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

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(Received September 28, 2010. Accepted November 16, 2010)

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, non-motile spores formed from the apex or side of conidiogenous cells. 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).

*Aspergillus 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-
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 fumigatus*. 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 C₂H₂ 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, metulae, 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 *Ani* *brlA* (but not *Afu* *brlA*) gene is a compound gene consisting of two overlapping transcriptional units designated *brlAa* and *brlb*. 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 *Afu* *brlA* 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 *Afu* *brlA* deletion completely eliminated asexual development in *Afu*, resulting in elongated aerial hyphae and increased hyphal mass in the colonies. These studies clearly demonstrated that *Afu* *brlA* 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 C₂H₂ 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

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**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. 3. Adapted and modified from references [2, 7].
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 brlAa, 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 autolize 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 fail 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-

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.
These results indicate that reduction of mycelial mass, and precocious cell death. Accelerated hyphal fragmentation, disintegration, a dramatic effect ofpared to the wild type. Tao and Yu [8] then examined the effects of \( \Delta \text{AfuwetA} \) on sporulation to various stressors and found that the \( \Delta \text{AfuwetA} \) conidia were much more sensitive to heat, and oxidative and osmotic stresses. As trehalose is essential for long-term spor viability and stress resistance [15, 27], Tao and Yu [8] determined that the \( \Delta \text{AfuwetA} \) conidia do not contain any trehalose, whereas wild type conidia harbor about 3.4 pg of trehalose per conidium. These results indicate that \( \text{Af}u\text{WetA} \) plays an essential role in trehalose biogenesis in conidia, which probably affects their viability and stress tolerance. This was the first report that \( \text{WetA} \) is essential for trehalose biogenesis in fungal conidia. Additionally, given that the \( \text{AfuwetA} \) 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 \( \text{AfuwetA} \) had an effect on spore germination and early vegetative growth. They found that after a 8-hr incubation in liquid submerged culture, the \( \Delta \text{AfuwetA} \) 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 \( \Delta \text{AfuwetA} \) mutant clearly showed a five-fold reduction in hynphal branching [8]. These results suggest that \( \text{Af}u\text{WetA} \) is associated with proper germ tube formation and vegetative growth. This is consistent with the previous finding that \( \text{AniwetA} \) overexpression in hyphae causes excessive branching in \textit{Ani} [26].

The \textit{BrlA-AbAA-WetA} cascade in \textit{Af}u. Tao and Yu [9] further dissected the genetic interactions among the central regulatory genes in \textit{Af}u. The absence of \textit{AfubrlA} eliminates the expression of all conidiation-specific genes tested, indicating that \( \text{Af}u\text{Br}lA \) functions upstream of \textit{AfubaAA}, \textit{AfuwetA}, \textit{AfivosA}, and \textit{AfurodA}. As \( \text{Af}u\text{AbAA} \) is necessary for activating \( \text{AfuwetA} \), \( \text{AfuwetA} \) mRNA accumulation is not detected in the \( \Delta \text{AfubaAA} \) mutant. These results corroborate the \( \Delta \text{AfubrlA} \rightarrow \Delta \text{AfubaAA} \rightarrow \Delta \text{AfuwetA} \) genetic pathway in \textit{Af}u. Moreover, levels of \( \text{AfivosA} \) mRNA decrease considerably in both \( \Delta \text{AfubaAA} \) and \( \Delta \text{AfuwetA} \) strains, indicating the role of these genes in proper \( \text{AfivosA} \) expression. However, as \( \text{AfivosA} \) is activated before \( \text{AfuwetA} \) in the wild type, it is proposed that \( \text{Af}u\text{AbAA} \) primarily activates \( \text{AfivosA} \) with the assistance of \( \text{Af}u\text{WetA} \). Importantly, \( \text{AfubrlA} \) is highly upregulated in the \( \Delta \text{AfubaAA} \) and \( \Delta \text{AfuwetA} \) mutants, particularly in conidia (aberrant conidiophores in \( \Delta \text{AfubaAA} \)) and early
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 potential transcriptional activation domain at the C terminus almost identical to that of VosA. The vosA mRNA is detected in the ΔAfubrlA mutant and AfuVeA levels are low in the ΔAfubrlA mutant, Tao and Yu [8] speculated that AfuVeA is primarily activated by AfuBrlA with the assistance of AfuAbaA. Accordingly, a genetic model regulating asexual development in Afu is proposed (Fig. 4).

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 C2H2 zinc fingers, and a cMyb-DNA binding domain, respectively. Thus, it has been thought that FlbB, FlbD, and FlbE 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, abA, and vosA, but not wetA. Kwon et al. [38] further reported that FlbC contains two C2H2 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 abA and vosA in the absence of brlA and abA, 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, abA, 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-
tual defects, enhanced autolysis, precocous 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 ∆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*, *Afu* flbE is somewhat constitutively expressed during the *Afu* lifecycle. Deletion of *Afu* flbE 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 *Afu*flbE, 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 *Afu*flbB produces two transcripts predicted to encode two b-zip polypeptides, *Afu*flbB (420 aa) and *Afu*flbBa (390 aa). *Afu*flbB deletion results in delayed and reduced conidiation, precocous cell death, the absence of conidiophore development in liquid submerged culture, altered expression of *Afu*brlA and *Afu*abaA, and the lack (or a reduction) in gliotoxin production. Importantly, they found that while introducing the wild type *Afu*flbB 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 *Afu*flbB 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 *Afu*flbB and *Afu*flbE are necessary for *Afu*flbD 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 *∆Afu*brlA 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 *Afu*fluG gene in *Afu* conidiation. As discovered in *Ani* [41], levels of the *Afu*fluG transcript are relatively constant throughout the lifecycle. The *Afu*fluG 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 *Afu*fluG deletion mutant did not produce conidiophores in liquid submerged culture, whereas *Afu* wild type strains sporulated abundantly at approximately 24 hr. Moreover, the *Afu*fluG deletion mutant showed reduced conidiation levels and delayed *Afu*brlA 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 *Afu*brlA expression. These findings led to the hypothesis that *Afu* has multiple pathways to activate *Afu*brlA 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-

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**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 *AfuFlbB* and *AfuFlbE* function upstream of *AfuFlbD* and cooperatively activate *AfuflbD*, which subsequently activates *AfuBr1A*. While the transcriptional activation of *AfuflbB* and *AfuflbE* is independent, it may be that the *AfuFlbB* and *AfuFlbE* proteins interact and form a functional complex as in *Ani* [36]. Furthermore, as *AfuflbC* expression is independent of *AfuFlbB* and *AfuFlbE*, *AfuFlbC* is predicted to function in a separate pathway [40]. Because the Δ*AfuBr1A* 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 *AfuBr1A* may play a direct positive role in gliotoxin biosynthesis. Further functional and molecular studies of *AfuflbC* and the identification of additional developmental regulators, including *AfuflgA* and *Afuwe1B*, 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; *Br1A*, 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.

Acknowledgements

I thank Dr. Kwang-Soo Shin, Dr. Nak-Jung Kwon, Dr. Min Ni, Peng Xiao, Li Tao, HeeSoo Park, and other fungal biologists for their wonderful contributions to a better understanding of fungal development. This work was primarily supported by National Science Foundation (IOS-0950850) grant to JHY.

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