

REVIEW

Developmental regulators in *Aspergillus fumigatus*

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The filamentous fungus *Aspergillus fumigatus* is the most prevalent airborne fungal pathogen causing severe and usually fatal invasive aspergillosis in immunocompromised patients. This fungus produces a large number of small hydrophobic asexual spores called conidia as the primary means of reproduction, cell survival, propagation, and infectivity. The initiation, progression, and completion of asexual development (conidiation) is controlled by various regulators that govern expression of thousands of genes associated with formation of the asexual developmental structure conidiophore, and biogenesis of conidia. In this review, we summarize key regulators that directly or indirectly govern conidiation in this important pathogenic fungus. Better understanding these developmental regulators may provide insights into the improvement in controlling both beneficial and detrimental aspects of various *Aspergillus* species.

Keywords: *Aspergillus fumigatus*, conidia, asexual development, regulation, signaling

Introduction

Aspergillus fumigatus is a saprophytic fungus that is most commonly found in the environment (Latge, 1999; Kwon-Chung and Sugui, 2013). This ubiquitous fungus produces a massive number of hydrophobic small asexual spores (conidia) that easily spread in the air (Dagenais and Keller, 2009; Ebbole, 2010). The airborne spores are inhaled by humans and can cause serious invasive pulmonary aspergillosis mainly in immunocompromised individuals with a mortality rate of over 50% (Latge, 1999, 2001; McCormick *et al.*, 2010; Cramer *et al.*, 2011). Conidia of this pathogenic fungus also contain potent allergens to which some people respond with hypersensitive reaction causing allergic bronchopulmonary aspergillosis (Stevens *et al.*, 2003; Tillie-

Leblond and Tonnel, 2005).

A. fumigatus can undergo asexual and sexual reproductive cycles (Adams *et al.*, 1998; Heitman *et al.*, 2014). While this fungus can produce sexual fruiting bodies cleistothecia, the majority of *A. fumigatus* are known to reproduce by asexual development (conidiation) (O’Gorman *et al.*, 2009; Yu, 2010; Dyer and O’Gorman, 2012; Alkhayyat *et al.*, 2015). Conidiation in *A. fumigatus* results in formation of conidia, which are produced on the asexual specific structures called conidiophores (Fig. 1A). These specialized developmental structures are important species-specific feature and can be used in taxonomy (Adams *et al.*, 1998; Bennett, 2010). The process of conidiation is an accurately timed and genetically programmed event responding to internal and external signals (Mirabito *et al.*, 1989; Timberlake, 1990; Adams *et al.*, 1998). The genetic control of conidiation in the model fungus *Aspergillus nidulans* has been intensively investigated over the past three decades (Adams *et al.*, 1998; Ni *et al.*, 2010; Park and Yu, 2012; Krijgsheld *et al.*, 2013). These studies have provided important knowledges for understanding conidiation in other aspergilli (Yu *et al.*, 2006; Yu, 2010; Alkhayyat *et al.*, 2015). In this review, we summarize known genetic components and the regulatory pathways governing conidiation in *A. fumigatus*.

Morphology of conidiophores

Conidiophore is a specialized multicellular developmental structure, which bears mitotically derived conidia (Adams *et al.*, 1998; Bennett, 2010). The formation of conidiophore is a highly intricate process, which can be divided into several differential stages (Timberlake, 1990; Ni *et al.*, 2010). Under proper conditions, the vegetative cells cease hyphal growth and start to form foot cells, thick-walled hyphal cells, which then extend into air to produce aerial hypha called a stalk. After extension of stalks stops, the tip of a stalk begins to swell and forms a multinucleate vesicle. The numerous buds, produced synchronously on the top of the vesicle, form one layer of uninucleate sterigmata termed phialides. Phialides, like stem cells, repeatedly produce differentiated cells via asymmetric mitotic division and form long chains of conidia which then undergo a maturation process including the delicate modification of the conidial wall, and a specific metabolic remodeling. These resulting multicellular structures contains up to 50,000 conidia and are called conidiospores (Yu, 2010) (Fig. 1A).

