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Regulation of Development in *Aspergillus nidulans* and *Aspergillus fumigatus*

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Members of the genus *Aspergillus* are the most common fungi and all reproduce asexually by forming long chains of conidiospores (or conidia). The impact of various *Aspergillus* species on humans ranges from beneficial to harmful. For example, several species including *Aspergillus oryzae* and *Aspergillus niger* are used in industry for enzyme production and food processing. In contrast, *Aspergillus flavus* produce the most potent naturally present carcinogen aflatoxins, which contaminate various plant- and animal-based foods. Importantly, the opportunistic human pathogen *Aspergillus fumigatus* has become the most prevalent airborne fungal pathogen in developed countries, causing invasive aspergillosis in immunocompromised patients with a high mortality rate. *A. fumigatus* produces a massive number of small hydrophobic conidia as the primary means of dispersal, survival, genome-protection, and infecting hosts. Large-scale genome-wide expression studies can now be conducted due to completion of *A. fumigatus* genome sequencing. However, genomics becomes more powerful and informative when combined with genetics. We have been investigating the mechanisms underlying the regulation of asexual development (conidiation) and gliotoxin biosynthesis in *A. fumigatus*, primarily focusing on a characterization of key developmental regulators identified in the model fungus *Aspergillus nidulans*. In this review, I will summarize our current understanding of how conidiation in two aspergilli is regulated.

KEYWORDS : *Aspergillus*, Conidiation, Gliotoxin, Fungi, Transcription factors

Conidia, often called conidiospores, are asexual, nonmotile spores formed from the apex or side of conidiogenous cells. Conidia are generated through mitosis followed by repeated asymmetric division of conidiogenous cells (phialides in *Aspergillus*). Conidia are generally haploid cells, isogenic to the haploid parent, capable of forming a new colony under appropriate conditions, and serve various biological functions including long-term viability [1]. Asexual reproduction in Ascomycetes (Phylum Ascomycota) involves conidia, which are formed on specialized developmental structures called conidiophores [2]. The morphology of these specialized asexual structures is the most important species-specific character and can, therefore, be used in taxonomy. In fact, the Italian priest and biologist Pier Antonio Micheli named the genus "Aspergillus" in 1729, because of the morphological similarity of conidiophores to the shape of an aspergillum (a holy water sprinkler) [3].

The *Aspergillus* asexual reproductive cycle can be divided into a vegetative growth phase and a developmental phase. The growth phase involves the germination of a conidium and the formation of an undifferentiated network of interconnected hyphal cells, which form the mycelium. After a certain period of hyphal growth and under proper conditions, some of the vegetative cells stop growing and begin asexual development, which includes conidiophore formation and spore maturation [2, 4]. Conidiophore formation starts from the thick-walled hyphal cells (foot cells), which extend into the air to produce stalks followed by the formation of a multinucleate vesicle. In *A. nidulans*, two layers of uninucleate reproductive cells, the metulae and phialides, are formed on top of vesicle in a subsequent budding-like process [2]. In *A. fumigatus*, conidiophores consist of a foot cell, stalk, vesicle, phialides (no metulae), and (up to 50,000) conidia [5, 6]. In both species, chains of conidia arise from repeated asymmetric mitotic divisions of phialides. The final multicellular conidia-bearing structures are called conidiophores (Fig. 1) [2, 7]. According to our recent transmission electron microscopy (TEM) studies [8], conidia sizes range from 2.4 to 2.7 mm in *A. nidulans* and from 1.3 to 1.8 mm in *A. fumigatus*, i.e., *A. nidulans* conidia are about 1.5 times larger in diameter than *A. fumigatus* conidia (Fig. 2).

A. fumigatus conidia are released into the air and are small enough to reach the alveoli after being inhaled by humans [9]. The conidia can germinate into invasive hyphae in immunocompromised hosts, which penetrate the vasculature and migrate to distal sites [5, 10]. Production of various fungal proteins, hydrophobins, melanins, and toxins, the rigidity of the hyphal cell wall, and the structural features of spores all aid the fungus to overcome the host's residual defense and, thus, contribute to the virulence of *A. fumigatus* [5].

