MINIREVIEW

Dimorphism in fungal plant pathogens

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Abstract

Fungi are mostly sessile organisms, and thus have evolved ways to cope with environmental changes. Many fungi produce ‘dormant’ structures, which allow them to survive periods of unfavorable conditions. Another ingenious active approach to a changing environment has been adopted by the ‘dimorphic fungi’, which simply shift their thallic organization as a way to adapt and thrive in the new conditions. Dimorphism is extensively exploited by both plant and animal pathogenic fungi, where the encounter with the host prompts a shift in the mode of growth. In this review, we focus on the phenomenon of dimorphism among plant pathogenic fungi through discussion of several relatively well-studied exemplar species.

Introduction

Dimorphism refers to the ability of certain fungi to switch between unicellular yeast and multicellular filamentous growth forms in response to changing environmental cues. During unicellular growth, fungal nuclei divide mitotically and cytokinesis proceeds either by a budding or a fission process, depending on the species (Alexopoulos et al., 1996; Madhani & Fink, 1998). Complete detachment of mother and daughter cells results in the establishment of a self-perpetuating population of single independent cells. In the filamentous stage, the fungal thallus consists of long continuous tubular structures generally divided by septae into compartments. Hyphal compartments form when septae are deposited behind the growing tip of the hypha (Alexopoulos et al., 1996). These compartments remain connected through pores, and thus maintain a cytoplasmatic connection along the hyphae. In some fungi, incomplete wall separation during cytokinesis of budding cells results in the formation of ‘pseudohyphae’. Despite the resemblance to a true filament, the septa of a pseudohypha contain no pores, and thus lack a cytoplasmatic connection between compartments.

Changes in the environment generate a variety of detectable signals. Dimorphic fungi perceive these signals and may undergo the morphogenetic shift as a way to adapt to the new prevailing conditions. The nature and intensity of the signals that trigger the switch varies with the fungal species. For example, several Mucor species exhibit dimorphism in response to both the type of carbon source present in the environment and the level of oxygen in the atmosphere. Mucor species grow as yeast only when a fermentable hexose is available. In addition, yeast growth is favored by anaerobic conditions, whereas an atmosphere rich in oxygen stimulates hyphal development (Orlowski & Ross, 1981; Orlowski, 1991; Wolff et al., 2002). Blastomyces dermatitidis and other members of a well-defined group of phylogenetically related mammalian pathogenic ascomycetes exist in the soil as saprobic mycelia. Within a host, they adopt a parasitic budding lifestyle triggered by the temperature change experienced when moving from soil (25 °C) to host (37 °C) (Maresca & Kobayashi, 1989; Nemecek et al., 2006). The maize pathogen Ustilago maydis switches between a saprobic yeast stage and a pathogenic filamentous one in response to nuclear condition and plant signals. Stable filamentous development takes place only within the maize plant, and it requires the mating of two compatible haploid cells. Because the resultant dikaryotic filaments can proliferate exclusively in planta, a signal(s) emanating from the living maize plant is hypothesized to be essential for triggering this behavior.

Dimorphism has often been defined as an environmentally controlled ‘reversible’ process, by which certain fungi
can switch between yeast and hyphal stages (Romano, 1966). This condition of 'reversibility' holds true for many dimorphic fungi, but not all. It is characteristic of the opportunistic animal pathogen Candida albicans, whose cells can change their morphology back and forth in response to environmental fluctuation. Importantly, for C. albicans, the ability to undergo morphological change is a crucial factor for virulence (Sanchez-Martinez & Perez-Martin, 2001). On the other hand, the switch is not reversible for some pathogenic dimorphic fungi such as U. maydis. For them, regaining a particular growth form implies mating and completion of the life cycle. Therefore, dimorphism, sexual development and virulence are often tightly associated in the life cycles of many of these fungi (Madhani & Fink, 1998).

