and the remainder of the germling, leaving the appressorium as a separate unicellular structure with a single nucleus. At this point, appressorial melanization occurs. As the appressorium matures, it strongly adheres to the host cuticle and generates the highest turgor pressure known in any living organism, as high as 8.0 MPa. This pressure, which is equivalent to the pressure felt at 800 m under water or 40 times that of an automobile car tire, is translated into mechanical force to puncture the plant cuticle with a penetration peg (Howard et al., 1991; de Jong et al., 1997; Ebbole, 2007). Appressorium function demands an autophagic process, regulated by at least the *MgATG8* gene, to recycle the contents of the appressorium-bearing conidium before plant penetration (Veneault-Fourrey et al., 2006).

Appressorial development can be induced in vitro and on various artificial surfaces, including cellophane, Teflon, Mylar, and polyvinyl chloride, which has been very useful in identifying conditions, signal molecules, or genes that regulate appressorium development (Valent, 1997; Talbot, 2003; Ebbole, 2007). For instance, exogenous addition of chemicals, such as secondary messengers or their analogs (cyclic AMP [cAMP] or diacylglycerol [DAG]), or plant components (cutin or lipid monomers) induces appressorium development by germinating conidia on noninductive hydrophilic surfaces, whereas some other chemicals suppress the developmental process on inductive hydrophobic surfaces (Lee and Dean, 1993; Thines et al., 1997). Oh et al. (2008) have identified 357 genes, referred to as appressorium-consensus genes, which are differentially expressed during appressorium formation in vitro.

The cAMP signaling pathway in eukaryotes involves production of cAMP by an adenylate cyclase and activation of a protein kinase A (PKA) by cAMP binding to its regulatory subunit. This releases the catalytic subunit of PKA (CPKA) for phosphorylation of target proteins. Mutants with targeted deletion of the *Magnaporthe* adenylate cyclase (*MAC1*) gene fail to form appressoria (Choi and Dean, 1997). The defect was restored by addition of cAMP or by a mutation in a regulatory subunit gene of PKA (*SUM1*, for suppressor of the Mac1$^-$ phenotype) that causes constitutive activation of PKA (Choi and Dean, 1997; Adachi and Hamer, 1998). Mutants with deletion of *CPKA* still produce appressoria, although they are small and nonfunctional (Mitchell and Dean, 1995; Xu et al., 1997). On a noninductive surface, the CpkA$^-$ mutants respond to exogenous cAMP, suggesting the presence of an additional catalytic subunit of PKA (Xu et al., 1997). The *CPKA/SUM1*-encoded PKA regulates turgor generation in appressoria through rapid degradation of lipid and glycogen reserves (Thines et al., 2000). Direct measurement of cellular cAMP in germ tubes showed significantly higher accumulation of cAMP in the germ tubes growing on an inductive surface than on a noninductive surface (Liu et al., 2007a), confirming the role of cAMP in appressorium morphogenesis. How the fungus senses the nature of the contact surface and triggers cAMP signaling is currently unclear, but this might involve surface proteins secreted during appressorium formation. Mutants lacking MPG1 (a fungal hydrophobin) or CBP1 (a chitin-binding protein) are inefficient in producing appressoria (<10% of wild-type frequencies), but an increase of cellular cAMP restores appressorium formation in respective mutants, suggesting their roles upstream of the cAMP signaling pathway (Talbot et al., 1993a, 1996; Beckerman and Ebbole, 1996; Kamakura et al., 2002).

One of the expanded gene families in *M. oryzae* encodes GPCRs, transmembrane receptors in eukaryotes that sense extracellular signals and activate signal transduction pathways. In fungi, GPCRs are involved in the regulation of contact-dependent morphogenesis (Kulkarni et al., 2005; Kumamoto, 2008). *M. oryzae* has 76 GPCR-like proteins (Kulkarni et al., 2005), among which PTH11 is proposed to have a role in contact surface recognition (DeZwaan et al., 1999). Mutation of *PTH11* causes a defect in appressorium maturation but does not impair any other stages of the life cycle. Appressorium formation in the *pth11*$^-$ mutants is restored by addition of cAMP and DAG, indicating that PTH11 acts upstream of cAMP/PKA and DAG/protein