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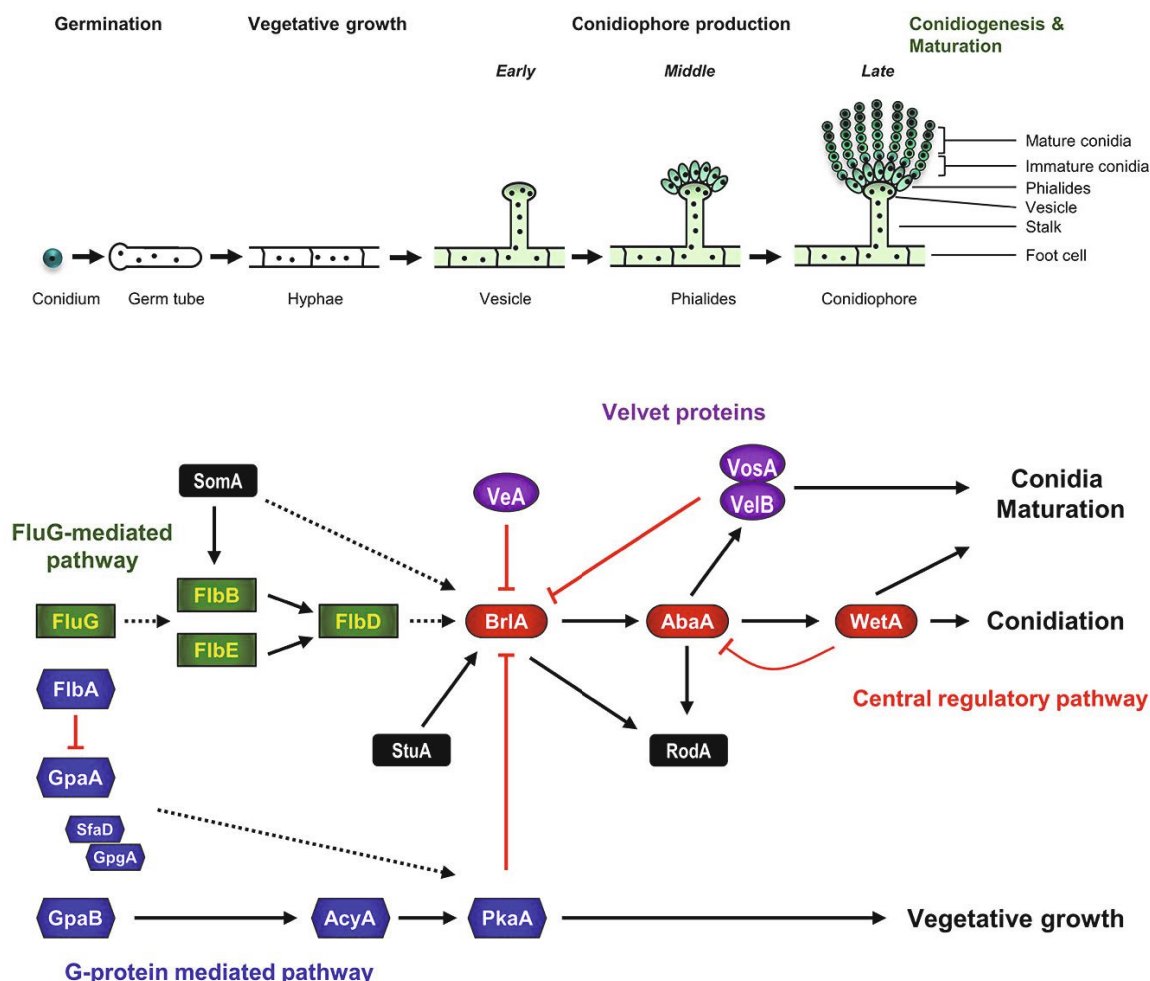


Fig. 1. Summary and regulation of conidiation in *Aspergillus fumigatus*. (A) The stages and progression of conidiation in *A. fumigatus*. (B) A genetic model for elements affecting the central regulatory pathway of conidiation in *A. fumigatus*.

Regulation of conidiation in *A. fumigatus*

Conidiation in *Aspergillus* involves many common themes including intra- and inter-cellular communications, temporal and spatial control of developmental-gene expression, and specialized differentiation of cells (Aguirre *et al.*, 1990; Aguirre, 1993; Adams *et al.*, 1998). The entire process of conidiophore production is genetically regulated and exerted by multiple activators and repressors (Mirabito *et al.*, 1989). These regulators govern the coordinated expression of distinct gene sets, which are required for progression of each stage (Etxebeste *et al.*, 2010; Ni *et al.*, 2010; Park and Yu, 2012). Known developmental regulators of *A. fumigatus* are summarized in Table 1.

Central regulators of conidiation

The central regulatory pathway controlling conidiation is highly conserved in *Aspergillus* (Yu, 2010; Alkhayyat *et al.*, 2015). This central regulatory pathway contains the three key elements BrlA, AbaA, and WetA that coordinate conidiation-specific gene expression, and determine the order of

gene activation during conidiophore formation and spore maturation (Boylan *et al.*, 1987; Mirabito *et al.*, 1989; Adams *et al.*, 1998).