Conserved Roles of BrlA-AbaA-WetA in *Aspergillus* Conidiation

Conidiation in *Aspergillus* involves many common devel-

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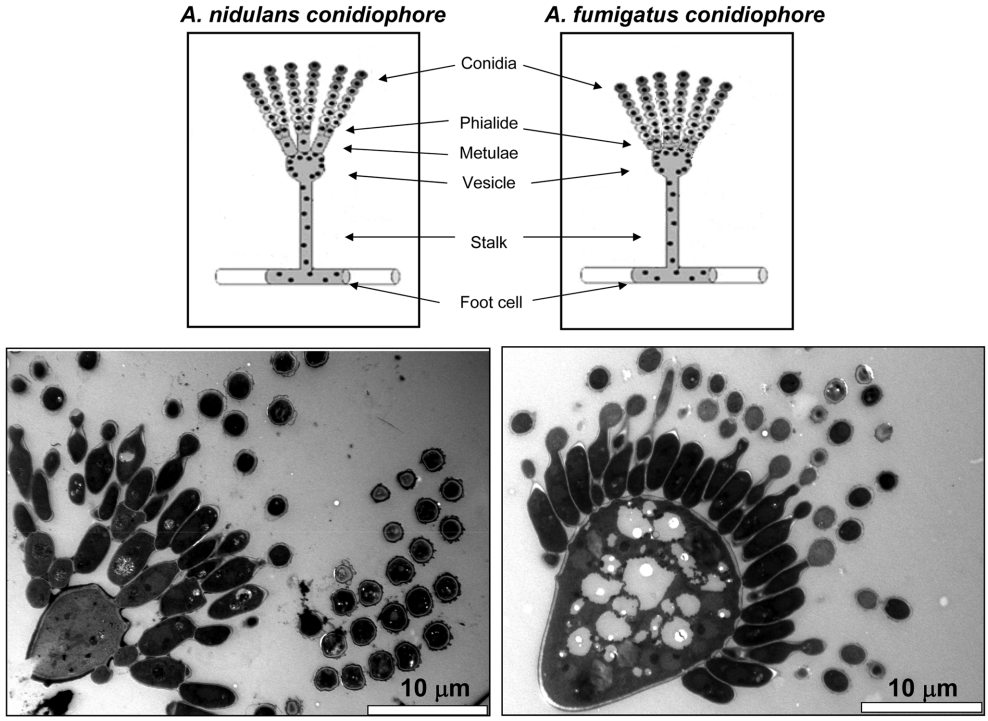

Fig. 1. Conidiophores of *Aspergillus nidulans* and *Aspergillus fumigatus*. Simplified illustrations (top panel) and transmission electron micrographs (bottom panel) of *A. nidulans* and *A. fumigatus* conidiophores are shown. Note the lack of metulae in the *A. fumigatus* conidiophore.

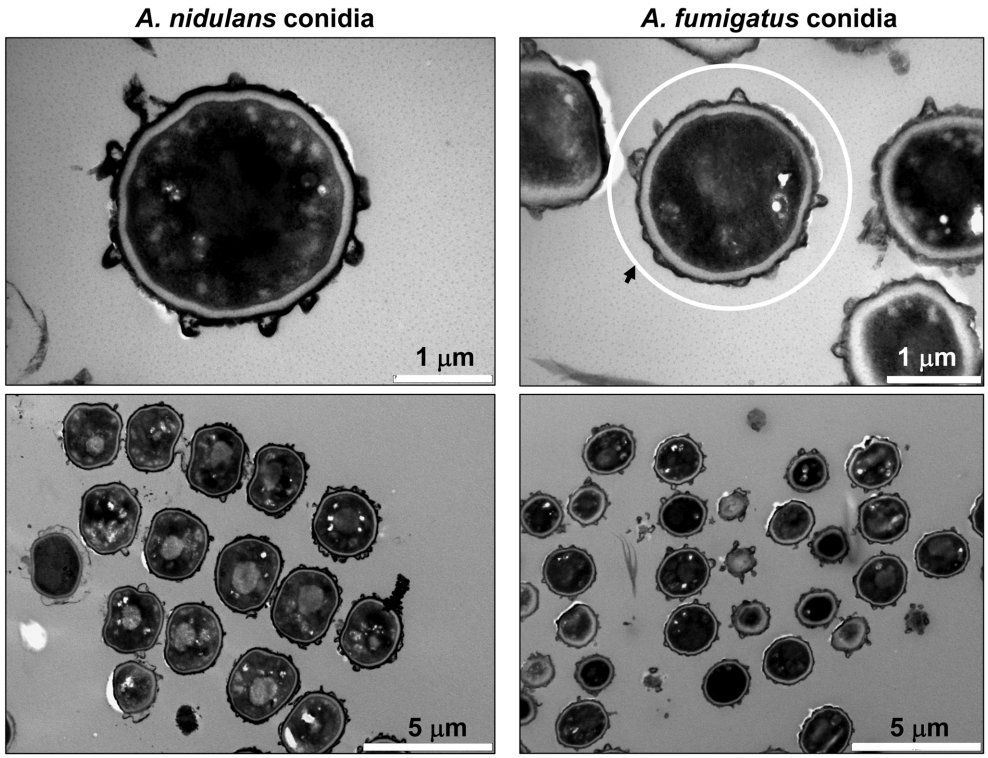

Fig. 2. Conidia of *Aspergillus nidulans* and *Aspergillus fumigates.* Transmission electron micrographs of *A. nidulans* and *A. fumigatus* conidia are shown. To aid the comparison, the size of an *A. nidulans* conidium (top left) is marked by a white circle surrounding an *A. fumigatus* conidium (arrow in top right panel). Note the size differences between the conidia of the two species.

opmental themes including spatial and temporal control of gene expression, specialized differentiation of cells, and intra and intercellular communications. Conidiation is regulated by an asexual developmental signaling pathway that directs expression control elements and other genes required for conidiophore assembly. As developmental mechanisms in *A. nidulans* have been intensively studied and results have provided important clues for understanding conidiation in other aspergilli [2, 4, 7], what is known in *A. nidulans* (*Ani*) will be described first followed by what has been found in *A. fumigatus* (*Afu*).