kinase C (PKC) signaling pathways. Heterotrimeric G proteins interact with GPCRs to relay signals to intracellular responders, such as adenylate cyclase, phospholipases, kinases, and ion channels (Li et al., 2007b). *M. oryzae* contains three Gα subunits (MagA, MagB, and MacC [Liu and Dean, 1997]), two Gβ subunits (Mgb1 and Mgb2 [Nishimura et al., 2003]), and one Gγ subunit (Liang et al., 2006). Among the Gα subunits, appressorium formation and virulence was impaired in *magB*$^-$ mutants but not in *magA*$^-$ and *magC*$^-$ deletion mutants (Liu and Dean, 1997; Fang and Dean, 2000). Disruption of the *magB* gene, however, caused pleiotropic effects, including defects in conidiation, sexual development, mycelial growth, and virulence, indicating a common involvement of G protein signaling in regulating cellular activities. All three Gα subunits interact with the regulator of G protein signaling, RGS1 (Liu et al., 2007a). Rgs1$^-$ mutants produce appressoria efficiently on both inductive and noninductive surfaces, which suggests that loss of RGS1 uncouples surface dependency during appressorial differentiation (Liu et al., 2007a). Similar to Rgs1$^-$ mutants, Rgs-insensitive or constitutively-active MagA strains accumulate high levels of cAMP and produce appressoria efficiently on inductive and noninductive surfaces. These results suggest a signaling pathway of MagA-dependent adenylate cyclase activation, resulting in an increase of cAMP.

The roles of mitogen-activated protein kinases (MAPKs) in fungal developmental processes and pathogenicity have been reviewed extensively (Xu, 2000; Zhao et al., 2007). In *M. oryzae*, three MAPKs, PMK1 (for pathogenicity MAPK), MPS1 (MAPK for penetration and sporulation), and OSM1 (for osmoregulation MAPK), are involved in infection-related development. PMK1, a functional homolog of the *Saccharomyces cerevisiae* FUS3/KSS1 kinases, regulates appressorium formation and is necessary for penetration and invasive growth (Xu and Hamer, 1996). PMK1 and two upstream components Mst7 (MAPK kinase) and Mst11 (MAPKK kinase) form the Mst11-Mst7-Pmk1 pathway. The adaptor protein Mst50 interacts with Mst11 and Mst7, and integrates diverse signals from Mgb1, Cdc42, Ras1, and Ras2 (Zhao et al., 2007). Activated PMK1 then moves into the nucleus and regulates target genes, including *MST12* (transcription factor) and *GAS1* and *GAS2* (encoding small proteins specifically expressed in appressoria) (Zhao et al., 2007). The PMK1 pathway also controls transfer of storage carbohydrates and lipids to the developing appressorium, where these storage reserves are rapidly degraded under control of the PKA pathway (Thines et al., 2000). *M. oryzae* MPS1 is required for appressorium function (Xu et al., 1998). MPS1 is a functional homolog of yeast SLT2, which regulates cell wall integrity. MPS1 appears to regulate cell wall remodeling during appressorium formation and polarity establishment required for producing a penetration peg. Mps1$^-$ mutants produce appressoria that are melanized but fail to penetrate and develop invasive hyphae. OSM1 appears to play a role in appressorium morphogenesis, but it is dispensable for plant infection (Dixon et al., 1999). OSM1 is a functional homolog of *S. cerevisiae* osmosensory MAPK HOG1 (for high-osmolarity glycerol) that regulates glycerol accumulation to maintain cellular turgor in response to hyperosmolarity. The *osm1*$^-$ mutants are sensitive to osmotic stress, but they do not have defects in glycerol accumulation and turgor generation in appressoria, or in plant infection, indicating that glycerol generation is not regulated by OSM1 in *M. oryzae*. Interestingly, *osm1*$^-$ mutants produce multiple appressoria under chronic hyperosmotic stress, suggesting a negative role of OSM1 in suppressing the PMK1 appressorium development pathway during stressful conditions.