BrlA is a key activator for developmental initiation in *Aspergilli* (Mah and Yu, 2006; Alkhayyat *et al.*, 2015). The *brlA* gene is specifically expressed during early phase of conidiation. The *brlA* null mutants mainly produce elongated aerial hyphae that fail to form asexual structure beyond the stalk stage of conidiogenesis, suggesting that BrlA is essential for the initiation of conidiophore formation. This key activator contains two C₂H₂ zinc finger domains and acts as a transcriptional activator. BrlA is necessary for proper expression of conidiation-related genes such as *abaA*, *wetA*, *vosA*, and *rodA* (Tao and Yu, 2011). These genes contain putative BrlA response elements (BREs; 5'-[C/A][G/A]AGGG[G/A]-3') in their promoter regions. BrlA is also required for other fungal biology. Deletion of *brlA* also alters expression of certain genes, which are related to conidial pigmentation, gliotoxin biosynthesis and ribosome pathway (Twumasi-Boateng *et al.*, 2009). The *brlA* deletion mutant exhibits reduced expression of the GliM and GliT proteins and fail to produce gliotoxin (Shin *et al.*, 2015).

Table 1. Summary of known genes involved in conidiation in *Aspergillus fumigatus*

Genes	Description (domain)	Functions	References
BrlA	C ₂ H ₂ zinc finger transcription factor	Initiation of conidiation	Mah and Yu (2006)
AbaA	Transcription activator (ATTS/TEA)	Conidiophore morphology, Phialides formation, Cell death and autolysis	Tao and Yu (2011)
WetA	Regulatory protein	Conidia wall formation Trehalose biosynthesis in conidia	Tao and Yu (2011)
FluG	Upstream activator of conidiation	Positively regulates conidiation	Mah and Yu (2006)
FlbB	basic leucine zipper transcription factor	Positively regulates conidiation	Xiao <i>et al.</i> (2010)
FlbE	Uncharacterized	Positively regulates conidiation	Kwon <i>et al.</i> (2010)
VeA	Velvet protein	Distinct roles in conidiation	Park <i>et al.</i> (2012a) Dhingra <i>et al.</i> (2012)
VelB	Velvet protein	Repressor of conidiation Required for conidia maturation	Park <i>et al.</i> (2012a)
VosA	Velvet protein	Repressor of conidiation Required for conidia maturation	Park <i>et al.</i> (2012a)
LaeA	Putative methyltransferase	Positively regulates conidiation	Bok <i>et al.</i> (2005)
GpaA	Gα subunit	Negatively regulates conidiation Positive regulates vegetative growth	Mah and Yu (2006)
GpaB	Gα subunit	Activates for vegetative growth and conidiation	Liebmann <i>et al.</i> (2003)
SfaD	Gβ subunit	Negatively regulates conidiation Positive regulates vegetative growth and conidial germination	Shin <i>et al.</i> (2009)
GpgA	Gγ subunit	Negatively regulates conidiation Positive regulates vegetative growth and conidial germination	Shin <i>et al.</i> (2009)
CpcB	Gβ-like protein	Required for vegetative growth, conidia germination, conidiation	Kong <i>et al.</i> (2013) Cai <i>et al.</i> (2015)
RicA	Putative GDP/GTP exchange factor	Putative activator of G protein signaling	Kwon <i>et al.</i> (2012)
FlbA	RGS protein	Attenuates GpaA-mediated signaling	Mah and Yu (2006)
AcyA	Putative Adenylate cyclase	Required for vegetative growth, conidiation, pigment formation	Liebmann <i>et al.</i> (2003)
PkaA	Catalytic subunit of protein kinase A	Required for vegetative growth, conidiation, pigment formation	Grosse <i>et al.</i> (2008)
PksP	Polyketide synthase	Conidial pigmentation	Jahn <i>et al.</i> (2002)
CnaA	Calcineurin catalytic subunit	Required for growth and conidiation	Steinbach <i>et al.</i> (2006)
CrzA	Calcineurin-responsive zinc finger transcription factor	Required for germination, growth and conidiation	Cramer <i>et al.</i> (2008) Soriani <i>et al.</i> (2008)
Hsp90	Heat-shock protein	Conidiation and cell wall integrity	Lamoth <i>et al.</i> (2012)
RasA	Monomeric GTPase protein	Required for conidiophore formation and morphology	Fortwendel <i>et al.</i> (2004)
RasB	Monomeric GTPase protein	Required for conidiophore formation and morphology	Fortwendel <i>et al.</i> (2004)
RhbA	Ras superfamily	Required for conidiophore in nitrogen source dependent	Panepinto <i>et al.</i> (2003)
StuA	APSES family protein	Required for conidiophore formation and morphology	Sheppard <i>et al.</i> (2005)
MedA	Developmental modifier	Required for conidiophore formation and morphology	Gravelat <i>et al.</i> (2010)
SrgA	Rab GTPase protein	Required for conidiophore formation phialides morphology	Powers-Fletcher <i>et al.</i> (2013)
SomA	Transcription factor (LUFS)	Upstream regulator of conidiation	Lin <i>et al.</i> (2015)
RodA	Hydrophobin	Essential for rodlet layer in conidia	Thau <i>et al.</i> (1994)
RodB	Hydrophobin	Essential for rodlet layer in conidia	Paris <i>et al.</i> (2003)
TpsA	Trehalose synthases	Conidial maturation and germination	Al-Bader <i>et al.</i> (2010)
TpsB	Trehalose synthases	Conidial maturation and germination	Al-Bader <i>et al.</i> (2010)
OrlA	Trehalose 6-phosphate phosphatase	Conidiospores production and conidiophore morphology	Puttikamonkul <i>et al.</i> (2010)