BrlA. In *Ani*, a key and essential step for conidiophore development is the activation of *brlA* encoding a C_2H_2 zinc finger transcription factor (TF), which induces expression of other genes required for conidiation [11]. Loss-of-function *brlA* mutants form structures that resemble conidiophore stalks (thus named "*bristle*"), except that they grow indeterminately and fail to produce vesicles, metuale, phialides, and conidia, indicating that BrlA controls the initiation of conidiophore development (Fig. 3) [2]. By contrast, *brlA* overexpression in vegetative cells causes termination of polar growth coupled with the commencement of abnormal sporulation leading to the formation of viable spores from hyphae [11]. No environmental signals, including nutrient limitations or various (osmotic and oxidative) stresses, can bypass the BrlA requirement for conidiation [2], indicating that activation of *brlA* expression early in conidiophore development represents a key and essential control point for commencing conidiation. The *AnibrlA* (but not *AfubrlA*) gene is a compound gene consisting of two overlapping transcriptional units designated *brlA*a and *brlA*b. Important information on the complex regulation of the two overlapping *brlA* transcription units is well described elsewhere [2].

*Afu*BrlA, the *Afu* BrlA homologue, shows 68% identity and 77% similarity to the *Ani* BrlA [12]. The *AfubrlA* gene encodes one 2.7 kb transcript that accumulates explicitly during asexual development [12]. Mah and Yu [12] tested whether BrlA-dependent conidiation is conserved in these two aspergilli using genetics and found that *AfubrlA* deletion completely eliminated asexual development in *Afu*, resulting in elongated aerial hyphae and increased hyphal mass in the colonies. These studies clearly demonstrated that *AfubrlA* is essential for conidiophore development in *Afu*, and that the role of the core downstream TF BrlA in conidiation is conserved in these aspergilli (Fig. 4).

BrlA is a putative TF (activator) with two C_2H_2 zinc finger motifs at the C-terminus [11]. Disruption of either one of the fingers by mutating the key cysteine to serine results in complete loss of BrlA activity [13]. Chang and Timberlake [14] further demonstrated that *brlA* expression in the budding yeast *Saccharomyces cerevisiae* results in *brlA* dependent activation of *Aspergillus* genes. Furthermore, they proposed the consensus BrlA binding sites (BrlA response elements [BREs]; 5'-(C/A)(G/A)AGGG(G/A)-3'). Although direct binding of BrlA to BREs remains to be verified *in vitro*, a number of developmental genes

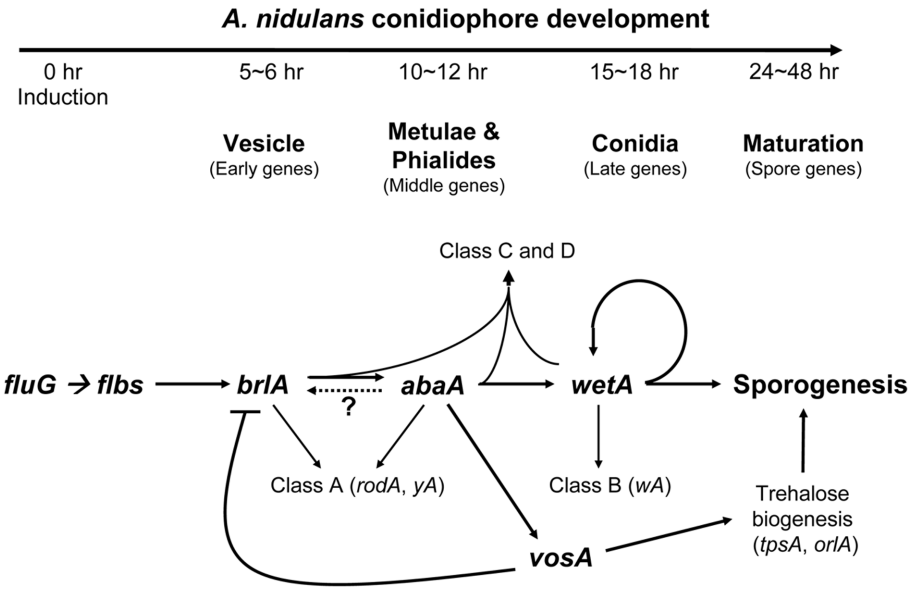

Fig. 3. Developmental regulation in *Aspergillus nidulans*. In this model, the *fluG* → Flbs upstream signal activates *brlA*, *abaA*, and *wetA*. Timeline for asexual development, associated cellular differentiation, and gene expression are indicated. *vosA* is activated by AbaA (and WetA) in conjunction with the formation of metulae, phialides, and conidia. VosA, in turn, represses *brlA*, activates trehalose biogenesis, and promotes maturation of conidia in cooperation with WetA [4, 15]. The activation of the class A, B, C, and D genes forms a conidiophore with the timing indicated in the top part of the Fig. 5. Adapted and modified from references [2, 7].