Melanin and glycerol play key roles in the generation of hydrostatic pressure in the appressorium. Melanin is a gray pigment produced by polymerization of the polyketide precursor 1,8-dihydroxynaphthalene (DHN) and is deposited between the appressorial cell wall and membrane (Howard and Valent, 1996). The high turgor pressure in melanized appressoria is generated by solute molecules, including glycerol, that accumulate to high levels through metabolism of glycogen, trehalose, and lipids, which are the most abundant storage products in conidia (de Jong et al., 1997; Wang et al., 2005). Autophagy is a primary intracellular catabolic mechanism for degrading and recycling organelles and proteins during cellular development (Levine and Klionsky, 2004). Mutants lacking each of six autophagy-related genes (*MgATG1*, *-2*, *-4*, *-5*, *-9*, and *-18*), which were identified based on homology to corresponding *S. cerevisiae ATG* genes, failed to penetrate the host due to lower levels of appressorial turgor pressure, providing evidence that glycerol production in appressoria requires the turnover of cytoplasmic contents of conidia (Liu et al., 2007b). Nonmelanized appressoria fail to generate turgor pressure, due to the lack of efficient accumulation of glycerol, thus resulting in nonpathogenicity (Chumley and Valent, 1990; Howard et al., 1991; de Jong et al., 1997). Melanin biosynthesis genes *ALB1* (MGG_07219.5), *RSY1* (MGG_05059.5), and *BUF1* (MGG_02252.5), respectively named for their albino, rosy, and buff pigmentation in culture (Chumley and Valent, 1990), encode a polyketide synthetase, scytalone dehydratase, and trihydroxynaphthalene reductase, respectively. All three genes are highly induced during appressorium formation but dramatically repressed when the fungus enters the host cell and establishes biotrophic invasion (Oh et al., 2008; Mosquera et al., 2009). This suggests that melanin biosynthesis is required for penetration but not for biotrophic colonization. Genes for two putative transcription factors, *PIG1* (MGG_07215.5) and *HTF* (MGG_07218.5), are linked to *ALB1* (MGG_07219.5) and the *BUF1* homolog *4HNR* (MGG_07216.5) on chromosome 1 (Oh et al., 2008; Valdovinos-Ponce, 2007). Mutants lacking the PIG1 transcription factor fail to produce melanin in vegetative hyphae, but the appressoria are still melanized, suggesting that a different mechanism regulates appressorial melanization (Tsuji et al., 2000). Deletion of *HTF* does not cause a defect in melanization of mycelia or appressoria or a reduction in pathogenicity (Oh et al., 2008; Valdovinos-Ponce, 2007). Regulatory genes for appressorial melanization remain to be determined.

Although mechanical pressure appears to be the primary means of plant cuticle penetration, cell surface-modifying enzymes have been implicated in facilitating penetration (Howard et al., 1991; Skamnioti and Gurr, 2007). The cuticle is the outermost barrier of plants, and fungi secrete cutinases, serine esterases that hydrolyze cutin, the main component of plant cuticle (Kolattukudy, 1985). *M. oryzae* has 17 putative cutinase genes, which is the largest number found among all fungal genomes sequenced so far (Dean et al., 2005; Skamnioti et al., 2008). For example, the *Fusarium graminearum* and *N. crassa* genomes contain 12 and 3 cutinases, respectively. Unlike the *CUT1* gene (Sweigard et al., 1992), which is not differentially expressed during appressorial development, the *CUT2* gene (MGG_09100.5) shows dramatic upregulation during penetration (Skamnioti and Gurr, 2007). The *cut2*$^-$ mutant is much less pathogenic than the wild type due to a defect in penetration peg formation. Surface adhesion and appressorium turgor generation are unaffected in the

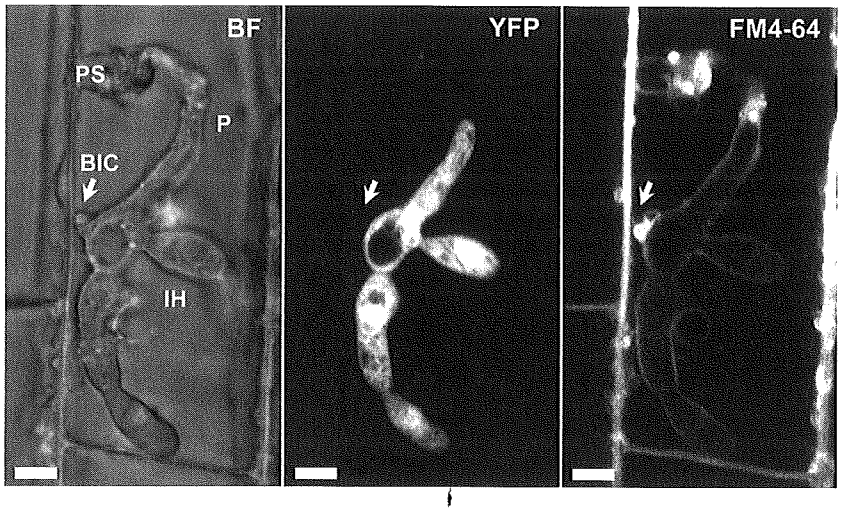

FIGURE 3 The EIHM tightly wraps the IH and prevents the endocytotic tracker dye FM4-64 from reaching IH membranes. At 29 hpi, an IH of a fungal transformant expressing cytoplasmic enhanced yellow fluorescent protein (YFP) is viewed by bright-field optics (left panel) and by YFP (middle panel) and FM4-64 (right panel) fluorescence (both shown as white). At this site, the primary hypha (P) extending from the appressorial penetration site (PS) had lost viability after IH formed (observed in ~50% of all infection sites). A BIC (arrow) beside the first IH cell is rich in FM4-64-stained membranes that are continuous with EIHM. Bars, 5 μm.

cut2^{-} mutant, providing direct evidence for a role for cutinase in penetration (Skamnioti and Gurr, 2007).

