MINIREVIEW

Growth and Developmental Control in the Model and Pathogenic Aspergilli

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The filamentous fungi comprise a ubiquitous group of heterotrophic organisms living as saprophytes, parasites, or symbionts. The basis for fungal vegetative growth is the continued and coordinated expansion of a series of fungal cell tips into a linear or complex structure. Fungi differentiate into a variety of structures including spores, which are the effective means of genome protection, survival, and propagation. Spores are also the primary agent for infecting host organisms for many human- and plant-pathogenic fungi. Asexual sporulation is a prevalent mode of reproduction for a diverse group of fungi, which results in the production of vast numbers of mitotically derived spores (reviewed in reference 2).

The genus Aspergillus represents the most widespread fungi in our environment, which all reproduce asexually by forming long chains of conidiospores (or conidia) radiating from a central structure known as a conidiophore (Fig. 1) (reviewed in reference 3). The impact of various Aspergillus species on humans runs the range from “good” to “bad.” For instance, several species such as Aspergillus oryzae and Aspergillus niger are used in industry for enzyme production and food processing. In contrast, Aspergillus flavus and Aspergillus parasiticus can produce the most potent naturally present carcinogen, aflatoxin, which can contaminate various foods and feeds (reviewed in reference 43). Moreover, the opportunistic human pathogen Aspergillus fumigatus has become the most prevalent airborne fungal pathogen, causing severe and usually fatal invasive aspergillosis in immunocompromised patients (reviewed in reference 22).

Aspergillus nidulans has served as an excellent model system for studying various biological questions, primarily due to the ease of genetic analysis through meiotic (sexual) recombination and the development of sophisticated molecular tools (32). These properties have provided a better understanding of the mechanisms controlling growth, development, secondary metabolism, and other aspects of cell biology in filamentous fungi (3, 35, 50, 57).

The availability of the genome sequences of several aspergilli facilitates comparative genomic, genetic, and functional studies. In particular, knowledge and information obtained from a model fungus can be effectively tested in less genetically tractable aspergilli. Recent studies of a number of Aspergillus species have proven that a model fungus can provide a useful framework for understanding the biology of agriculturally and/or medically relevant aspergilli (6, 7, 38, 41, 49, 57). For instance, the novel nuclear protein LaeA has been shown to function as a global regulator of secondary metabolism as well as a regulator of morphogenetic virulence factors in the genus Aspergillus (6, 7). This review summarizes our current understanding of the genetic mechanisms controlling asexual development (conidiation) and vegetative growth in the model (A. nidulans) and pathogenic (A. fumigatus) aspergilli.

KEY DOWNSTREAM ACTIVATORS OF CONIDIATION IN A. NIDULANS

Conidiation in A. nidulans involves many common developmental themes including spatial and temporal regulation of gene expression, specialized cellular differentiation, and intercellular communication. The asexual reproductive cycle in A. nidulans can be divided into two distinct phases: vegetative growth and development. The growth phase involves germination of an asexually derived spore called a conidium and formation of an undifferentiated network of interconnected hyphal cells that form the mycelium (reviewed in reference 2). After a certain period of vegetative growth, under appropriate conditions, some of the hyphal cells stop normal growth and begin conidiation by forming conidiophores that bear multiple chains of conidia (Fig. 1) (reviewed in reference 3).

A key and essential step for conidiophore development is activation of the brlA gene encoding a C2H2 zinc finger transcription factor, which induces expression of other genes required for asexual development (1, 12). Loss-of-function brlA mutants form structures that resemble conidiophore stalks (thus named “bristle”; Fig. 1 and 2), except that they grow indeterminately and fail to produce the other specialized cell types needed for sporulation (8, 13). By contrast, overexpression of brlA in vegetative cells causes termination of polar growth coupled with the commencement of abnormal conidiation leading to formation of viable spores from hyphae (1). No environmental signals such as nutrient limitations or various (osmotic and oxidative) stresses have been shown to bypass the need for BrlA in conidiation (reviewed in reference 3). These studies demonstrated that activation of brlA expression early in conidiophore development represents a major and essential
control point for initiating conidiation. The \textit{brlA} gene is a compound gene consisting of two overlapping transcription units designated \textit{brlA}A and \textit{brlA}B. While the \textit{brlA}A and \textit{brlA}B polypeptides have redundant functions, the \textit{brlA}A and \textit{brlA}B transcription units are individually needed for normal development (reviewed in reference 3). Moreover, it has been shown that these units are regulated by different mechanisms and it has been proposed that the \textit{brlA} locus has evolved to achieve differential responses to the multiple regulatory inputs throughout development (see reference 3 and references therein). Additional information for complex regulation of the two overlapping \textit{brlA} transcription units is well described in reference 3.