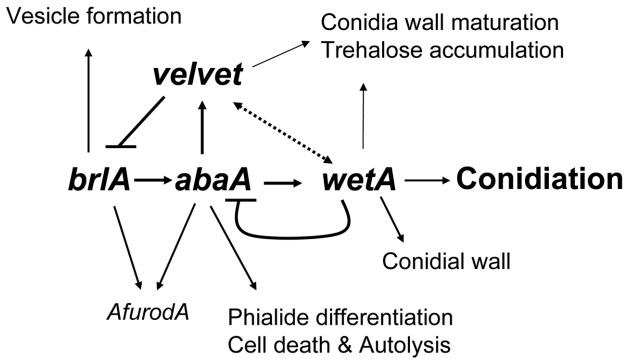

Fig. 4. Central regulatory pathway in *Aspergillus fumigatus* conidiation. BrlA, AbaA, WetA, and the *velvet* regulators including VosA play distinct roles in *A. fumigatus* asexual development. The *A. nidulans* model presented in Fig. 3 is generally applicable to the downstream regulation of *A. fumigatus* conidiation.

including *abaA*, *wetA*, *rodA*, and *yA* have multiple BREs in their promoter regions [2]. Importantly, Tao and Yu [8] reported that multiple BREs are present in the promoter regions of *Afu brlA*, *abaA*, *wetA*, *rodA* and the *velvet* regulators [15], implying a potentially conserved regulatory circuit in the two aspergilli.

AbaA and WetA. The *abaA* gene, activated by BrlA during the middle stages of conidiation when metulae and phialides are being formed, functions in the differentiation of phialides. Abacus (*abaA*) mutants have nearly normal conidiophores bearing abacus-like structures with swellings at intervals instead of chains of conidia [16]. Morphological studies have revealed that the metulae of *abaA* mutants produce supernumerary tiers of cells with properties of metulae, not phialides, indicating that *abaA* is essential for the differentiation and functionality of phialides as conidiogenous cells [17]. *abaA* expression is dependent on *brlA* activity [18], and BREs are found in the *abaA* promoter region [14], suggesting that BrlA directly activates *abaA*. The *abaA* gene encodes a developmental regulator, which begins to be expressed when phialides form at 15~18 hr after developmental induction (Fig. 3), and loss of *abaA* function results in abnormal expression of a number of developmentally regulated genes [18].

abaA overexpression in vegetative hyphae causes growth cessation and enhances cellular vacuolization but not spore formation in *Ani* [19]. *abaA* overexpression activates the expression of *wetA* and *brlA*, and it is thought that *abaA* induces the expression of *brlA* at certain times of development. However, the genetic interaction between *abaA* and *brlA* seems more complex, as *brlA* mRNA levels increase in *abaA* null mutants [15, 20], which might involve VosA (Fig. 3).

The AbaA protein contains an ATTS/TEA DNA-binding motif [21, 22] and a leucine zipper for potential dimerization [19]. Expression studies in both *Ani* and *S. cerevisiae* have demonstrated that AbaA binds to the *cis*-regulatory elements upstream of the *yA* gene [23]. Results of a gel mobility shift assay indicate that AbaA binds to the consensus sequence 5'-CATTCY-3' (AbaA response element [ARE] where Y is pyrimidine) [23]. In fact, multiple AREs are found in the promoter regions of developmentally regulated genes, including *brlA*α, *wetA*, *yA*, *rodA*, and *abaA*. Importantly, Park *et al.* [24] demonstrated that AbaA binds to the chitin synthase gene promoter, *chsC*, suggesting that AbaA also regulates chitin biosynthesis during conidiophore development by controlling the expression of certain chitin synthetases.

Wet-white (*wetA*) mutants produce colorless conidia that completely autolyze within a few days, leading to the formation of liquid droplets (wet) on the tops of conidial heads [16]. Sewall *et al.* [25] have shown that the *wetA* gene is required for the synthesis of crucial cell wall components late in the development of the inner C4 layer (Fig. 3). Similar to *brlA*, the *Ani wetA* gene encodes two transcription units, and *wetA* transcription is predicted to be complex and subjected to developmental control. AbaA, but not *brlA*, activates *wetA* expression, as *abaA* overexpression activates *wetA* in the absence of wild type *brlA* [19]. Furthermore, *wetA* is auto-activated, as it is not expressed in *wetA* temperature-sensitive mutants (Fig. 3) [18, 19].

