1 Molecular Biology of Asexual Sporulation in Filamentous Fungi

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CONTENTS

I. Introduction

Filamentous fungi are of great importance to humankind as pathogens, environmental recyclers, industrial producers, and agricultural aids. Fungi produce spores as the primary means of reproduction, propagation, and infectivity (Ebbole 2010). Fungi use asexual sporulation as a main reproductive mode (Adams et al. 1998; Ni et al. 2010). Asexual nonmotile spores, called conidia (conidiospores), are formed on the specialized developmental structure called conidiophore. The entire processes of the initiation, progression, and completion of asexual sporulation are regulated by various positive and negative genetic elements that direct the expression of genes required for the proper assembly of the conidiophore and the formation and maturation of conidiospores (Adams et al. 1998; Park and Yu 2012). This chapter presents a snapshot of the up-to-date molecular biology of conidiation in the three model fungi *Aspergillus nidulans*, *Penicillium marneffei*, and *Fusarium graminearum*.

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II. Asexual Sporulation in *Aspergillus nidulans*

A. Morphology of Asexual Structure

A conidiophore is a specialized asexual structure, which is the most remarkable characteristic of a specific fungal species (Adams et al. 1998; Yu 2010). The formation of conidiophores in *A. nidulans* is highly sophisticated and can be divided into several differential stages (Fig. 1.1) (Mims et al. 1988; Timberlake 1990). First, conidiation starts from the foot cells, thick-walled cells, which elongate into the air to produce aerial stalks. After the extension of stalks ceases, the tip of stalks then starts to swell and form apical vesicles, which contain multiple nuclei. Through a budding-like process, 60 metulae are formed on the surface of the vesicle and then each metula produces 2 buds that develop into uninucleate sterigmata, termed phialides. Phialides undergo repeated asymmetric mitotic divisions to generate chains of conidia (about 120 conidia per

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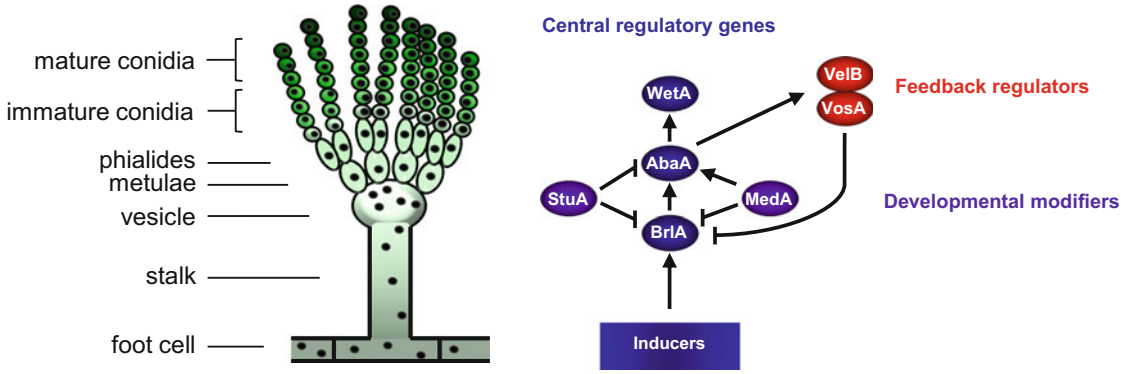

Fig. 1.1 Conidiophore and the central regulators of conidiation in *A. nidulans*. Simplified illustration of the conidiophore (*left*) and a genetic model of the central regulatory pathway of conidiation (*right*) are shown

phialide) (Adams et al. 1998; Mims et al. 1988). Conidia are haploid cells and capable of forming a new colony under appropriate conditions. Soon after conidia are produced, they undergo a maturation process, involving the modification of the conidial wall and trehalose biogenesis for the establishment of conidial dormancy (Ni et al. 2010; Sewall et al. 1990b; Timberlake 1990). The whole process of conidiation is genetically programmed, and various regulators are responsible for the progression of each stage (Adams et al. 1998).

B. Regulators of Asexual Development

1. Central Regulators of Conidiation

a) BrlA

An essential initiation step of conidiation is the activation of *brlA* encoding a C_2H_2 zinc-finger transcription factor (TF) (Adams et al. 1988, 1990). The conidiophores of *brlA* null mutants fail to form a vesicle and other asexual structures and continue to grow conidiophore stalks (thus termed "bristle") (Clutterbuck 1969). Contrarily, the overexpression of *brlA* causes termination of hyphal growth coupled with the formation of spores directly from the hyphal tips (Adams et al. 1988), suggesting that *brlA* is sufficient to induce the transition from polar hyphal growth to asexual sporulation.