AbaA is a key regulator for the differentiation and function of phialides (Sewall *et al.*, 1990a; Tao and Yu, 2011). The absence of *abaA* causes formation of atypical conidiophores exhibiting long chains of cylinder-like cells without conidia. While the conidiophores of the *abaA* deletion mutant can produce apical hyphal cells, the cylinder-like elongated phialides are not capable to undergo vegetative growth (Tao and Yu, 2011). The *abaA* mRNA highly accumulates during the middle phase of asexual development and then disappears after formation of phialides. The *abaA* expression is dependent on BrlA, but not WetA. AbaA contains an ATTS/TEA DNA-binding domain and is required for expression of spore-specific genes, including *wetA*, *vosA*, and *velB*, that contain

putative AbaA response elements (AREs, 5'-CATTCTY, where Y is a pyrimidine) in their promoter regions (Andrianopoulos and Timberlake, 1991, 1994; Tao and Yu, 2011). Taken together, these data demonstrate that AbaA is a transcriptional factor regulating expression of genes associated with late phase of conidiation. In addition to this function, AbaA also functions in autolysis and cell death. Deletion of *abaA* results in delayed hyphal mass loss and prolonged cell viability compared to wild type, whereas overexpression of *abaA* induces hyphal fragmentation, reduction of mycelial mass, and precocious cell death (Tao and Yu, 2011).

WetA is essential for the completion of conidiation (Sewall *et al.*, 1990b; Tao and Yu, 2011). The *wetA* gene is activated

by AbaA in the middle to late phases of conidiation, and its mRNA is highly accumulated in the late phase of conidiation and in conidia, implying that WetA mainly functions in conidia. The absence of *wetA* results in the formation of the white conidia with defective conidia cell wall. Conidia of the $\Delta wetA$ mutant lack both crenulation of the electron-dense C1 layer and condensation of the electron-light C2 layer. The *wetA* mutant conidia exhibit reduced tolerance to several stresses and spore viability, and do not contain any trehalose (a necessary cellular protectant), suggesting that WetA plays a critical role in trehalose biogenesis in conidia, which may affect conidia viability and stress tolerance. Additionally, WetA functions in spore germination and early phase of fungal growth (Tao and Yu, 2011). The deletion of *wetA* results in delayed germ-tube formation and decreased hyphal branching.

To dissect the genetic relationship between the central regulatory genes in *A. fumigatus*, Tao and Yu (2011) carried out a series of Northern blot analyses. The $\Delta brlA$ mutant fails to accumulate mRNA of certain conidiation-specific genes, indicating that BrlA functions upstream of *abaA*, *wetA*, *vosA*, and *rodA*. Accumulation of *wetA* mRNA is not detected in the $\Delta abaA$ mutant, suggesting that AbaA is necessary for the activation of *wetA*. These results confirm the $BrlA \rightarrow AbaA \rightarrow WetA$ activatory pathway in *A. fumigatus*. Importantly, *brlA* mRNA is highly accumulated in the $\Delta abaA$ or $\Delta wetA$ mutant during late phase of conidiation and early phase of vegetative growth, suggesting that AbaA and WetA somehow confer negative feedback regulation of *brlA*. Based on these results, a genetic model regulating conidiation in *A. fumigatus* is proposed (Fig. 1B).