Despite the great diversity of triggering signals that exist for dimorphic fungi, the cell signaling pathway network governing the dimorphic/pathogenic development has been well conserved. Two main pathways, the cAMP-dependent protein kinase A (PKA) and the mitogen-activated protein kinase (MAPK), are at the heart of this network, integrating input signals and regulating fungal dimorphism and pathogenicity. These pathways are not exclusive to pathogenic dimorphic fungi; they also are critical for developmental events in nonpathogenic fungi. For example, in Saccharomyces cerevisiae and Schizosaccharomyces pombe, they control pseudohyphal differentiation and sexual development, respectively (DeVoti et al., 1991; Mochizuki & Yamamoto, 1992; Neiman et al., 1993; Pan et al., 2000; Schwartz & Madhani, 2004). Cross-talk between the cAMP and MAPK pathways has been shown to be vital for the correct development of fungal pathogens, including Magnaporthe grisea (Hamer & Talbot, 1998), U. maydis (Gold et al., 1994; Kruger et al., 1998; Mayorga & Gold, 1998; Kahmann et al., 1999; Andrews et al., 2000), C. albicans (Leberer et al., 2001) and others.

In this review, we focus on the phenomenon of fungal dimorphism among plant pathogens, and we present a brief summary of the current knowledge about dimorphic plant pathogenic fungi. We begin by discussing the extensively studied model plant pathogen U. maydis. We then describe several other known dimorphic plant pathogenic fungi, comparing them with the U. maydis system when appropriate.

**Ustilago maydis**

*Ustilago maydis* is a basidiomycete phytopathogenic fungus responsible for corn smut disease. The formation of 'tumor-like' structures called galls on the maize plant is the distinctive hallmark symptom of this disease. *Ustilago maydis* is a dimorphic fungus with a saprobic, haploid, unicellular phase (the sporidial stage) outside the plant and a parasitic dikaryotic filamentous stage within the plant. Because of the extensive repertoire of available genetic tools and the easy cultivation of its haploid phase in the laboratory, *U. maydis* serves as an excellent model for studying fungal pathogenicity and dimorphism.

In the saprophytic phase, the haploid sporidia divide by budding and are incapable of infecting maize. Only when the mating of compatible sporidia occurs is the fungus capable of parasitic growth. The mating-type *a* locus consists of two tightly linked genes: *mfa1* encoding a lipopeptide pheromone and *prf1* encoding a seven-transmembrane receptor (Spellig et al., 1994). This pheromone–receptor system accounts for cell recognition and fusion (Bolker et al., 1992; Gold et al., 1994; Banuett, 1995). Pheromone binding to its receptor mediates cell recognition and initiates sexual development. At this point, cells arrest in the G2 phase of the cell cycle and prepare to form conjugation tubes (Garcia-Muse et al., 2003).

When conjugation tubes fuse, plasmogamy occurs, but karyogamy is delayed until a later developmental stage, leading to the establishment of a dikaryotic filament. Maintenance of a stable dikaryon requires heterozygosity at the *b* mating-type locus. The *b* locus consists of two tightly linked genes, encoding two homeodomain proteins, *bE* and *bW*, that heterodimerize to form a functional transcription factor (Gillissen et al., 1992; Brachmann et al., 2001). An active *bE/*bW transcription factor assembles only when *bE* and *bW* are derived from different *b* alleles (Kamper et al., 1995). Although establishment of the *b*-dependent filament can be induced in culture, beyond cell elongation, no further proliferation of this initial dikaryon has been observed outside the plant. The nuclei in this early dikaryon are subject to a G2/M arrest and thus unable to progress through the cell cycle. Because further growth of the initial dikaryon has only been observed *in planta*, a maize signal(s) is hypothesized to act to bypass this G2/M arrest checkpoint. However, the nature of the plant-derived signal(s) remains a mystery (Fig. 1).

The pheromone-signaling pathway induces the transcription of several pheromone-induced genes, among which are the *a* and *b* locus genes, and *prf1* (Hartmann et al., 1996; Urban et al., 1996; Brachmann et al., 2001). *Prf1* is a key element in this pathway, integrating the initial pheromone signal with gene expression. *Prf1* is a high mobility group (HMG)-domain transcription factor that recognizes pheromone-response elements (PREs) in the regulatory regions of pheromone-induced genes. *prf1* itself contains two PREs and is therefore subject to autoregulation (Hartmann et al., 1996, 1999). Basal expression of *prf1* occurs in cells without pheromone induction, guaranteeing basal expression of *a* locus genes.