The *Ani wetA* gene is predicted to encode a 60 kDa protein rich in serine (14%), threonine (7%), and proline (10%) [26]. While no known DNA binding domains are present in WetA, it has been proposed to function as a regulator of spore-specific gene expression [26]. This is based on observations that *wetA* alone is sufficient to activate many sporulation-specific genes [26], and that the *wetA* mutants fails to accumulate many sporulation-specific mRNAs [18]. Furthermore, *wetA* overexpression in vegetative cells inhibits hyphal growth, resulting in excessive hyphal branching and the activation of spore-specific genes [26]. However, *wetA* overexpression does not result in *brlA* or *abaA* activation or lead to precocious conidiation. Taken together, WetA is proposed to activate a set of genes required for spore formation and maturation, which may function to complete the final two conidial wall layers and/or direct their assembly (Fig. 3). Together, the *brlAabaAwetA* cascade has been proposed to define a central regulatory pathway that controls temporal and spatial expression of conidiation-specific genes during conidiophore development and spore maturation (Fig. 3) [4, 18, 19]. Recently, the novel regulator VosA has been identified in a genetic screen. VosA is a multifunctional regulator that acts in concert with the central regulatory genes, couples trehalose biogenesis and conidia maturation, and exerts negative feedback regulation of *brlA*, thereby com-

pleting conidiation in *Ani* (Fig. 3) [15].

*Afu*AbaA (EAL88194, 60% identity, 74% similarity to *Ani*AbaA) and *Afu*WetA (EAL89470, 58% identity, 68% similarity) were previously identified through a genome search [7]. The *AfuabaA* open reading frame (ORF) is composed of 2,518 bp with three exons and two introns and is predicted to encode a 797 aa-length protein containing an ATTS/TEA DNA-binding domain [8]. The *AfuwetA* ORF comprises 1,701 bp with no introns and is predicted to encode a 566 aa-length protein with a *conserved C-terminal* domain. Tao and Yu [8] examined the levels of the *AfuabaA* and *AfuwetA* transcripts throughout the lifecycle and found that *AfuabaA* and *AfuwetA* are highly expressed during asexual development. *AfuabaA* mRNA starts to accumulate at 6 hr post-developmental induction, reaches its highest level at 12 hr, and disappears at 48 hr. *AfuwetA* mRNA begins to accumulate at 12 hr post-developmental induction, reaches its highest level at 48 hr post induction, and is present in conidia. Further genetic studies have revealed that *AfuabaA* and *AfuwetA* expression is dependent on *Afu*BrlA [8].

Deletion of *AfuabaA* results in the formation of aberrant conidiophores exhibiting reiterated cylinder-like terminal cells without conidia [8], indicating that, as in *Ani*, *Afu*AbaA is essential for differentiation and functionalization of *Afu* phialides as conidiogenous cells. Importantly, Tao and Yu [8] found that the most distal cells of the Δ*AfuabaA* conidiophores are capable of apical hyphal growth, whereas the fractions of cylinder-like elongated phialides do not undergo vegetative growth. They also examined hyphal dry weights and found that the absence of *AfuabaA* causes delayed hyphal mass loss compared to that in the wild type. When cell viability was examined by determining the percent Alamar Blue reduction, which represents living cell mitochondrial activity, the Δ*AfuabaA* mutant clearly exhibited prolonged cell viability compared to the wild type. Tao and Yu [8] then examined the effects of *AfuabaA* overexpression and found that it caused accelerated hyphal fragmentation, disintegration, a dramatic reduction of mycelial mass, and precocious cell death. These results indicate that *Afu*AbaA functions in *Afu* autolysis and cell death.

The *AfuwetA* gene is subsequently activated by *Afu*AbaA in the middle to late phases of conidiation [8]. Deletion of *AfuwetA* causes the formation of colorless conidia with imperfect spore walls. Furthermore, TEM analyses of the *AfuwetA* mutant revealed that *wetA* conidiophores exhibit an interconnected conidia phenotype, i.e., incomplete conidial separation and maturation. As *Afu*WetA plays an essential role in completion of the conidial wall, Tao and Yu [8] hypothesized that the absence of *AfuwetA* may affect spore viability and/or integrity, and found that the Δ*AfuwetA* mutant exhibits dramatically reduced viability starting from day 10, whereas wild type conidia maintain high viability until day 20. Moreover, TEM studies revealed that, even at day 2, about 20% of the Δ*AfuwetA* conidia appear to lack cytoplasm and exhibit sheared conidial walls. These results indicate that *Afu*WetA is essential for both viability and integrity of conidia. Tao and Yu [8] then examined the effects of Δ*AfuwetA* on spore tolerance to various stressors and found that the Δ*AfuwetA* conidia were much more sensitive to heat, and oxidative and osmotic stresses. As trehalose is essential for long-term spore viability and stress resistance [15, 27], Tao and Yu [8] determined that the Δ*AfuwetA* conidia do not contain any trehalose, whereas wild type conidia harbor about 3.4 pg of trehalose per conidium. These results indicate that *Afu*WetA plays an essential role in trehalose biogenesis in conidia, which probably affects their viability and stress tolerance. This was the first report that WetA is essential for trehalose biogenesis in fungal conidia. Additionally, given that the *AfuwetA* gene is activated during the late stage of conidiation, and that its mRNA accumulates preferentially in mature conidia, Tao and Yu [8] further determined whether the absence of *AfuwetA* had an effect on spore germination and early vegetative growth. They found that after a 8-hr incubation in liquid submerged culture, the Δ*AfuwetA* mutant conidia formed only one unidirectional germ tube without branching, which resulted in loosened mycelial aggregates, whereas conidia of wild type and complemented strains produced bi- or multi-directional germ tubes that formed branches. Moreover, after a 14-hr incubation, the Δ*AfuwetA* mutant clearly showed a five-fold reduction in hyphal branching [8]. These results suggest that *Afu*WetA is associated with proper germ tube formation and vegetative growth. This is consistent with the previous finding that *AniwetA* overexpression in hyphae causes excessive branching in *Ani* [26].