Upstream regulators of conidiation

Activation of *brlA*, a key step for conidiation initiation, is regulated by various upstream activators and repressors (Etexbeste *et al.*, 2010; Alkhayyat *et al.*, 2015). Previously, six upstream developmental activators, *fluG*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE*, were identified via genetic analyses of recessive mutations in *A. nidulans* (Wieser *et al.*, 1994). The deletion of each one of these upstream developmental activators causes *fluffy* phenotypes with reduced *brlA* expression. The *A. fumigatus* homologues of six upstream developmental activators were identified, and FluG, FlbA, FlbB, and FlbE were characterized (Yu *et al.*, 2006). FluG acts as an upstream regulator of asexual development (Mah and Yu, 2006). The *fluG* deletion mutant fails to produce conidiophores in liquid medium, whereas wild type (WT) strains form asexual structure under the same condition. Moreover, the *fluG* deletion mutant exhibits decreased conidiospore production and delayed *brlA* expression during synchronous asexual developmental induction. However, the *fluG* deletion mutant produces similar amount of conidiospores in air-exposed culture. These results imply that the presence of air bypasses the need for FluG in asexual development, and that FluG plays a certain activating role in *brlA* expression and conidiophore production. In addition, these findings led to the hypothesis that multiple pathways are involved in activation of *brlA* expression (Mah and Yu, 2006).

Two FLBs, *flbB* and *flbE*, are required for proper conidiation in *A. fumigatus* (Kwon *et al.*, 2010; Xiao *et al.*, 2010).

Deletion of *flbE* results in decreased conidiation and delayed *brlA* and *vosA* expression. FlbE is also essential for salt induced development in liquid submerged culture (Kwon *et al.*, 2010). Distinct from *A. nidulans*, the *A. fumigatus flbB* gene produces two distinct transcripts predicted to encode two different basic leucine zipper domain polypeptides, FlbB β and FlbB α (Xiao *et al.*, 2010). The deletion of *flbB* results in reduced and delayed conidiation, altered *brlA* and *abaA* expression, the lack of conidiophore development in liquid submerged culture, and the absence (or reduction) of gliotoxin production. Importantly, disruption of the ATG start codon for either one of the two FlbB polypeptides causes reduced conidiation, suggesting that both polypeptides are required for wild type developmental phenotype. Northern blot analyses demonstrated that both *flbB* and *flbE* are essential for *flbD* expression, suggesting that FlbB and FlbE function upstream of FlbD as proposed in *A. nidulans* (Xiao *et al.*, 2010).

The Velvet family proteins

The Velvet regulators, including VeA, VelB, VelC, and VosA, coordinate fungal growth, differentiation, pathogenesis, and secondary metabolism in many fungi (Ni and Yu, 2007; Bayram and Braus, 2012). These Velvet family proteins define a class of fungi-specific transcription factors that contain the Velvet domain with the DNA-binding ability (Ahmed *et al.*, 2013; Beyhan *et al.*, 2013). Importantly, the Velvet proteins form dynamic complexes that play differential roles in regulating various processes in filamentous and dimorphic fungi (Bayram *et al.*, 2008; Park *et al.*, 2012b, 2014). While VeA is required for proper asexual development, two different studies presented opposite results (Dhingra *et al.*, 2012; Park *et al.*, 2012a). First, Dhingra *et al.* (2012) showed that the deletion of *veA* causes decreased the number of conidiospores. They also demonstrated that overexpression of *veA* causes reduced conidial production, suggesting that VeA is required for proper conidiation. Park and colleagues (2012a) presented different results that the absence of *veA* results in the plentiful production of conidiophores and highly increased *brlA* mRNA accumulation, indicating that VeA acts as a negative regulator in conidiation. VelB and VosA also negatively control asexual development (Park *et al.*, 2012a). The absence of *velB* or *vosA* results in increased formation of conidiophores and elevated *brlA* accumulation in liquid submerged culture and developmentally induced condition. Taken together, these three Velvet regulators function as repressors of conidiation (Park *et al.*, 2012a).

VosA and VelB play an additional role in conidia maturation and viability (Park *et al.*, 2012a). The *vosA* or *velB* null mutant strains exhibit a significant reduction in conidia viability, conidial trehalose amount, conidial tolerance against oxidative stress, and elevated conidial germination. Genetic studies demonstrated that VosA and VelB play an interdependent role in conidia maturation. The *vosA* and *velB* mRNA levels were high in conidia and regulated by AbaA during middle phase of conidiation. Overall, these results propose that VosA and VelB, in coordination with the central regulatory pathway, complete conidiogenesis in *A. fumigatus* (Park *et al.*, 2012a).