Additional studies identified and characterized the \textit{abaA} and \textit{wetA} genes that are regulated by \textit{BrlA}. Several \textit{BrlA} response elements [(C/A)(G/A)AGGG(G/A)] are present in the upstream cis-regulatory regions of \textit{abaA} and \textit{wetA} (and other developmentally regulated genes including rodA and yA; see reference 3 and references therein). The \textit{abaA} gene encodes a developmental regulator that is activated by \textit{brlA} during the middle stages of conidiophore development after metula differentiation (Fig. 1; Table 1) (4, 5). The \textit{wetA} gene functions in the late phase of conidiation for the synthesis of crucial cell wall components (31, 47). These three genes have been proposed to define the central regulatory pathway that acts in concert with other genes to control conidiation-specific gene expression and determine the sequence of gene activation during conidiophore development and spore maturation (34; reviewed in reference 3). Mutations in any one of these three genes block conidiation at a specific developmental stage and prevent expression of a broad group of developmentally regulated mRNAs (class A to D; Fig. 1). While interactions of these three sequentially expressed regulators control temporal and spatial specificity of conidiation (34), the molecular mechanisms balancing the activities of \textit{brlA}, \textit{abaA}, and \textit{wetA} remain to be uncovered. Other developmental modifiers including \textit{StuA} and \textit{MedA} are reviewed in reference 3.

**UPSTREAM DEVELOPMENTAL ACTIVATORS IN A. NIDULANS**

Six genes (\textit{fluG}, \textit{flbA}, \textit{flbB}, \textit{flbC}, \textit{flbD}, and \textit{flbE}) required for proper activation of \textit{brlA} expression were identified by investigating a large number of fluffy mutants with severe defects in \textit{brlA} expression (\textit{FLB} mutants [\textit{fluffy low-\textit{brlA}} expression] [52; reviewed in reference 3]). These \textit{FLB} mutants were further subdivided into three categories based on their developmental phenotypes. Loss-of-\textit{fluG} function mutants are extremely fluffy and entirely aconidial on complete medium (Fig. 2) but conidiate to a low degree when grown on minimal medium (24). \textit{flbA} mutants are distinguished from the others by the fact that they...
undergo autolysis as the colonies mature (Fig. 3) (23, 52). The major role of FlbA in conidiation is discussed below in this review. Finally, flbB, flbC, flbD, and flbE mutants exhibit delayed conidiation (52, 53).

The genetic interactions between these flb genes were examined by assessing the phenotypes of various double mutants (52) and by testing the requirement for submerged sporulation observed following overexpression of fluG, flbA, or flbD (25, 28).

The GpgA and AfGpgA proteins are deduced based on the study by Seo et al. (44).
53). Because wild type and other fluffy mutants could rescue conidiation of fluG mutants in an extracellular manner, it was proposed that FluG is required for production of an extracellular signal (smaller than 6,000 to 8,000 Da) that is predicted to trigger the initiation of conidiation (24). The FluG protein is similar to a bacterial glutamine synthetase I (see below). Two of the delayed-conidiation loci, flbC and flbD, are predicted to encode DNA binding proteins that may define potential direct activators of brlA expression (Table 1) (reviewed in reference 3). Because mutations in flbC and flbD have additive effects on conidiation, these genes are proposed to control independent steps in a nonlinear pathway. The flbB gene is predicted to encode a b-Zip-like transcription factor, and flbE encodes a novel protein conserved in various fungi (Table 1) (reviewed in reference 3). They apparently function in the flbD branch of the conidiation pathway because flbD−/flbB− and flbD−/flbE− double mutant strains exhibited phenotypes identical to those of each single mutant strain. The gene order flbE→flbD→flbB was proposed based on the requirement of individual genes for flbD-induced submerged sporulation (53). As fluG functions first in this regulatory cascade, overexpression of fluG requires the activities of flbC, flbA, and the flbD pathway independently for activating brlA and conidiation in submerged culture (25, 53).