The BrlA-AbaA-WetA cascade in *Afu*. Tao and Yu [9] further dissected the genetic interactions among the central regulatory genes in *Afu*. The absence of *AfubrlA* eliminates the expression of all conidiation-specific genes tested, indicating that *Afu*BrlA functions upstream of *AfuabaA*, *AfuwetA*, *AfuvosA*, and *AfurodA*. As *Afu*AbaA is necessary for activating *AfuwetA*, *AfuwetA* mRNA accumulation is not detected in the Δ*AfuabaA* mutant. These results corroborate the *AfubrlA* → *AfuabaA* → *AfuwetA* genetic pathway in *Afu*. Moreover, levels of *AfuvosA* mRNA decrease considerably in both Δ*AfuabaA* and Δ*AfuwetA* strains, indicating the role of these genes in proper *AfuvosA* expression. However, as *AfuvosA* is activated before *AfuwetA* in the wild type, it is proposed that *Afu*AbaA primarily activates *AfuvosA* with the assistance of *Afu*WetA. Importantly, *AfubrlA* is highly upregulated in the Δ*AfuabaA* and Δ*AfuwetA* mutants, particularly in conidia (aberrant conidiophores in Δ*AfuabaA*) and early

(6 hr) and late (24 hr) phases of vegetative growth, suggesting that *Afu*AbaA and *Afu*WetA are necessary for proper negative regulation of *AfubrlA* upon completion of conidiation and during certain phases of vegetative growth. *Afu*RodA is a small, moderately secreted hydrophobic polypeptide that forms the outermost rodlet layer of conidia. Conidia of *AfurodA* mutants lack the external rodlet layer and are hydrophilic [28]. As no *AfurodA* mRNA is detected in the Δ*AfubrlA* mutant and *AfurodA* levels are low in the Δ*AfuabaA* mutant, Tao and Yu [8] speculated that *AfurodA* is primarily activated by *Afu*BrlA with the assistance of *Afu*AbaA. Accordingly, a genetic model regulating asexual development in *Afu* is proposed (Fig. 4).

VosA and the *velvet* regulators. A recent genetic study identified the novel regulator VosA, which functions in maturation of conidia and completion of *Ani* development [15]. The *vosA* mRNA(s) accumulates specifically during the formation of sexual and asexual spores. Importantly, *vosA* deletion results in the lack of trehalose in both types of spores, resulting in a rapid disappearance (evaporation) of the cytoplasm, disintegration of cellular organelles, and loss of long-term spore viability. Moreover, the *vosA* mutant spores exhibit dramatically reduced tolerance to heat and oxidative stress. As VosA mainly localizes in the nucleus of mature conidia and contains a potential transcriptional activation domain at the C terminus [15], it may be a TF that primarily controls the late process of sporulation, including trehalose biogenesis. The VosA protein expressed in metulae and phialides and later localized to the nucleus of the conidia plays two chief roles: 1) activation of genes involved in spore maturation; 2) negative feedback regulation of *brlA* and developmental specific genes (Fig. 3). VosA studies clearly divide conidiophore formation and conidia maturation into two distinct genetic phases.

VosA has been identified with three other *Ani* proteins sharing high levels of similarity; VeA [29], VelB (EF540815), and VelC (EF540816). These, together with VosA, define the *velvet* regulators, and they are highly conserved in many filamentous and dimorphic fungi, sharing at least one highly conserved domain [15, 30]. Importantly, recent studies have shown that VeA, VelB, and VosA bridge light-responding development and secondary metabolism by forming trimeric complexes with the nuclear master regulator of secondary metabolism, LaeA, in *Ani* [30, 31].

The *AfuvosA* gene shows an mRNA accumulation pattern almost identical to that of *AnivosA* [15]. To begin to understand the roles of the *Afu velvet* proteins, we generated *A fu* mutants lacking *AfuveA*, *AfuvelB*, *AfuvosA*, and *AfuvelC*, and found that, while the deletion of *AfuvosA* caused an approximate 50% reduction in the spore trehalose content and viability, it did not result in uncontrolled activation of conidiation in *Afu* (Park and Yu, unpublished) [15]. Our recent unpublished data suggest that the feedback regulation of conidiation in *Afu* is primarily exerted by VelB and VeA, whereas trehalose biogenesis is conferred by both *Afu*VosA and *Afu*VelB (additive roles) (Fig. 4) (Park and Yu, unpublished).