To test the conservation of interaction and function of VeA

in *A. nidulans* and *A. fumigatus*, the *A. fumigatus* *ΔveA* mutant strains expressing the *A. nidulans* VeA protein fused with a TAP tag were generated, and examined for their phenotypes and protein-protein interaction. The results demonstrate that *A. nidulans* VeA can fully complement *A. fumigatus* *ΔveA* and can interact with the *A. fumigatus* VelB and LaeA proteins. Based on these and other findings, we proposed that both function and interaction of the Velvet complexes, including VelB-VeA-LaeA, are conserved in other *Aspergillus* species (Park *et al.*, 2012a). LaeA (loss of *alfR* expression A), the Velvet interacting partner, is also required for proper conidiation (Bok *et al.*, 2005). In liquid submerged culture, the *ΔlaeA* mutant was impaired in production of conidiophores and conidiospores.

Heterotrimeric G protein signaling pathways

Heterotrimeric G proteins (G proteins) are conserved in eukaryotes and involved in most fungal biology, including growth, differentiation, sporulation, and metabolism (Harnett and Klaus, 1988; Simon *et al.*, 1991; Yu, 2006; Li *et al.*, 2007). The G protein signaling system consists of G protein-coupled receptor (GPCR), a G protein composed of α , β and γ subunits, and variety of effectors (Birnbaumer, 1990, 2007).

In *A. fumigatus*, the two $G\alpha$ subunits GpaA and GpaB have been characterized (Liebmann *et al.*, 2003; Mah and Yu, 2006). GpaA activates vegetative growth but represses conidiation. (Mah and Yu, 2006). The *gpaA* mRNA mainly accumulated in vegetative stage, and the dominant activating GpaA_{Q204L} mutation led to increased hyphal proliferation with reduced conidiation. The dominant interfering GpaA_{G203R} mutation suppressed the decreased conidiation phenotype caused by loss of *flbA*, suggesting that GpaA is the primary target of FlbA, an RGS (regulator of G protein signaling) protein (Mah and Yu, 2006). The *ΔgpaB* mutant exhibits reduced conidiation, implying that GpaB is required for proper conidiation (Liebmann *et al.*, 2003). Liebmann *et al.* (2003) further investigated the relationship between GpaB and the cAMP-PKA pathway, and demonstrated that GpaB acts as an upstream activator of the cAMP-PKA signaling cascades. The components of the cAMP-PKA signaling pathway, AcyA and PkaA are also required for growth and conidiation (Liebmann *et al.*, 2003). In addition, AcyA and PkaA control conidial pigmentation via regulating expression of the polyketide synthase gene *pksP* (Jahn *et al.*, 2002; Grosse *et al.*, 2008).

Like the role of $G\alpha$ subunits, the $G\beta$ and $G\gamma$ subunits (SfaD and GpgA, respectively) play a vital role in controlling vegetative growth, spore germination, conidiation, and gliotoxin biosynthesis (Shin *et al.*, 2009). Deletion of *sfaD* or *gpgA* results in impairment in hyphal growth, and conidial germination. The *ΔsfaD* or *ΔgpgA* mutant exhibits enhanced conidiospores production, whereas overexpression of *sfaD* or *gpgA* results in reduced levels of conidiation in liquid submerged cultures, demonstrating that SfaD and GpgA play a negative role in conidiospore production. Based on these results and the prior knowledge, it has been speculated that two $G\alpha$ subunits (GpaA and GpaB) work with the $G\beta\gamma$ (SfaD::GpgA) heterodimer, and these hetero-trimeric complexes control cAMP-PKA signaling pathway and repress conidiation (Grice *et al.*, 2013).

A $G\beta$ -like protein CpcB (cross pathway control B) also gov-

erns fungal growth, development and gliotoxin production (Kong *et al.*, 2013). The *cpcB* deletion mutant exhibits severe growth defect, decreased conidiation, and attenuated virulence (Kong *et al.*, 2013; Cai *et al.*, 2015). Cai *et al.* (2015) found that the *A. nidulans* CpcB can functionally substitute for conidiation defect caused by the absence of *A. fumigatus* *cpcB*, indicating that the role of CpcB is likely conserved in *Aspergilli*. The *ΔcpcB ΔgpaB* double mutants significantly exacerbated the conidiation defect compared to WT or single deletion mutants, indicating that CpcB plays an additional role compared to the GpaB (Cai *et al.*, 2015).

RicA, a putative GDP/GTP exchange factor for G proteins, is required for proper vegetative growth and conidiation in *A. fumigatus* (Kwon *et al.*, 2012). The *ΔricA* mutant exhibits severely restricted colony growth, formation of abnormal and minuscule of conidia, and delayed accumulation of *brlA*, *abaA*, and *wetA* mRNAs. In addition, the *ΔricA* conidia exhibit reduced and delayed germination (Kwon *et al.*, 2012).