The fluG gene encodes a cytoplasmically localized protein that is present at relatively constant levels throughout the life cycle, implying that the amount of the FluG factor may increase gradually during vegetative growth. The C-terminal half of FluG contains a glutamine synthetase I-like domain (24), and the N-terminal half is similar to the product of the early nodulin gene MtN6 in Medicago truncatula (33), yet the N-terminal half of FluG is dispensable for conidiation (14). Overexpression of the full length or the C-terminal half of FluG in vegetatively grown hyphae was shown to be sufficient to cause activation of brlA and development of conidiophores in liquid-submerged culture (14, 25).

Loss of fluG function also results in the absence of production of the mycotoxin sterigmatocystin (ST), the penultimate precursor of aflatoxin (19). Later, the role of FluG in ST production was found to be a potential posttranscriptional activation of FlbA, which in turn attenuates vegetative growth signaling mediated by a heterotrimeric G protein (19; reviewed in references 57 and 58; see below). However, the precise role of FluG in activating conidiation and ST production remained to be unveiled, and recent studies (42, 46) have provided some important clues on the FluG-dependent developmental transition and toxin production.

**FluG-MEDIATED CONIDIATION OCCURS VIA DEREPRESSION IN A. NIDULANS**

The molecular event(s) responding to FluG activity was investigated by isolating 40 suppressor mutations (SFGs [suppressors of fluG]) that restored conidiation and ST production in the haploid ΔfluG mutant (42). These recessive sfg mutations defined four loci, where 31 mutations mapped to sfgA, six mapped to sfgB, and one each mapped to sfgC and sfgD (42). Isolation of 31 sfgA mutations implied that SfgA might play a pivotal role in regulating conidiation downstream of FluG and led to the identification of sfgA as the first priority.

The sfgA gene was cloned via complementation employing a wild-type library. The sfgA gene is predicted to encode a novel 601-amino-acid protein containing the fungus-specific Zn(II)$_2$Cys$_6$ binuclear cluster DNA binding motif at the N terminus, suggesting that SfgA is likely a transcription factor (46). Sequence analyses of the sfgA coding region from all 31 sfgA mutants identified various (loss-of-function) mutations in all mutants. The deletion and 31 other sfgA mutant alleles bypassed the need for fluG in conidiation and production of ST. Moreover, deletion and 12 other sfgA mutant alleles resulted in the formation of conidiophores in liquid-submerged cultures even in the absence of fluG function. Furthermore, both the ΔsfgA and ΔsfgA ΔfluG mutations resulted in the identical phenotypes in growth, conidiation, and ST production. These results led to the hypothesis that the principal role of FluG in conidiation is to remove repressive effects imposed by SfgA. In line with the proposed repressive role of SfgA, the elevated level of sfgA mRNA was sufficient to inhibit conidiation and expression of development and ST-specific genes. Double mutant analyses revealed that SfgA functions downstream of FluG but upstream of transcriptional activators (FlbD, FlbC, FlbB, and BrlA). Due to the incomplete suppression of ΔflbE by ΔsfgA and the production of ST by an flbE− mutant (19), FlbE and SfgA are proposed to function at the same level (Fig. 4A). In conjunction with the observation that
most C6 factors function as transcriptional activators (reviewed in reference 51), a working model in which SfgA activates a group of genes (designated SARs for SfgA-activated repressors of conidiation) that repress conidiation and FlbE competes with SfgA to inhibit expression of SARs has been proposed (Fig. 4A) (46).