Upstream regulators of asexual development. Identification and characterization of six upstream genes (*fluG*, *flbA*, *flbB,* *flbC*, *flbD*, and *flbE*) required for proper expression of *brlA* in *Ani* have illuminated genetic regulatory cascades for activating conidiation [2]. Among these, *flbB*, *flbC*, *flbD*, and *flbE* are defined by mutants exhibiting the fluffy delayed conidiation phenotypes [32]. FlbB, FlbC, and FlbD are putative TFs containing a basic leucine zipper (b-zip), two C_2H_2 zinc fingers, and a cMyb-DNA binding domain, respectively. Thus, it has been thought that FlbB, FlbB, and FlbD likely function as DNA binding proteins that may control the transcriptional activation of other developmental regulators, such as *brlA*, in response to sporulation signals [2]. Detailed functional studies of these potential TFs have demonstrated that FlbB/C/D activate *brlA* expression [33-38]. Moreover, b-zip TF FlbB is necessary for the activation of *flbD* expression, while FlbB and FlbD cooperatively activate *brlA* [37].

Kwon *et al*. [38] recently characterized FlbC in *Ani*. They found that *flbC* mRNA is present throughout the lifecycle at relatively high levels during vegetative growth and during early asexual and late sexual developmental phases. Deletion of *flbC* causes a delay/reduction in sporulation, *brlA* and *vosA* expression, and conidial germination. While *flbC* overexpression does not cause conidiophore development, it inhibits hyphal growth and activates expression of *brlA*, *abaA*, and *vosA*, but not *wetA*. Kwon *et al.* [38] further reported that FlbC contains two C_2H_2 zinc-fingers at the C-terminus and a putative activation domain at the N-terminus. They also found that FlbC localizes to the nuclei of both hyphae and developmental cells. Localization and expression of FlbC is not affected by the absence of FlbB or FlbE and vice versa. Importantly, as *flbC* overexpression inhibits growth and activates *abaA* and *vosA* in the absence of *brlA* and *abaA*, respectively, Kwon *et al.* [38] proposed that FlbC plays a direct activating role in the expression of these genes. Consistent with this idea, an *in vitro* DNA binding assay revealed that FlbC binds to the promoter regions of *brlA*, *abaA*, and *vosA*, but not to that of *wetA*.

The *AniflbE* gene is predicted to encode a 201 aa-length polypeptide with two conserved yet uncharacterized domains, and it has been demonstrated that FlbE and FlbB are functionally interdependent, that they physically interact *in vivo*, and co-localize to the hyphal tip in *Ani* [36]. Our recent studies [39] revealed that both deletion and overexpression of *flbE* in *Ani* results in developmen-

tal defects, enhanced autolysis, precocious cell death, and delayed expression of *brlA*/*vosA*, suggesting that balanced FlbE activity is crucial for proper growth and development. The N-terminal portion of FlbE exhibits a transactivation ability in budding yeast, whereas the C-terminal half negatively affects this activity. Site-directed mutagenesis of certain conserved N-terminal amino acids abolishes transactivation ability, overexpression-induced autolysis, and complements the null mutation. These results suggest that the conserved N-terminal domain might be crucial for the functionality of FlbE. Finally, *flbD* overexpression, but not that of *flbB* or *flbC*, restores conidiation in *Ani* D*flbE*, generally supporting the current genetic model of developmental regulation [39].

In a series of recent studies, we characterized the functions of FlbE and FlbB in *Afu* [39, 40]. The predicted *Afu* FlbE protein is composed of 222 aa in length. While *flbE* is transiently expressed during the early growth phase in *Ani*, *AfuflbE* is somewhat constitutively expressed during the *Afu* lifecycle. Deletion of *AfuflbE* causes reduced conidiation and delayed expression of *brlA* and *vosA* in both species. Moreover, *Afu*FlbE is necessary for salt-induced development of *Afu* in a liquid submerged culture. As the *Ani flbE* null mutation can be fully complemented by *AfuflbE,* the developmental function of FlbE appears to be conserved in aspergilli [39].

Xiao *et al*. [40] conducted a series of molecular analyses and found that *AfuflbB* produces two transcripts predicted to encode two b-zip polypeptides, *Afu*FlbBb (420 aa) and *Afu*FlbBa (390 aa). *AfuflbB* deletion results in delayed and reduced conidiation, precocious cell death, the absence of conidiophore development in liquid submerged culture, altered expression of *AfubrlA* and *AfuabaA*, and the lack (or a reduction) in gliotoxin production. Importantly, they found that while introducing the wild type *AfuflbB* allele fully complemented these defects, disruption of the ATG start codon to either one of the *Afu*FlbB polypeptides led to partial complementation, indicating that both polypeptides are needed for wild type level sporulation and gliotoxin biogenesis. Consistent with these observations, introducing *Ani flbB* encoding one polypeptide (426 aa) into the *AfuflbB* null mutant partially restores asexual and chemical development. Xiao *et al*. [40] also found that the presence of 0.6 M KCl in a liquid submerged culture suppresses the defects caused by the lack of one, but not both, of the *Afu*FlbB polypeptides, suggesting a genetic prerequisite for *Afu*FlbB in *Afu* development. Northern blot analyses revealed that both *AfuflbB* and *AfuflbE* are necessary for *AfuflbD* expression, suggesting that FlbD functions downstream of FlbB and FlbE in both aspergilli. Xiao *et al*. [40] further suggested that *Afu*BrlA might be necessary for gliotoxin biosynthesis, which was based on the observations that the Δ*AfubrlA* mutant lacks gliotoxin production, and that multiple BREs are present in the promoter regions of many gliotoxin biosynthetic and regulatory genes.