Upon exposure to the pheromone stimulus, full activation of *Prf1* is achieved by posttranslational phosphorylation. *Prf1* contains six MAPK phosphorylation sites...
(Kaffarnik et al., 2003). Genetic components of an MAPK module have been isolated and characterized. Some MAPK pathway members have been shown to be essential for manifestation of filamentous growth triggered by defective PKA signaling. This MAPK pathway consists of the MAPKKK Ubc4/Kpp4, the MAPKK Fuz7/Ubc5, the MAPK Ubc3/Kpp2 and Ubc2 (Banuett & Herskowitz, 1994; Mayor-Gas and Gold, 1999; Muller et al., 1999, 2000; Andrews et al., 1999, 2003; Andrews et al., 2003). Ubc2 is structurally related to S. cerevisiae Ste50p, a protein that physically interacts with Ste11p (a Ubc4 ortholog) to regulate its activity in the pheromone-response pathway (Wu et al., 1999). Therefore, Ubc2 was proposed to act as an adaptor protein upstream of Ubc4 (Mayor-Gas and Gold, 2001). Recent studies confirm a physical interaction between Ubc2 and the Ubc3 MAPKK (Klosterman et al., 2008). Interestingly, Ubc2 has a basidiomycete-specific carboxy-terminal region possessing two SH3 domains that are lacking in Ste50p and other ascomycete counterparts. This C-terminal region is dispensable for dimorphism but is essential for virulence (Klosterman et al., 2008). The MAPK module diverges downstream of Ubc3, leading to Prf1 pheromone-dependent gene expression and to conjugation-tube formation, a Prf1-independent process (Muller et al., 2003).

Morphogenesis and pathogenesis in U. maydis are also regulated by a cAMP signaling pathway (Gold et al., 1994). This pathway consists of the heterotrimeric G protein α subunit Gpa3, Uac1 and the cyclic AMP-dependent PKA (Gold et al., 1997; Durrenberger et al., 1998; Kruger et al., 1998). Although not directly proven, indirect evidence indicates that U. maydis PKA behaves similarly to other eukaryotic PKA. The eukaryotic PKA holoenzyme is a tetramer consisting of two catalytic subunits (Adr1) and two type II-regulatory subunits (Ubc1). Binding of the regulatory subunits to the catalytic subunits prevents PKA activity. cAMP binds to cAMP-binding sites present in the regulatory subunits and consequently induces a conformational change that causes the release of the active catalytic subunits (Taylor et al., 1990; Gold et al., 1994; Durrenberger et al., 1998). Mutations in uac1 or adr1, which lower PKA activity, cause a haploid-budding strain to express constitutive filamentous growth (Gold et al., 1994; Durrenberger et al., 1998).

Exposure of the uac1 mutant to exogenous cAMP restored the budding phenotype but as expected, it had no effect on an adr1 mutant (Durrenberger et al., 1998; Martinez-Espinoza et al., 2004). Based on these facts, it is evident that budding growth in U. maydis requires an active PKA. Inhibition of PKA by a decrease in the intercellular cAMP level or mutations in adr1 leads to filamentous growth. Consistent with this model, the ubc1 mutant (predicted to have a high PKA activity) exhibits a multiple-budding phenotype similar to that encountered when cells are exposed to high levels of exogenously added cAMP.

Cross-talk between the MAPK and cAMP signaling pathways regulates fungal mating and dimorphism (Fig. 2). In U. maydis, integration of both pathways involves Prf1. In addition to MAPK phosphorylation mentioned above, Prf1 contains five putative PKA phosphorylation sites, which are essential and sufficient for the induction of genes at the b locus. On the other hand, induction of b genes requires Prf1 activation through phosphorylation by MAPK as well as PKA. These facts led to the proposal that the phosphorylation state of Prf1 allows the transcription factor to discriminate between promoters (Kaffarnik et al., 2003). For a more full description of the functions of both pathways among fungi, please refer to Lengeler et al. (2000).