In summary, the current hypothesis for A. nidulans developmental transition is as follows: (i) during the early vegetative growth phase the level of the FluG factor in cells is low and SfgA-mediated repression of conidiation is predominant, and (ii) accumulation of the FluG factor beyond a certain level in cells removes SfgA-mediated negative control of conidiation, which allows the initiation of conidiophore development. These findings have provided a new concept for the FluG-dependent regulatory mechanism of conidiation and ST biosynthesis in A. nidulans.

**ROLES OF AfFluG IN A. FUMIGATUS CONIDINATION**

With the information obtained from studying A. nidulans developmental regulation, comparative functional studies in A. fumigatus were carried out (30). As shown in Table 1, developmental regulators in the two aspergilli share relatively high amino acid level identity and similarity. Among these genes, functions of key upstream (AfFluG) and downstream (AfbrlA) controllers in A. fumigatus were examined. The levels of the AfFluG transcript are relatively constant throughout the life cycle as shown in A. nidulans (30). However, distinct from A. nidulans, the AfFluG deletion mutant can sporulate normally like wild type in air-exposed culture (solid medium) conditions (Fig. 2), indicating that activation of A. fumigatus conidiation in the presence of air does not require the activity of AfFluG. On the other hand, the AfFluG deletion mutant did not produce conidiophores in liquid-submerged culture, where A. fumigatus wild-type strains sporulated abundantly within 24 h. Moreover, the AfFluG deletion mutant exhibited delayed/reduced conidiation and expression of AfbrlA under the synchronized developmental induction conditions. These results indicate that, while the presence of air can bypass the need for AfFluG in conidiophore development, AfFluG plays a certain role in A. fumigatus conidiation through influencing expression of AfbrlA. These findings led to the hypothesis that A. fumigatus has more than one pathway activating expression of AfbrlA independently from AfFluG (Fig. 4B).

**REQUIREMENT OF AfbrlA IN A. FUMIGATUS CONIDINATION**

AfbrlA, the A. fumigatus BrlA homologue, shows 68% identity and 77% similarity to the A. nidulans BrlA protein (Table 1). The AfbrlA gene encodes a 2.7-kb transcript that accumulates explicitly during the progression of asexual development (30). The observation implying the presence of a different upstream regulatory mechanism(s) for the activation of sporulation in A. fumigatus led Mah and Yu (30) to test whether downstream regulation of conidiation by BrlA is also divergent in the two aspergilli. They found that deletion of AfbrlA completely eliminated asexual development in A. fumigatus, resulting in elongated aerial hyphae and increased hyphal mass in the colonies (Fig. 2). The result clearly demonstrates that AfbrlA is also essential for conidiophore development in A. fumigatus and that the role of the core downstream transcription factor BrlA in conidiation is conserved in the two aspergilli.

**Gα AND RGS COORDINATE GROWTH AND DEVELOPMENT IN A. NIDULANS**

The biggest difficulty in dissecting upstream regulation of A. nidulans development was determining the position of flbA in the FluG-dependent conidiation pathway. This was clarified by understanding the role of fadA encoding a Gα subunit for a heterotrimeric G protein (55). The fadA (fluffy autolytic dominant) gene was identified by studying a dominant activating mutation (G42R) that caused the fluffy-autolytic phenotype almost identical to that resulting from loss of flbA function (Fig. 3) (55). Genetic studies revealed that FadA-mediated signaling promotes vegetative growth and inhibits both asexual and sexual development as well as production of ST and that FadA signaling is in part transduced via cyclic AMP (cAMP)-dependent protein kinase A (PKA) (Fig. 4A) (19, 48, 55). FbA is an RGS (regulator of G protein signaling) protein, and its primary role is to negatively control FadA-mediated vegetative growth signaling, likely by enhancing the intrinsic GTPase activity of FadA (55). Thus, loss of flbA function and constitutively active FadA mutations (G42R, R178C, and Q204L) derived from the loss of the intrinsic GTPase activity of FadA result in the similar hyperproliferation autolytic phenotype (23, 54, 55, 56). On the other hand, overexpression of flbA or the dominant interfering FadA>G203R mutation results in restricted hyphal growth and hyperconidiation (23, 55). Importantly, the deletion and dominant interfering FadA mutations (G203R and R205H) suppress the fluffy-autolytic phenotype caused by ΔflbA and restore conidiation and ST production (19, 54, 55). These findings corroborate the hypothesis that the FadA-dependent growth signaling pathway is negatively controlled by FbA and that at least partial inhibition of growth signaling is required for the commencement of development and ST production.