Biotrophic Invasive Hyphae Successively Colonize Live Rice Cells

Excellent cytological and ultrastructural analyses have been reported for the blast fungus interacting with host plant cells after penetration (reviewed by Kankanala et al., 2007). Once inside the epidermal cell, the penetration peg expands to form a narrow filamentous primary hypha that differentiates into thicker, bulbous IH in the compatible interaction (Heath et al., 1990). New details of biotrophic cell invasion have been described based on live-cell imaging in optically clear, excised rice leaf sheaths (Koga et al., 2004; Kankanala et al., 2007). Fungal and host cellular components were visualized with fluorescent dyes and reporter proteins. For example, studies with the endocytotic tracer dye FM4-64 answered the long-standing question of whether blast biotrophic hyphae breach the host plasma membrane to grow in direct contact with cytoplasm of the invaded cell or if they remain separated from the cytoplasm by invaginated plant plasma membrane (Kankanala et al., 2007). FM4-64 dye inserts into a cell's plasma membrane, moves by lateral diffusion in the membrane, and is actively internalized into the cell by the endocytotic pathway. The dye inserted into membranes of the rice sheath cells and into membranes of the primary hyphae, which appeared to invaginate the rice plasma membrane. In contrast, bulbous IH were precisely outlined by the dye (Fig. 3). The IH appeared to have been protected from FM4-64 insertion in their plasma membranes, presumably because they were sealed in plant membrane, named the extrainvasive-hyphal membrane (EIHM). Transmission electron microscopy of infected sheath samples, prepared by high-pressure freezing and freeze substitution to allow better membrane preservation, confirmed the presence of EIHM around IH. Apparently, differentiation of the primary hypha to IH included sealing the hyphae in an EIHM-bound compartment, where FM4-64 could not reach IH cellular membranes.

Live-cell imaging confirmed that sequential rice cell invasions were biotrophic, because invaded cells appeared to be healthy and plasmolyzed in sucrose solution, indicating an intact plasma membrane in the cell (Kankanala et al., 2007). Host cells failed to plasmolyze shortly before IH moved into neighbor cells, indicating that invaded host cells were no longer viable at that time.

Confocal imaging combined with time-lapse experiments demonstrated that IH appear to search along the plant cell wall for specific locations to cross into neighboring cells (Kankanala et al., 2007). Once found, the IH swell and send tiny penetration peg-like structures, the IH peg, across the cell wall (Fig. 2B). The extreme degree of constriction that IH undergo as they cross the plant cell wall is shown in Fig. 4. Additional transmission electron microscopy showing correlations of wall crossing points and pit fields with clustered plasmodesmata, failure of IH to enter guard cells that lack plasmodesmata, and additional observations strongly suggest that IH exploit plasmodesmata for movement into living neighbor cells (Kankanala et al., 2007).

Studies of in planta secretion of fluorescently labeled blast AVR effectors led to the discovery of a complex, pathogen-induced structure, the biotrophic interfacial complex (BIC), which accumulates these secreted proteins (R. Berruyer, C. H. Khang, P. Kankanala, S.-Y. Park, K. Czymmek, S. Kang, and B. Valent, unpublished data). The experiments involved the AVR-Pita1, PWL1, and PWL2 effectors, which are predicted to be translocated into the cytoplasm of living rice cells. Effector secretion was observed in planta using fungal transformants that expressed translational fusions of various fluorescent proteins at the C termini of different effector polypeptides under control of their native promoters. This analysis showed that BICs develop in two stages coupled to differentiation of intracellular filamentous hyphae into IH (Fig. 2). First, fluorescent effector proteins were secreted into the dome-shaped "membranous caps," extensions of the EIHM at the tips of primary hyphae in first-invaded cells (Fig. 2B, 22 to 25 h postinoculation [hpi]) and of filamentous IH in subsequently invaded cells (Fig. 2B, 36 to 40 hpi).