Recently, the Crk1 kinase was described as a new class of MAPK with crucial roles in U. maydis dimorphism and pathogenicity. A major role of Crk1 in morphogenesis is evident because in a haploid background, crk1 overexpression prompts filamentous growth while deletion of crk1 suppresses the filamentous phenotype of an adr1 mutant (Garrido & Perez-Martin, 2003).Δcrk1 mutants exhibit a loss of virulence, a probable consequence of the lack of prf1 expression observed in these strains. Importantly, activation of Crk1 by phosphorylation at its T-loop TXY motif relies, at least in part, on MAPKK Fuz7/Ubc5 (Garrido et al., 2004).

Once the dikaryotic mycelium is well established inside the plant, it divides and grows intra- and intercellularly, inducing the formation of galls. Galls result from abnormal
plant–cell development stimulated by the fungus and they are the site of teliospore production.

**Ceratocystis ulmi**

*Ceratocystis* (*Ophiostoma*) *ulmi*, the causal agent of Dutch elm disease, is another relatively well-studied dimorphic fungal plant pathogen. The yeast–hyphal switch is regulated by environmental factors and occurs only in haploid isolates with no apparent involvement of mating processes (Pereira *et al*., 2000). Kulkarni & Nickerson (1981) found that the nitrogen source is critical in determining the growth form; *C. ulmi* develops in the yeast phase in media containing proline but as mycelium with ammonium, arginine or asparagine as the nitrogen source. Both blastospores and conidiospores have dimorphic developmental potential upon germination (Kulkarni & Nickerson, 1981). An interesting aspect of the environmental regulation of dimorphism in *C. ulmi* is that, in the medium with proline, growth form is affected by inoculum density; concentration of cells $\geq 10^6$ cells mL$^{-1}$ consistently resulted in budding growth. However, lower concentrations produced a transient mycelial state, with the hyphal length being inversely proportional to the initial inoculum concentration (Kulkarni & Nickerson, 1981). This implicates quorum-sensing mechanisms in the regulation of the dimorphic switch. Hornby *et al.* (2004) further analyzed quorum sensing in *C. ulmi* and determined that the inoculum effect is mediated by a secreted molecule that induces a shift from mycelial to budding growth. Among the environmental parameters that regulate dimorphism, cell density is among the less well characterized (Nickerson *et al*., 2006) and *C. ulmi* could provide an interesting model system for its study among the fungal plant pathogens.

Dimorphic growth in *C. ulmi* has not been studied widely at the molecular level, and the information available on this
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T. deformans involves the budding of ascospores while still within the ascus. This process generates sacs stuffed full of small yeast cells (Webster & Weber, 2007). Inoculation of host leaves triggers the budding to filamentous growth transition in this fungus, which is homothallic as indicated by its capacity to generate ascii and ascospores after inoculation with a single budding cell. This is unlike some other Taphrina species that are heterothallic. Once on the peach leaf, a mitotic nuclear division establishes a binucleate condition. This provides an interesting genetic question as to whether the dikaryon in T. deformans, albeit completely homozygous, triggers pathogenic development as seen in U. maydis, which, in contrast, requires heterozygosity of the paired nuclei for pathogenic development. The sparse filamentous form ramifies subcuticularly and/or intercellularly. Subcuticular asci burst through the cuticle to release their spores. Little is known regarding the triggers of dimorphism in Taphrina but it is likely a result of host signals and/or ploidy considerations.

Mycosphaerella graminicola

Mycosphaerella graminicola, one of the most important pathogens of wheat, has a dimorphic growth pattern in which growth form is associated with pathogenicity. It switches from a yeast-like form to an infectious filamentous form that penetrates leaf tissue through stomata. This transition can be mimicked in culture by switching from a rich medium that promotes yeast-like growth to a poor medium that induces filamentation (Mehrabi & Kema, 2006). This in vitro triggered switch considerably facilitates analysis of dimorphism at the molecular level. A recent study analyzed the role of the conserved cAMP signaling pathway in regulating dimorphism in M. graminicola by disrupting the catalytic and regulatory subunit of PKA, termed MgTpk2 and MgBcy1, respectively (Mehrabi & Kema, 2006). This study showed that MgTpk2 has a positive role in regulating filamentous growth. However, although MgTpk2 mutants exhibited a defect in filamentation on potato dextrose agar, they were still able to germinate and filament on water agar, which mimics the poor nutritional conditions of the foliar surface. MgTpk2 mutants were still able to penetrate plant tissue and colonize the mesophyll. Analysis of an osmosensing MAPK ortholog-encoding gene, MgHog1, also revealed a positive role for this pathway in filamentous growth. Nevertheless, MgHog1 mutants exhibited a more dramatic defect in filamentous growth than did MgTpk2 mutants; filamentous growth was fully impaired in these strains even on water agar (Mehrabi et al., 2006). Consistent with this result, on plant tissue, MgHog1 mutants were unable to initiate the formation of germ tubes and were therefore nonpathogenic (Mehrabi et al., 2006). These data revealed that the two signaling pathways in which these
genes are components play different roles in regulating filamentous growth in *M. graminicola*.