Regarding the upstream region of FlbB/C/D/E, Mah and Yu [12] examined the functions of the *AfufluG* gene in *Afu* conidiation. As discovered in *Ani* [41], levels of the *AfufluG* transcript are relatively constant throughout the lifecycle. The *AfufluG* deletion mutant conidiated normally, similar to wild type on solid medium, indicating that activation of *Afu* conidiation in the presence of air does not require *Afu*FluG. However, the *AfufluG* deletion mutant did not produce conidiophores in liquid submerged culture, whereas *Afu* wild type strains sporulated abundantly at approximately 24 hr. Moreover, the *AfufluG* deletion mutant showed reduced conidiation levels and delayed *AfubrlA* expression upon induction of synchronized asexual development. These results led Mah and Yu [12] to conclude that while the presence of air bypasses the need for *Afu*FluG in conidiophore development, *Afu*FluG plays a particular role in *Afu* conidiation and *AfubrlA* expression. These findings led to the hypothesis that *Afu* has multiple pathways to activate *AfubrlA* expression (Fig. 5).

Model and Conclusions

Our further studies suggest that *Afu*FlbD is essential for proper asexual development in *Afu* (Xiao and Yu, unpub-

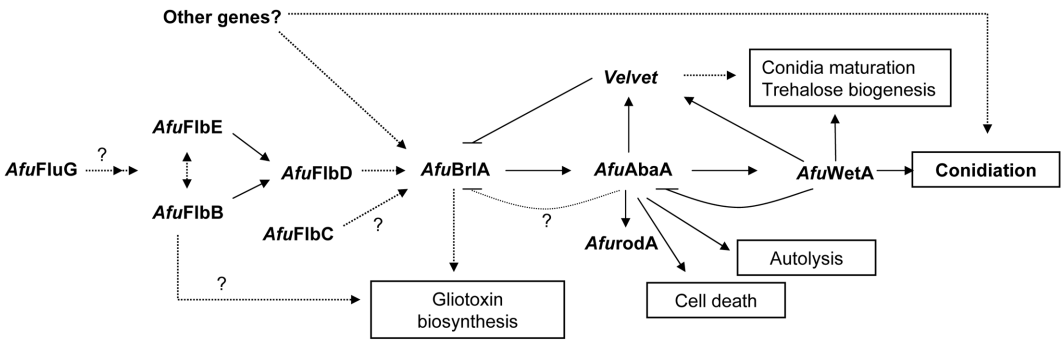

Fig. 5. Comprehensive model for upstream and downstream regulation of *Aspergillus fumigatus* asexual and chemical development.

lished data). To examine potential genetic interactions among upstream developmental regulators in *Afu*, Xiao *et al*. [40] conducted a series of expression studies and proposed that *Afu* has an upstream regulatory cascade slightly different from the one proposed for *Ani* (Fig. 5). As *AfuflbB* and *AfuflbE* expression is independent, and both are required for proper *AfuflbD* expression, Xiao *et al*. [40] proposed that *Afu*FlbB and *Afu*FlbE function upstream of *Afu*FlbD and cooperatively activate *AfuflbD*, which subsequently activates *AfubrlA*. While the transcriptional activation of *AfuflbB* and *AfuflbE* is independent, it may be that the *Afu*FlbB and *Afu*FlbE proteins interact and form a functional complex as in *Ani* [36]. Furthermore, as *AfuflbC* expression is independent of *Afu*FlbB and *Afu*flbE, *Afu*FlbC is predicted to function in a separate pathway [40]. Because the Δ*AfubrlA* mutant is defective in gliotoxin production and multiple BREs are present in the promoter regions of many gliotoxin biosynthetic and regulatory genes, Xiao *et al*. [40] speculated that *Afu*BrlA may play a direct positive role in gliotoxin biosynthesis. Further functional and molecular studies of *AfuflbC* and the identification of additional developmental regulators, including *AfusfgA* and *AfuwetB*, are in progress in my laboratory.

In this review, I have briefly summarized our current understanding of the upstream and downstream regulation of *Aspergillus* conidiation focusing on key TFs. In summary, these two aspergilli have common essential downstream activators of conidiophore development; BrlA, AbaA, and WetA. Moreover, *velvet* regulator functions are generally conserved in these aspergilli. However, as clear differences in the upstream regulation of conidiation between the two species exist, much remains to be learned. Further genetic and genomics studies aimed at identifying and characterizing the unique regulators and control networks in *Afu* will illuminate the complex mechanisms of development in this opportunistic pathogenic fungus.

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