Later, a series of studies identified SfaD (Gβ) (40), GpgA (Gγ) (44), and PhnA (a phosducin-like protein acting as a Gβγ activator) (45), which all function in vegetative growth signaling (Table 1). In addition, these G protein components are all found to be essential for sexual fruiting body formation in A. nidulans in a somewhat dominant manner (reviewed in reference 58). While mutual inactivation of any one of these G protein components resulted in restricted vegetative growth and suppression of the fluffy-autolytic phenotype caused by ΔflbA, no mutations could bypass the need for FluG in asexual development. These results further clarified that the role of FbA in asexual development is indirect and led to the current model showing that the vegetative growth and conidiation pathways are independent and that both inhibition of growth signaling and activation of development-specific functions must occur in order for development to proceed in A. nidulans (Fig. 4A). The second Gα-RGS pair (Gα1B-RGSα) has been shown to play a key role in regulating conidiation, conidial germination, and stress response (Fig. 4A) (11, 18). Details of functions and characteristics of individual G protein signaling components and RGSs in A. nidulans are described in references 10, 21, and 58 and are not discussed in this review.
CONSERVED ROLES OF Gα AND RGS IN A. FUMIGATUS DEVELOPMENT

As G protein components are highly conserved in eukaryotes, the corresponding A. fumigatus homologues of the above-mentioned A. nidulans signaling elements show extremely high amino acid level identity (Table 1) (21). To test whether the two aspergilli have the conserved signaling mechanisms for balancing growth and development, the functions of AfFlbA and GpaA (FadA homologue [28]) were examined in A. fumigatus (30). Mah and Yu generated the null AfflbA mutant and also isolated 14 additional loss-of-function AfflbA mutants following chemical mutagenesis. Phenotypic analyses of various AfflbA- mutants revealed that the absence (or reduction) of AfflbA function is associated with the reduced levels of conidiation and conidial pigmentation. Furthermore, loss of AfflbA function caused increased hyphal proliferation during the early phase of colony growth (up to 2 days) and resulted in the colonies exhibiting an expanded growing edge with delayed conidiation (Fig. 3). In addition, AfflbA- mutants failed to produce conidiophores in liquid-submerged culture conditions, whereas wild-type and AfflbA-complemented strains produced conidiophores copiously. Finally, AfFlbA is found to be necessary for proper expression of AfbrlA and normal progression of conidiation. Together, Mah and Yu proposed that AfFlbA down-regulates hyphal proliferation, which in turn stimulates development in A. fumigatus. However, it is important to note that, distinct from A. nidulans, loss of AfflbA function does not abolish conidiation completely or lead to hyphal disintegration (autolysis) in A. fumigatus (compare Fig. 3 bottom panels). In an effort to explain this critical difference, Mah and Yu speculated that the potential presence of multiple mechanisms activating conidiation in A. fumigatus might circumvent the need for AfFlbA in conidiation and allow AfflbA mutants to produce spores, thereby avoiding hyphal disintegration.

Mah and Yu further tested whether the FadA homologue GpaA (28) is the primary target of AfFlbA. This was accomplished by generating the constitutively active GpaA G203R and dominant interfering GpaA G203R mutant alleles. They found that, similar to the effects caused by ΔAfFlbA, the presence of the GpaA G203R allele in wild type, i.e., heterozygous for gpaA, caused elevated hyphal proliferation and reduced sporulation in a dominant manner without autolysis (Fig. 3). Moreover, the ectopic integration of the gpaA G203R allele restored sporulation in an AfflbA- mutant to the wild-type level in both air-exposed and liquid-submerged culture conditions, indicating that inactivation of GpaA signaling could bypass the role of AfFlbA in proper progression of conidiation. Collectively, the study demonstrated that GpaA and AfFlbA constitute a Gα-RGS pair, which coordinates vegetative growth and development in A. fumigatus, and that the primary roles of FadA (GpaA) and FlbA (AfFlbA) are conserved in the two aspergilli.