As a key TF, BrlA is necessary for activating the expression of development-related genes during the early phase of conidiation (Adams et al. 1988; Boylan et al. 1987; Clutterbuck 1990; Mirabito et al. 1989; Stringer et al. 1991; Timberlake 1991). The *brlA* mutants fail to accumulate transcripts of certain development-specific genes, including *abaA*, *wetA*, *rodA*, and *yA* (Birse and Clutterbuck 1991; Boylan et al. 1987; Mayorga and Timberlake 1990; Stringer et al. 1991). BrlA contains two C_2H_2 zinc-finger motifs (Adams et al. 1988), which are important for DNA binding and BrlA TF activity (Adams et al. 1990). Mutation in either one of the two motifs results in a complete loss of BrlA activity (Adams et al. 1990). This central developmental TF interacts with the **BrlA response elements** (BREs; 5′-(C/A)(G/A)AGGG(G/A)-3′) and activates mRNA expression of multiple developmental genes (Chang and Timberlake 1993). Chang and Timberlake demonstrated that *brlA* expression in *Saccharomyces cerevisiae* caused activation of the *brlA*-dependent gene, and multiple BREs were present in the promoter regions of several developmentally regulated genes in *A. nidulans* (Chang and Timberlake 1993).

Expression of *brlA* is subject to complex regulation. The *brlA* gene consists of two overlapping transcripts, designated *brlA*α and *brlA*β, which are regulated at different levels and are individually required for proper development (Han and Adams 2001; Han et al. 1993; Prade and Timberlake 1993). The *brlA*β transcript encodes two open reading frames (ORFs), *brlA*β ORF, and a short upstream ORF (*brlA*β μORF) (Han et al. 1993). Expression of *brlA*α is only regulated at the transcriptional level and requires both BrlA and AbaA (Han et al. 1993). Expression of *brlA*β is regulated at both the transcriptional and translational levels. For

transcriptional control, there are multiple *cis*-acting regulatory sequences involved in the activation or repression of *brlA*β mRNA (Han and Adams 2001). In vegetative cells, translation of the *brlA*β μORF represses BrlA translation and thereby prevents premature development (Han and Adams 2001; Han et al. 1993).

b) AbaA

AbaA is a presumed TF that is induced by BrlA during the middle phases of asexual development after differentiation of metulae and is required for proper formation and function of phialides (Boylan et al. 1987; Sewall et al. 1990a). The *abaA* null mutants bear an abacus-like structure with swellings at intervals in place of chains of conidia, suggesting its possible role in cytokinesis (Clutterbuck 1969; Sewall et al. 1990a). Non-sporulating conidiophores of *abaA* mutants are differentiated from sterigmata but do not form conidiogenous phialides, suggesting that *abaA* is required for proper function of phialides as sporogenous cells. The overexpression of *abaA* in vegetative cells results in growth termination and cellular vacuolization but no spore formation (Adams and Timberlake 1990; Mirabito et al. 1989).

AbaA is a member of TEF-1 (Transcriptional Enhancer Factor-1) family that contains an ATTS (AbaA, TEC1p, TEF-1 sequence)/TEA DNA-binding domain with a potential leucine zipper for dimerization (Andrianopoulos and Timberlake 1991, 1994; Mirabito et al. 1989). Results from a gel shift assay showed that AbaA interacts with the consensus sequence 5′-CATTCY-3′ (**AbaA response element** (ARE), where Y is a T or C) (Andrianopoulos and Timberlake 1994). One or multiple AREs are present in the promoter regions of several developmental genes such as *yA*, *rodA*, *wA*, and *wetA*, the upstream gene *brlA* and *abaA* itself (Andrianopoulos and Timberlake 1994) (Aramayo and Timberlake 1993; Mayorga and Timberlake 1990; Mirabito et al. 1989; Stringer et al. 1991). Further studies demonstrated that AbaA regulates the chitin synthase gene *chsC* (Ichinomiya et al. 2005; Park et al. 2003), the *velvet* genes *vosA* and *velB* (Ni and Yu 2007; Park et al. 2012b), and a component of the axial bud site marker Axl2 (Si et al. 2012) during conidiation.