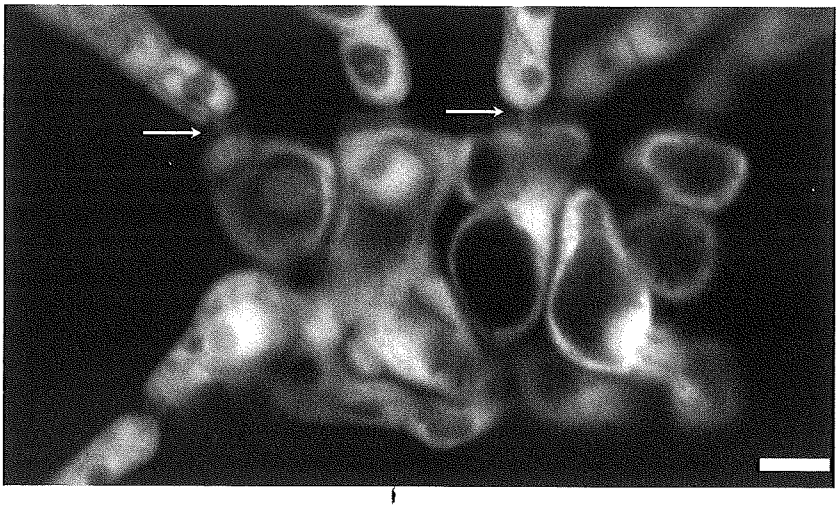

FIGURE 4 IH exhibit extreme constriction as they cross the rice cell wall at 32 hpi. YFP fluorescence in the fungal cytoplasm (in white) is shown alone to highlight the constriction (arrows). Bar, 5 μm. Reproduced with permission from Kankanala et al., 2007.

When these filamentous hyphae first swelled into IH, fluorescent EIHM caps moved to form a visible structure beside the differentiating IH cell (Fig. 2B, 26 to 30 hpi and 36-40 hpi). Fluorescent effectors remained localized to the BIC region as long as IH continued to grow in the rice cell (Fig. 2B). Secreted effector fusions partially colocalized with an aggregation of plant endocytotic membranes that labeled with FM4-64 (Fig. 3, arrows). Host cytoplasmic dynamics were focused around both the membranous cap- and IH-BICs during the early hyphal differentiation stage. Correlative light and electron microscopy showed that BICs corresponded to the complex aggregations of lamellar membranes and diverse vesicles previously reported between the IH cell wall and EIHM (Kankanala et al., 2007). Accumulation of secreted effectors and dynamic connections with host cytoplasm suggested that BICs are a hub of communication between IH and the host cell, with a likely role in effector secretion into the host cytoplasm.

Other than *AVR* effector genes and a nucleus-localized protein, MIR1 (Li et al., 2007a), genes that were specifically expressed by biotrophic IH have not been identified. Identification of fungal and rice genes expressed during biotrophic invasion in leaves has been difficult because so few host cells have encountered the pathogen at early infection stages (Berruyer et al., 2006). After enriching for IH RNA (up to 20% of the total) in infected rice sheath tissue, Mosquera et al. (2009) identified many genes that were highly upregulated in IH, including many encoding novel biotrophy-associated secreted (BAS) proteins that are excellent candidate effectors. Functional analyses of these candidate effector genes and of the host "effector-triggered-susceptibility" genes will begin to associate molecular mechanisms with the cell biology of biotrophic invasion of rice.

Necrotrophic Growth and Sporulation

Necrotrophic hyphae in blast disease presumably follow invasion by biotrophic IH (Berruyer et al., 2006), perhaps around the time that visible symptoms occur, in order to utilize host cell walls and other nutrients before sporulation. Extensive evidence suggests that necrotrophic hyphae do play a role in tissue colonization. Cytological studies have shown extensive destruction of mesophyll cells in some fungus-plant interactions (Heath et al., 1990; Rodrigues et al., 2003). The *M. oryzae* genome contains many genes that encode known and hypothetical plant cell wall-degrading enzymes, and these genes belong to expanded gene families (Dean et al., 2005). For example, there are 20 putative xylanase encoding genes in the *M. oryzae* genome (Wu et al., 2006). Mutants lacking individual *XYL1*, *XYL2*, *XYL6*, or both *XYL1* and *XYL2* genes were as pathogenic as the wild type, indicating that they are not individually required for pathogenicity. How these enzymes contribute when the fungus infects plant tissues remains to be determined (Wu et al., 1997, 2006).

The rice blast fungus produces a variety of phytotoxic metabolites, including tenuazonic acid (TA), picolinic acid, piricularine, pyriculol, pyriculariol, coumarin, tyrosol, and 9,12-octadecadienoic acid (Valent and Chumley, 1991; Park et al., 2008), which have been associated with blast lesion development. When dropped on a leaf surface, TA induces local necrosis resembling blast lesions, and TA-deficient mutants produce fewer lesions than the wild-type strain (Aver'yanov et al., 2007). The blast fungus, strictly known as a monocot pathogen in the field, was recently shown to infect certain ecotypes of *Arabidopsis thaliana*, a dicot model plant (Park et al., 2008). Unlike hemibiotrophism on rice and other grass hosts, infection of *A. thaliana* appeared to be necrotrophic and occurred with the aid of phytotoxic metabolites, including 9,12-octadecadienoic acid. Some of the blast fungal metabolites, such as TA and picolinic acid, were demonstrated to be hypersensitive-response elicitors, inducing resistance responses in rice (Zhang et al., 2004; Aver'yanov et al., 2007). Further studies are needed to identify the biosynthetic pathways for these metabolites and to understand their roles in contributing to pathogenicity or to resistance responses.