The Ca^{2+} -Calcineurin pathway

The Ca^{2+} -Calcineurin pathway is conserved from human to fungi, and acts as multi-function unit for fungal biology (Klee *et al.*, 1979; Rusnak and Mertz, 2000; Shibasaki *et al.*, 2002; Steinbach *et al.*, 2007). In response to external and internal stresses, Ca^{2+} concentration is increased and the Ca^{2+} ions bind to calmodulin. The Ca^{2+} -calmodulin complex activates calcineurin, and then activated calcineurin controls several target proteins, including NFAT and Crz1 in human and *Saccharomyces cerevisiae*, respectively, by dephosphorylation (Cyert, 2003; Hogan *et al.*, 2003).

In *A. fumigatus*, the Ca^{2+} -Calcineurin pathway is required for proper growth, development and virulence (Juvvadi *et al.*, 2014). The mutants lacking the calcineurin catalytic subunit *cnaA* exhibit defective growth and conidiation (Steinbach *et al.*, 2006). In addition, the *ΔcnaA* conidia appear to lack the surface rodlet and clumping needed to be formed between individual conidia (Steinbach *et al.*, 2006; Juvvadi *et al.*, 2008). One of calcineurin target protein, CrzA (calcineurin-responsive zinc finger A), is also required for proper growth and conidiation. Deletion of *crzA* leads to significant defects in conidial germination, hyphal growth, and asexual development (Cramer *et al.*, 2008; Soriani *et al.*, 2008). One of calcineurin interacting protein, Hsp90 (heat-shock protein), orchestrates conidiation and cell wall integrity (Lamoth *et al.*, 2012). Genetic repression of *hsp90* causes decreased conidiation and spore viability, severe defects of conidial germination, down-regulated the conidiation-specific genes.

The Ras mediated signaling pathway

The Ras family proteins are a group of monomeric GTPases that are conserved in eukaryotes (Rojas *et al.*, 2012). In *A. fumigatus*, two Ras proteins, RasA and RasB, have been identified, and shown to play crucial roles in hyphal morphogenesis, polarized growth, and asexual development (Norton and Fortwendel, 2014). The dominant activate *rasA* or *rasB* mutation leads to reduced conidiation with malformed conidiophores. The dominant negative *rasA* or *rasB* mutant shows a reduced germination rate or a delay in initiation of germination. (Fortwendel *et al.*, 2004). The *ΔrasA* mutant

forms a compact colony accompanied by near absent conidiation, and produces aberrant conidiophores (Fortwendel *et al.*, 2008). Deletion of *rasB* leads to production of abnormal conidiophores (Fortwendel *et al.*, 2005). Overall, these results indicate that the two *ras* genes are required for proper production and formation of conidiophores.

The RhbA protein, a member of the Ras superfamily, is also required for asexual development in a nitrogen source dependent manner. Transcript of *rhbA* is detected throughout the asexual developmental cycle, and highly accumulated in response to nitrogen starvation (Panepinto *et al.*, 2002). The *rhbA* mutant can produce conidiophores in liquid submerged culture under ammonium-excess condition whereas WT did not produce conidiophores (Panepinto *et al.*, 2003).

Other factors

StuA is an APSES (Asm1p, Phd1p, Sok2p, Efg1p, and StuAp) family protein, required for proper asexual development (Sheppard *et al.*, 2005). The Δ *stuA* mutant produces small numbers of markedly abnormal dysmorphic conidia. The results of a whole genome transcriptomic analysis demonstrate that StuA regulates mRNA expression of certain genes, which are associated with morphology, development, or biosynthesis of putative secondary metabolites (Sheppard *et al.*, 2005; Twumasi-Boateng *et al.*, 2009).

MedA is a developmental modifier that regulates temporal expression of central regulatory genes in *A. nidulans* (Clutterbuck, 1969; Aguirre, 1993; Busby *et al.*, 1996). Deletion of *medA* leads to dramatically decreased conidiation and production of aberrant conidiophores. Unlike the Δ *medA* mutant in *A. nidulans*, however, the Δ *medA* mutant in *A. fumigatus* cannot produce classic medusoid conidiophores.

In addition, MedA is not necessary for expression of the central regulatory genes, indicating that MedA plays a different role in two *aspergilli* (Gravelat *et al.*, 2010).

The SrgA protein is a Rab GTPase closely related to Sec4 and highly accumulated at hyphal tips and mature conidiophores (Powers-Fletcher *et al.*, 2013). The Δ *srgA* mutant exhibits attenuated conidiophores and dysmorphic phialides, suggesting that SrgA plays a certain role in asexual developmental program.