Genetic interaction between *abaA* and *brlA* is complicated (Aguirre 1993; Aguirre et al. 1990). The overexpression of *abaA* in hyphae induces expression of *brlA*, whereas the *abaA* null mutant also activates *brlA* expression (Aguirre 1993), indicating that AbaA acts as both an activator and a repressor of *brlA*. Further studies proposed that AbaA exerts its repressive role in *brlA* expression via activating VosA, a key feedback negative regulator of *brlA* (Han and Adams 2001; Ni and Yu 2007).

c) WetA

The *wetA* gene is activated by sequential expression of *brlA* and *abaA* during asexual sporulation, and WetA is essential for the modification of the conidial wall resulting in the impermeable and resilient conidia (Marshall and Timberlake 1991; Sewall et al. 1990b). The phenotype of *wetA* null mutants is described as "wet-white" because these mutants produce colorless and autolytic conidia (Clutterbuck 1969; Sewall et al. 1990b). Conidia of the *wetA* null mutant lack both the condensation of the C2 wall layer and the formation of C3 and C4 layers (Sewall et al. 1990b). The *wetA* gene is sufficient for the activation of the conidium-specific gene, including *wA*, and the *wetA* null mutant fails to accumulate mRNAs of conidium-specific genes, indicating that WetA may act as a (transcriptional) regulator of conidium-specific genes (Marshall and Timberlake 1991; Sewall et al. 1990b). The overexpression of *wetA* in hyphae inhibits hyphal growth and causes excessive branching without causing the activation of *brlA* and *abaA* expression or the induction of precocious conidiation (Marshall and Timberlake 1991). Overall, WetA is required for spore maturation and is proposed to be an activator of a set of conidium-specific genes.

d) StuA and MedA

StuA (stunted) and MedA (medusa) function as developmental modifiers that are necessary for the precise organization of conidiophores (Adams et al. 1998). The StuA is a sequence-specific TF, which contains the APSES domain (Dutton et al. 1997). Mutational inactivation of *stuA* causes production of abnormal conidiophores with the lack of metulae and phialides.

However, some *stuA* mutants can produce viable conidia directly from vesicle (Clutterbuck 1969). The *stuA* gene encodes two transcription units, *stuA*α and *stuA*β, and transcription of both mRNAs dramatically increases during the establishment of developmental competence (Miller et al. 1991, 1992). StuA is required for proper activation of *brlA* and repression of *abaA* (Dutton et al. 1997). Apposite *stuA* expression is required for the proper conidiophore morphology, but StuA may not affect the temporal development of cell types (Wu and Miller 1997). Mutational inactivation of *medA* results in aberrant conidiophores with multiple layers of sterigmata before conidia formation (Clutterbuck 1969). While StuA is essential for spatial expression of *brlA*, MedA is required for proper temporal expression of *brlA*α and *brlA*β. In addition, MedA acts as a coactivator required for *abaA* expression (Busby et al. 1996).

2. Controllers of the Central Regulators

a) FluG-Mediated Signaling Pathway

Developmental transition from vegetative growth is highly complex and involves various positive elements in response to internal and external cues (Fig. 1.2) (Adams et al. 1998). Two decades ago, Wieser et al. carried out genetic analyses of recessive mutations and identified six upstream developmental activators, including *fluG*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE* (Wieser et al. 1994). The deletion of any one of these genes leads to the fluffy phenotypes (cotton-like colonies) coupled with decreased *brlA* expression (Adams et al. 1998; Wieser et al. 1994). A series of follow-up studies has revealed the molecular mechanisms and genetic relationships of these genes and proposed a model for the roles of the upstream developmental activators in conidiation (Adams et al. 1998; Etxebeste et al. 2010; Park and Yu 2012; Yu 2010).

FluG contains a glutamine synthetase I like domain and is a key activator of asexual development in *A. nidulans* (Lee and Adams 1994). The *fluG* deletion mutant cannot produce normal conidiophores under standard culture conditions, whereas the overexpression of *fluG* causes conidiophore formation and *brlA* activation in liquid submerged culture (D'Souza et al. 2001; Lee and Adams 1996). Lee and Adams proposed that FluG is required for the synthesis of an extracellular sporulation inducing factor (ESIF, also known as the FluG factor), which signals the activation of conidiophore development (Lee and Adams 1994). Later studies by Rodriguez-Urra and colleagues have revealed that the FluG factor is a diorcinol-dehydroaustinol adduct, which can rescue the conidiation defects caused by the deletion of *fluG* (Rodriguez-Urra et al. 2012). The FluG-initiated developmental signaling then leads to two independent regulatory processes: (1) inhibition of hyphal growth via activation of FlbA and (2) activation of developmental-specific regulatory cascades (FlbB–FlbE) (Adams et al. 1998). Further genetic studies have revealed that both FluG-mediated processes occur via the removal of repression of conidiation imposed by SfgA (Seo et al. 2003, 2006). SfgA is an upstream repressor of conidiation with a Gal4-type $Zn(II)_2Cys_6$ binuclear DNA-binding domain. Double mutant analyses indicate that SfgA functions downstream of FluG but upstream of FlbA–FlbD and BrlA (Seo et al. 2006).