PKS-NRPS, hybrids of polyketide synthases and nonribosomal peptide synthetases, are newly discovered fungal enzymes involved in secondary metabolite biosynthesis. *M. oryzae* contains 9 PKS-NRPS genes (Collemare et al., 2008). Appressorium-specific expression of *ACE1* (the *AVR* gene described below), *SYN2*, and *SYN8* suggests that the resulting metabolites play a role during penetration, possibly by inhibiting defense responses of the epidermal cells. *SYN6* was also suggested to play a role in colonization. Single-deletion mutants lacking *ACE1*, *SYN2*, and *SYN6* were as pathogenic as the wild type on susceptible rice, suggesting that

the corresponding metabolites are not required for infection or that functionally redundant metabolites are produced.

The rice blast fungus produces elliptical lesions with grey centers (from abundant conidiophores) under sporulating conditions of high humidity (Fig. 1). Conidiation requires a period of darkness as a cue for conidial development, regulated by a blue-light receptor, mgwc-1 (MG03538.4; ortholog of *N. crassa* white collar-1), and mature conidia are released in the dark (Barksdale and Asai, 1961; Lee et al., 2006). Previous genetic studies have identified several loci (e.g., *CON*, *ACR*, and *SMO*) that control conidiation and conidial morphology (Hamer et al., 1989; Shi and Leung, 1995; Lau and Hamer, 1998). Strains with mutations of *CON5* and *CON6* fail to produce conidia. *CON5* was found to be epistatic to *CON6* because the *con5*$^-$ mutant produces no conidiophores, but the *con6*$^-$ mutant produces conidiophores that do not bear conidia (Shi and Leung, 1995). Four other genes, *CON1*, *CON2*, *CON4*, and *CON7*, appear to act downstream from *CON5* and *CON6*, because these mutants show defects in conidiation (Shi and Leung, 1995). CON7 encodes a transcription factor regulating expression of genes involved in morphogenesis, including the GPCR gene *PTH11* and genes involved in cell wall formation (Odenbach et al., 2007).

Mutations that affect conidiation are often associated with defects in appressorium formation and pathogenicity, as observed in a variety of mutants, including Con$^-$, Acr1$^-$, Smo$^-$, Cut2$^-$, MagB$^-$ (Hamer et al., 1989; Shi and Leung, 1995; Liu and Dean, 1997; Lau and Hamer, 1998; Liu et al., 2007a; Skamnioti and Gurr, 2007), Mac1$^-$ (Choi and Dean, 1997), and Mps1$^-$ (Xu et al., 1998) mutants. This can be explained, at least partly, by the fact that conidiation and infection structure differentiation in fungi are regulated by G-protein-mediated signaling (Li et al., 2007b). In *M. oryzae*, Rgs1 and MagB in fact control conidiation (Liu et al., 2007a). Overexpression of Rgs1 inhibits conidiation, and the deletion of Rgs1 leads to hyperconidiation. Consistent with this, RGS-insensitive MagB also shows hyperconidiation.

Jeon et al. (2007) analyzed 21,070 transferred-DNA-tagged mutants by using a high-throughput screening system for seven phenotypes. Many of the mutants that are impaired in pathogenicity (559 mutants) are also impaired in asexual development, i.e., conidiation and conidial morphology (207 mutants; 37%), and 66% of the latter mutants are also defective in appressorium formation. This study identified a putative GTPase (MGG_02731; RAC homolog from *Colletotrichum trifolii*) and a protein involved in protein trafficking through the Golgi network (MGG_02423; ERD2 homolog from *S. cerevisiae*) that play roles in asexual development.