DIFFERENTIAL ROLES OF PKA IN REGULATING CONIDINATION

In fungi, the G protein-cAMP-dependent PKA signaling pathway plays a critical role in controlling vegetative growth, development, nutrient sensing, mating, stress response, secondary metabolism, and pathogenicity (reviewed in references 15, 26, and 27). In A. nidulans, PkaA (primary PKA) and PkaB (secondary PKA) represent the sole PKA catalytic subunits and play overlapping and opposite roles in diverse biological processes (36, 48). FadA-dependent vegetative growth signaling is in part transduced via PkaA, and PkaB functions as a backup unit for hyphal growth. The fact that GanB, SfaD::GpaA, and PkaA are required for proper germination of conidia (11, 20) indicates that PkaA is likely activated by both GanB and FadA (Fig. 4A). As PkaA is a key downstream element in the FadA-mediated growth signaling pathway, the absence of pkaA function resulted in restricted vegetative growth coupled with hyperactive conidiation and suppressed the fluffy-autolytic phenotype caused by ΔflbA and the dominant activating fadA G42R mutation (48). In addition, overexpression of pkaA led to elevated hyphal proliferation and reduced sporulation and ST production (48). Collectively, it has been proposed that a CAM and PKA signaling cascade plays a major role in activation of vegetative growth and repression of conidiation downstream of G proteins in A. nidulans (Fig. 4A).

As found in A. nidulans, two PKA catalytic subunits have been found in A. fumigatus, where PkaCl (showing 86% identity to PkaA) (Table 1) plays a predominant role in controlling growth and development (29). Deletion of pkaCl resulted in reduced growth rate and delayed germination, indicating that, as found in A. nidulans, PkaCl is necessary for proper vegetative growth and germination. However, distinct from A. nidulans, the lack of PkaCl function caused reduced sporulation. Liebmann et al. proposed that GpaB, AcyA (adenylate cyclase), and PkaCl constitute a major signaling cascade controlling vegetative growth, development, and virulence (28, 29). Particularly, it was shown that the cAMP-PKA network is necessary for proper expression of pksP encoding a polyketide synthase that is involved in the biosynthesis of the conidial pigment 1,8-dihydroxynaphthalene-like pentaketide melanin conferring resistance to phagocytic cell destruction in the host. Thus, deletion of pkaCl causes dramatically lowered expression of pksP, which contributes to the reduced virulence of the mutant (28, 29).

In summary, despite high amino acid level identity, the role of a G protein (GanB and GpaB) and PKA in controlling asexual development is opposite in the two aspergilli. The potential participation of PkaCl in the GpaA signaling branch remains to be investigated. We present a current model depicting regulation of vegetative growth and development in A. fumigatus (Fig. 4B).

CONCLUSIONS AND PROSPECTS

As A. nidulans and A. fumigatus are distantly related (17, 37), these fungi have distinct reproductive modes and structures. A. nidulans (teleomorph Emericella nidulans) can reproduce in both asexual and sexual manners. However, despite the extant full genome potential for sexual reproduction (39; reviewed in reference 16), the experimental evidence that A. fumigatus can undergo the sexual life cycle remains to be presented. Moreover, whereas the conidiophore of A. nidulans is composed of vesicle, primary sterigmata (metulae), secondary sterigmata
indicating that the imperfect fungus
ment through AfBrlA. In summary, while the two
being) sequenced and are available online (http://www.ncbi.nlm
conidia can survive at various temperatures up to 70°C (re-
temperatures, where growth can occur up to 55°C, and its
lacks metulae (reviewed in reference 22). In addition to the
fungi. Conclusively, whereas model fungi can provide a useful
mous potential of the comparative and functional genomic stud-
ferences between the two species in that AfFlbA or AfFluG is
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