FluG-initiated developmental-specific regulatory cascades (FlbB–FlbE) consist of at least two separated pathways, FlbC and FlbE/FlbB/FlbD, for activation of *brlA* (Etxebeste et al. 2010; Park and Yu 2012). FlbC is a C_2H_2 zinc-finger protein that directly binds to the promoter region of *brlA* and activates *brlA* expression (Kwon et al. 2010a). Kwon et al. also demonstrated that FlbC also binds to the *cis*-element of *abaA* and *vosA* and activates their expression (Kwon et al. 2010a). FlbE contains two uncharacterized conserved domains and co-localizes with FlbB, a basic leucine zipper (b-zip) TF, at the hyphal tip (Etxebeste et al. 2009; Garzia et al. 2009; Kwon et al. 2010b). FlbE and FlbB together induce *flbD* expression interdependently (Garzia et al. 2009; Kwon et al. 2010b). Then, FlbB cooperates with

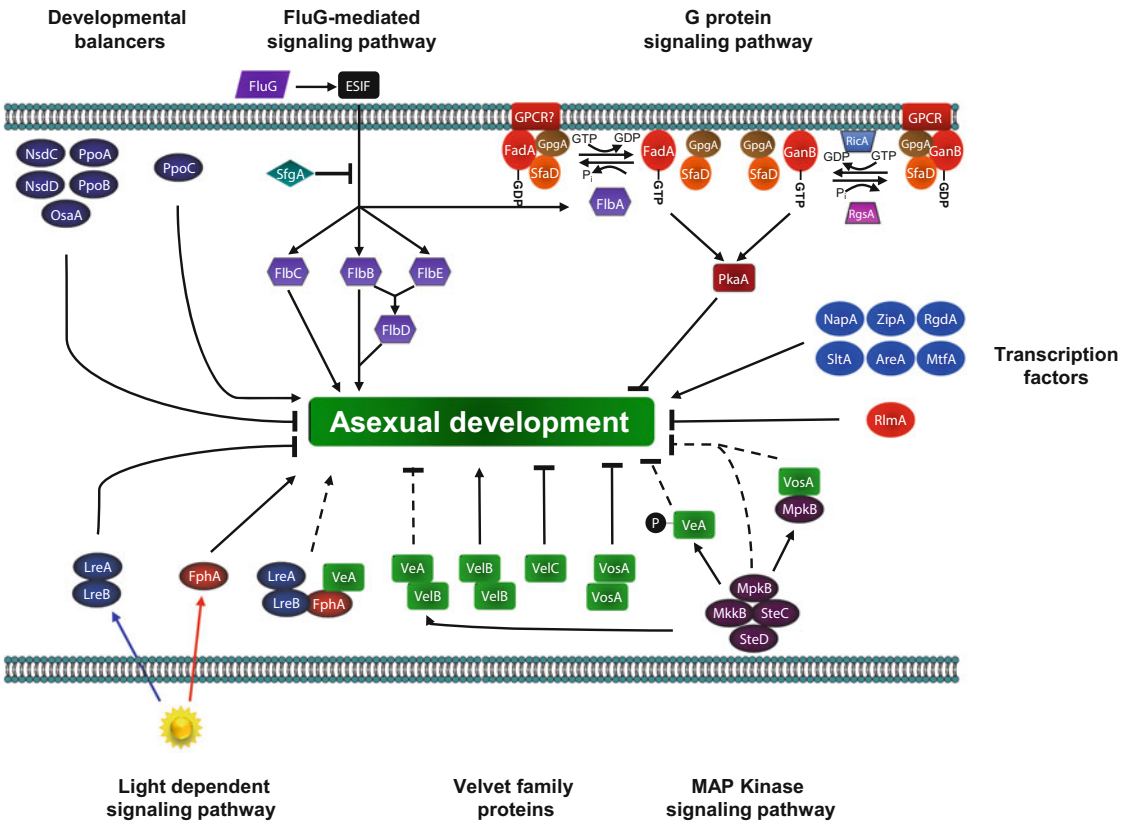

Fig. 1.2 A comprehensive model for the regulation of asexual sporulation in *A. nidulans*. This model includes almost all elements that influence conidiation

FlbD (a cMyb-type transcription factor) for *brlA* activation (Garzia et al. 2010). Overall, FlbB~E play independent or interdependent roles and are necessary for full activation of *brlA*.