SomA is a transcription factor with LUG/LUH-Flo8-single-stranded DNA binding (LUFS) domain (Lin *et al.*, 2015). The absence of *somA* leads to delayed hyphal growth and blocked conidiation. Genetic repression of *somA* leads to decreased mRNA expression of conidiation-related genes, such as *brlA*, *medA*, *stuA*, and *rodA*. Taken together, SomA acts as a key transcription factor regulating formation of conidiospores and expression of conidiation-related genes.

The *rodA* gene encodes a hydrophobin and is essential for formation of the rodlet layer and hydrophobicity in conidia (Thau *et al.*, 1994). Conidia of the Δ *rodA* mutant lack the rodlet layer and are hydrophilic. The other hydrophobin RodB is highly homologous to RodA, but is not required for formation of the conidial rodlet layer. The conidia of the Δ *rodA* Δ *rodB* double mutant produce an amorphous layer of conidia (Paris *et al.*, 2003).

Trehalose is a key protectant against desiccation and various environmental stresses (Paul *et al.*, 2008). Trehalose biosynthesis and breakdown play crucial roles in conidial maturation and germination. The two trehalose synthases TpsA and TpsB are identified and found as required for conidial viability, conidial wall synthesis, stress tolerance, and virulence (Al-Bader *et al.*, 2010). Trehalose 6-phosphate phos-

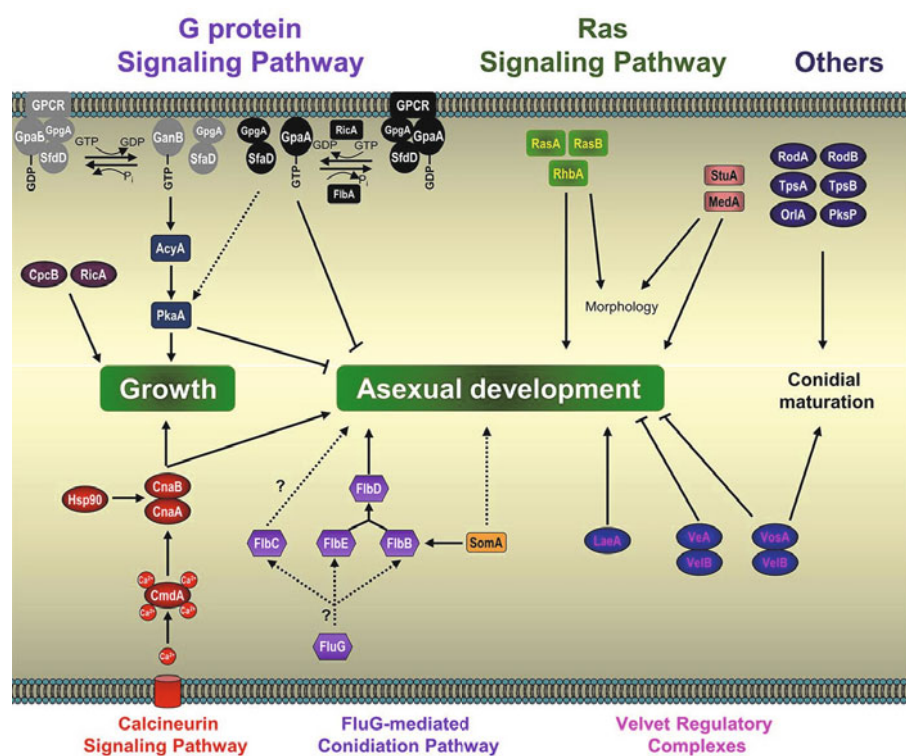


Fig. 2. Direct and indirect regulators influencing asexual development in *Aspergillus fumigatus*.

phatase OrlA is also necessary for conidiospore production and conidiophore morphology. The $\Delta orlA$ mutant exhibits the lack of conidia production and severe morphological defects, which could be rescued by addition of osmotic stabilizers (Puttikamonkul *et al.*, 2010).

Conclusion

Intense studies over the past decades have revealed much about the positive and negative factors that control the intricate process of conidiation in *A. fumigatus*. In this review, we have summarized our current knowledge of key regulators of conidiation in *A. fumigatus* (Fig. 2). Importantly, these multifactorial pathways involved in conidiation are also linked in secondary metabolism and pathogenicity (Hohl and Feldmesser, 2007; Abad *et al.*, 2010; Dolan *et al.*, 2015). It is anticipated that a better understanding of the genetic regulatory mechanisms of conidiation in an opportunistic human pathogen will illuminate new approaches to control fungal disease caused by *A. fumigatus*, and provide an opportunity to develop novel anti-fungal drugs.

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