HOST SPECIFICITY IN RICE BLAST DISEASE

Effector Action in Pathogenicity and Resistance

Blast effector proteins are presumed to be delivered to the cytoplasm of living host cells to control host defenses and promote disease. *AVR* genes, whose products trigger hypersensitive resistance in invaded cells, encode effectors that happen to be recognized by corresponding *R* genes. Map-based cloning strategies based on measuring this AVR activity resulted in cloning of four *AVR* genes (Ebbole, 2007). Two of these are *AVR-CO39*, encoding a small polypeptide (Farman et al., 2002), and *ACE1*, encoding a PKS with a C-terminal NRPS that is expressed only in appressoria (Böhnert et al., 2004). The *PWL* genes are *AVR* genes that confer host species specificity by preventing the fungus from infecting weeping lovegrass (Kang et al., 1995; Sweigard et al., 1995). The *PWL2* gene occurs in rice-infecting strains, and it encodes a 145-amino-acid, secreted, glycine-rich, hydrophilic protein. *PWL1* encodes the *PWL2* ortholog from finger millet-infecting strains, and it encodes a 147-amino-acid secreted protein with 75% amino acid identity to PWL2 protein. *AVR-Pita1* stands out as the strongest candidate for a fungal effector gene. *AVR-Pita1* (Orbach et al., 2000; Khang et al., 2008), the *AVR* gene corresponding to the blast resistance gene *Pi-ta* (Bryan et al., 2000), encodes a putative zinc metalloprotease that is specifically expressed by biotrophic hyphae in planta. In yeast two-hybrid assays and in in vitro membrane binding assays, the mature protease binds specifically to the leucine-rich domain at the C terminus of the Pi-ta resistance protein (Jia et al., 2000). A rice biolistic transient expression assay indicated that the mature AVR-Pita1 metalloprotease functions in rice cells to trigger Pi-ta-mediated hypersensitive resistance (Jia et al., 2000). These results, together with the probable localization of *Pi-ta* in the plant cytoplasm, suggest that the blast fungus delivers the mature protease inside rice cells. How AVR-Pita1 functions as an effector inside the host cells remains to be discovered.

It is important to understand how the fungus so quickly defeats *R* genes deployed for disease control. In laboratory studies, some blast *AVR* genes undergo frequent spontaneous mutations and deletions due to flanking repetitive sequences and to location in the unstable regions near telomeres (Sweigard et al., 1995; Orbach et al., 2000). Examples of spontaneous transposon-mediated insertional mutation events that inactivated *AVR* genes and changed the cultivar specificity of rice blast strains have been documented for *AVR-Pita1* in laboratory studies (Kang et al., 2001) and for *AVR-Pita1* and *ACE1* in the field (Fudal et al., 2005; Zhou et al., 2007). Clearly, the abundant repetitive sequences in *M. oryzae* contribute to rapid genome evolution, and the resulting loss of gene function can change host cultivar specificity.

Although there is no direct evidence, circumstantial evidence suggests that AVR-Pita and PWL effectors function in pathogenicity for hosts lacking corresponding *R* genes (Kang et al., 1995; Sweigard et al., 1995, Khang et al., 2008). The *AVR-Pita* and *PWL* genes are members of multigene families that are widely distributed among isolates from diverse host species, and some members lack AVR activity, suggesting functional divergence as well as positive selection for maintenance in pathogen populations. The presence of rice-specific functional AVR-Pita members among strains nonpathogenic to rice also suggests they have roles during infection. In planta specific expression of *AVR-Pita* and *PWL* genes also supports a role in pathogenicity.

Host Species Specificity

The recent emergence of wheat blast and GLS as serious disease problems highlights the importance of understanding the molecular and mechanistic bases of host species specificity in the *M. grisea* species complex. Multiple examples of single genes that confer host species specificity have been described since the original report by Yaegashi in 1978 (Yaegashi, 1978). These include *PWL1* from finger millet pathogens and *PWL2* from rice pathogens, which prevent these strains from infecting weeping lovegrass (Kang et al., 1995; Sweigard et al., 1995). Tosa and colleagues crossed a wheat isolate with a *Setaria* isolate, an oat isolate, and a rice isolate and identified five loci that impact pathogenicity toward wheat. The *PWT1* and *PWT2* genes were identified from both rice and *Setaria* pathogens (Murakami et al., 2000;

Tosa et al., 2006), and the *PWT3* and *PWT4* genes were identified from the oat pathogen (Takabayashi et al., 2002). A fifth gene, *PWT5*, was identified only in the rice pathogen (Tosa et al., 2006). Interestingly, these genes induced different physiological responses in wheat; *PWT1* and *PWT5* induced a hypersensitive response, and *PWT2* induced papillae that block fungal entry into the host tissue. The *PWT3* gene from the oat isolate appeared to be a temperature-sensitive *AVR* gene (Takabayashi et al., 2002). The other gene, *PWT4*, exhibited wheat cultivar specificity, and identification of a corresponding wheat *R* gene confirmed a gene-for-gene relationship in wheat blast (Takabayashi et al., 2002). Similar results were reported for specificity towards *Setaria* spp. (Murakami et al., 2003). An independent study identified two *AVR* genes from a *Digitaria* isolate, *PRE1* and *AVR2*, which control rice cultivar specificity (Chen et al., 2006). Not all differences in host species specificity are due to *AVR*-like genes. One study showed polygenic segregation of genes from a finger millet pathogen controlling the extent of lesion development on rice, as well as *AVR* genes corresponding to different rice cultivars (Valent et al., 1991). However, *AVR* genes are clearly associated with host species specificity. The simple genetic differences between species-adapted isolates suggested that these strains are genetically close and that they could evolve to infect different grass species.