In filamentous fungi, FlbA is the first studied regulator of G-protein signaling (RGS) protein (Lee and Adams 1994; Yu et al. 1996). RGS proteins are a group of proteins containing a conserved ~130 amino acid RGS box that interacts with an activated GTP-Gα subunit and increases its intrinsic GTPase activity, thereby facilitating the hydrolysis of GTP-Gα (active form) to GDP-Gα (inactive form) (Chidiac and Roy 2003; McCudden et al. 2005). FlbA is the cognate RGS of the FadA Gα protein, and together they govern the upstream regulation of vegetative growth, development, and biosynthesis of secondary metabolites (Hicks et al. 1997; Tag et al. 2000; Yu and Keller 2005; Yu et al. 1996). As an RGS, FlbA is presumed to enhance the intrinsic GTPase activity of FadA (Yu et al. 1996, 1999). Loss of *flbA* function results in a similar fluffy autolytic phenotype without sporulation caused by dominant mutations in FadA (Hicks et al. 1997; Lee and Adams 1994; Yu et al. 1996).

b) Heterotrimeric G Protein Signaling Pathways In *A. nidulans*, two main G heterotrimeric G protein signaling pathways involving FadA and GanB Gα subunits play a predominant role in controlling growth, development, stress response, and secondary metabolism (Yu 2006). For more information on G protein signaling pathways, please refer to Chap. 7. FadA (fluffy autolytic dominant A) was identified and characterized by studying dominant activating mutants (G42R, R178L, G183S, R178C, and Q204L) that exhibit the fluffy autolytic phenotype (Wieser et al. 1994; Yu et al. 1996, 1999). Dominant interfering mutation (G203R) in FadA caused hyperactive sporulation and reduced hyphal growth (Hicks et al. 1997; Yu

et al. 1996). This FadA-mediated signaling needs to be attenuated by FlbA (RGS) for proper asexual and sexual development to occur (Hicks et al. 1997; Wieser et al. 1997). Similar to FadA, GanB (G protein alpha subunit in *A. nidulans*) negatively controls asexual development (Chang et al. 2004). While the *ganB* null or dominant interfering (G207R) mutants produce conidiophores in liquid submerged culture, constitutively active GanB mutations (Q208L and R182L) cause severe defects in asexual development (Chang et al. 2004).

GanB-mediated signaling is in part activated by the putative GDP/GTP exchange factor, RicA (Kwon et al. 2012), and is negatively controlled by RgsA (Han et al. 2004a). These two Gα subunits work with the SfaA(Gβ): GpgA(Gγ) dimer (Lafon et al. 2005; Rosen et al. 1999; Seo et al. 2005) and inhibit asexual development in part via the cyclic AMP (cAMP)-dependent protein kinase PkaA (Shimizu and Keller 2001). Recently, Kong and colleagues characterized the Gβ-like protein B CpcB that is required for proper conidiation and *brlA* expression in *A. nidulans* (Kong et al. 2013). Overall, these results indicate that individual G protein components are associated with asexual development and may play differential roles in conidiation (Yu 2006).

c) MAP Kinase Signaling Pathways

Mitogen-activated protein kinase (MAPK) pathways respond to various environmental cues and amplify the signals, leading to appropriate cellular responses in fungi (Gustin et al. 1998). *A. nidulans* contains four MAPK genes: *mpkA*, *mpkB*, *mpkC*, and *hogA* (Galagan et al. 2005). Among these, MpkB, a homologue of Fus3p in *S. cerevisiae*, is associated with conidiation, sexual development, and secondary metabolism (Atoui et al. 2008; Bayram et al. 2012; Jun et al. 2011; Kang et al. 2013; Paoletti et al. 2007). The MAPK kinase kinase SteC, a component of the MAP kinase module Ste11–Ste7–Fus3 complex, is also required for proper formation of conidiophore (Wei et al. 2003). The deletion of *mpkB* causes increased *brlA* expression and the formation of conidiophores in liquid submerged cultures (Kang et al. 2013). Moreover, the Δ*mpkB* mutant produces abnormal conidiophores and exhibited a decreased number of conidia (Bayram et al. 2012; Jun et al. 2011; Kang et al. 2013). Interestingly, the deletion of *mpkB* results in decreased VeA phosphorylation and formation of the VeA–VelB heterodimer, which acts as a key and essential activator of sexual development (Bayram et al. 2012). MpkB also interacts with VosA in the nucleus. These results suggest that MpkB is also involved in the regulation of the *velvet* proteins' activities, thereby drastically affecting fungal development (Bayram et al. 2012). In-depth information on MAP kinase pathways is found in Chap. 6.