**Holleya sinecauda**

*Holleya sinecauda*, a pathogen of mustard seeds, is another dimorphic ascomycete. In plant lesions, the fungus is isolated almost exclusively in the yeast form (Holley et al., 1984). This is also the prevalent form at early stages of growth on solid medium; however, when grown for longer than 6 days on plates, a network of true hyphae is formed (Holley et al., 1984). Filamentous growth is very prominent at the edges of colonies, whereas in the center growth remains yeast-like or pseudohyphal (Schade et al., 2003). In liquid media, although short-branched pseudohyphae are observed, the formation of true mycelium is not prominent (Holley et al., 1984; Schade et al., 2003), suggesting that surface cues/adhesion are required to induce the dimorphic switch. *Holleya sinecauda* is closely related to *S. cerevisiae*, which can grow as yeast and pseudohyphae, and to the strictly filamentous fungus *Ashbya gossypii* (Kurtzman, 1995; Prillinger et al., 1997). Molecular characterization of *H. sinecauda* budding and filamentous growth, and comparison with *A. gossypii* could help elucidate which factors are determinants of these two modes of growth. An advance that will greatly aid molecular characterization of *H. sinecauda* dimorphism is the development of a transformation system Schade et al. (2003), based on sequences from *A. gossypii*. This method was utilized to delete a novel Rho-type GTPase in *A. gossypii* and *H. sinecauda*. Although no phenotype was observed in the *H. sinecauda rho* mutant strain, the successful deletion of the gene confirms the efficacy of the transformation system (Walther & Wendland, 2005).

**Verticillium**

Verticillium species, including the wide-spread economically damaging species *Verticillium dahliae* and *Verticillium albo-atrum*, spread to distal portions of plants at a rate too rapid to be explained by hyphal growth. Conidia have been implicated in this rapid dissemination within the host vascular tissue in diverse plant species (Pegg, 2002). Vascular conidia of *V. albo-atrum* have long been known to be present in infected vessels (Pegg, 2002). Conidia may be produced from phialides or as yeast-like blastoconidia as reported in cotton (Schnathorst, 1981) and may accumulate to very high concentrations in susceptible hosts. *Verticillium albo-atrum* produces spores in liquid culture. Reminiscent of the above discussion on *C. ulmi*, Keen et al. (1971) showed that it produces spores exclusively if cultures are inoculated at a high concentration. If inoculated at a lower rate, the cultures grow predominantly as hyphae. Partially successful attempts were made to use inhibitory compounds to generate exclusively filamentous cultures to inhibit the disease.

**Concluding remarks**

Dimorphic fungi shift from yeast to hyphal phases in response to environmental and/or genetic cues. In *C. albicans*, a dimorphic fungal animal pathogen, the yeast phase is important for systemic spread through the blood stream within the host while the hyphal phase gains entry into the vascular system and other tissues by its penetration capacity. Similarly, several vascular pathogens such as *Ceratoctysis* and *Verticillium* species used yeast cells for passive transport to distal portions of the plant through the vascular system first penetrated by the hyphal form. In the model Basidiomycete plant pathogen, *U. maydis*, dimorphism is controlled by genetic factors in which the hyphal form is generated by postmating heterozygosity of the *b* mating type locus in the resultant dikaryon. This dikaryon, however, is a dead-end cell type due to cell-cycle arrest unless a heretofore unknown host-plant signal releases this arrest. Our understanding of the triggers and roles of dimorphism in fungal plant pathogens is still very limited but experimental exploration of economically important pathogens and the emerging model systems described above, among others, will provide for a deeper understanding of this interesting and important process.

**References**