Effector Secretion

Biotrophic or hemibiotrophic fungi must deliver effector proteins across plant cell membranes into living plant cells in order to block defenses and control cell metabolism. This process is not understood for any fungus, even though extensive research is focused on the type III secretion system used by bacteria for delivering effectors inside host cells (Alfano and Collmer, 2004) and on the roles for protein translocation motifs (RXLR) in delivering oomycete effectors into the host cytoplasm (Morgan and Kamoun, 2007). For rice blast disease, the increasing numbers of *AVR*-like genes that control host specificity and the large number of R proteins that are predicted to be localized in the rice cytoplasm are consistent with the hypothesis that *M. oryzae* translocates many effectors into the host cytoplasm. With the exception of ACE1, known effector proteins have N-terminal signal peptides for entry into the endoplasmic reticulum (ER)-mediated secretion pathway. Involvement of ER-mediated secretion was confirmed by Yi et al. (2009) when they demonstrated that LHS1, the ER-localized heat shock protein 70 (HSP70) chaperone with an important role in ER import and protein folding, is required for effector secretion. Differential accumulation of known effectors (Berruyer et al., unpublished) and some BAS proteins (Mosquera et al., 2009) in BICs at the hypha-rice cell interface, together with characterization of Golgi-localized APT2 with a role in effector secretion (Gilbert et al., 2006), suggested that the effector secretion pathway might diverge after entry into the ER. The disproportionate need for proper protein secretion during biotrophic tissue colonization, demonstrated with *lhs1*$^-$ mutants (Yi et al., 2009), shows the importance of understanding protein secretion mechanisms in *M. oryzae*.

After secretion from the fungus, a subset of BAS proteins colocalize with effectors in BICs (Mosquera et al., 2009), supporting a hypothesized role for BICs in translocation of effectors into the host cytoplasm. Recent results showed that indeed, fluorescent effector proteins that accumulate in BICs are delivered to the cytoplasm of invaded cells (C. H. Khang and B. Valent, unpublished results). Interestingly, these fluorescent effectors also enter neighboring cells that are not yet invaded by the fungus. The secreted protein BAS4, which accumulates uniformly throughout the interfacial matrix surrounding IH, was not translocated to the rice cytoplasm. Thus, the way is open to understand effector translocation mechanisms, to identify fungal translocation motifs, and to identify the set of effectors in the *M. oryzae* genome.

FUTURE PROSPECTS

The rice blast system offers unique opportunity for basic research on fungal hyphal development and host interactions impacting a major plant disease and for applied research on developing effective strategies for achieving durable resistance. Towards these goals, major efforts must go into identifying the entire sets of effectors required for disease, into understanding how these effectors are delivered into living host cells, and into defining effector functions in promoting disease or triggering resistance. These studies are now being facilitated by the growing blast genome and cell biological resources. Great progress has been made in understanding pathogenicity mechanisms, especially before penetration into the host tissue, but much remains to be learned. For example, although signal transduction pathways are relatively well understood, little is known about the initiating signaling cues or the downstream morphogenetic responses. Interesting questions concern how the fungus executes its amazing biology associated with appressorial penetration pegs breaching the tough plant surface and then gently colonizing living cells, and associated with IH pegs, perhaps co-opting plasmodesmata to enter living neighbor cells. Much remains to be learned in understanding biotrophic invasion, the switch to necrotrophic growth, and sporulation.

The *M. grisea* species complex is a thriving collection of host plant-adapted populations. The rice-infecting population has so far eluded decades of effort on sustainable control strategies, and the recently emerged diseases on wheat and on perennial ryegrass appear to be just as difficult to control. In addition to understanding mechanisms of rice cultivar specificity, the *M. grisea* species complex presents exciting opportunities for understanding the mechanisms of host species specificity and fungal population shifts to specialize on new plant species. Increasing evidence suggests that, like rice cultivar specificity, host species specificity is often due to *R*-gene-mediated recognition of blast effector proteins. Comparative genomics should help identify effector sets that confer host species specificity as well as host cultivar specificity. Understanding how *AVR* effector genes are evolving in the field holds great potential for discovering novel routes to durable disease resistance.

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