d) The *Velvet* Family Proteins

The *velvet* family proteins, mainly VeA, VelB, VelC, and VosA, play a central and global role in coordinating fungal growth, development, virulence, and metabolism in ascomycetes and basidiomycetes (Bayram and Braus 2012; Ni and Yu 2007). These proteins define a new fungi-specific TF class that contains the *velvet* domain with DNA-binding activity (Ahmed et al. 2013; Ni and Yu 2007). Interestingly, the *velvet* regulators form diverse complexes that play differential roles in coordinating various biological processes in fungi (Bayram et al. 2008; Park et al. 2012b, 2014; Sarikaya Bayram et al. 2010). VeA is the founding member of the *velvet* regulators playing a key role in the light-dependent developmental control. The *veA1* mutation abolishes the light affected developmental changes and causes severely restricted sexual development with increased conidiation (Kafer 1965; Mooney and Yager 1990). Further studies demonstrated that the nuclear localization and complex formation of VeA are important for the regulation of light-controlled development (Bayram et al. 2008; Stinnett et al. 2007). In the dark, VeA predominantly localizes in the nucleus and forms the VeA–VelB heterodimer, which controls sexual development or the VelB–VeA–LaeA heterotrimeric complex that regulates secondary metabolism (Bayram et al. 2008). VeA also interacts with the light complex components, which balance asexual and sexual development (Purschwitz et al. 2008, 2009). Recently, Bayram et al. demonstrated that phosphorylation of VeA is regulated by the MAP kinase MpkB (AnFus3) (Bayram et al. 2012). Similar to VeA, VelB is

required for sexual development and production of certain secondary metabolites (Bayram et al. 2008). In addition, VelB functions as a positive regulator of conidiation (Park et al. 2012b). The *velB* deletion mutant exhibits the decreased conidiospore number, whereas the overexpression of *velB* results in enhanced conidial production (Park et al. 2012b). Another multifunctional regulator VosA functions as a key repressor of conidiophore formation and an essential activator of trehalose biogenesis in conidia (Ni and Yu 2007). VosA directly binds to the promoter of *brlA* and represses *brlA* expression in vegetative cells (Ahmed et al. 2013; Ni and Yu 2007). VosA physically interacts with VelB, VosA, or VelC, and each complex is presumed to control different sets of genes thereby regulating various aspects of fungal development (Park et al. 2012b, 2014; Sarikaya Bayram et al. 2010). VelC is important for balancing asexual and sexual development, and acts as an activator of sexual development, likely by binding to VosA during the early stages of sexual development thereby leading to the increased formation of VelB–VeA that is essential for the initiation of sexual fruiting (Park et al. 2014). As a consequence, VelC indirectly inhibits asexual development in *A. nidulans*.

e) Light and Signals

Light is a key environmental cue affecting diverse cellular processes, including fungal development and secondary metabolism in *A. nidulans* (Bayram et al. 2010; Mooney and Yager 1990). For example, light induces conidiation but represses sexual development and ST production (Bayram et al. 2010). In fungi, several photoreceptors play a crucial role in light-response processes (Bayram et al. 2010). The red-light sensor FphA is a **fungal phytochrome** that stimulates asexual development (Blumenstein et al. 2005). The deletion of *fphA* causes reduced mRNA accumulation of the *brlA* and *fluffy* genes and decreased conidial production (Blumenstein et al. 2005; Ruger-Herreros et al. 2011). LreA and LreB are main components of the blue light-sensing system, and they form complexes with FphA (Purschwitz et al. 2008). The deletion of *lreA* or *lreB* causes slightly increased conidiospore production in light and dark conditions, suggesting that LreA and LreB repress asexual development (Purschwitz et al. 2008). These sensors form the photoreceptor complex LreA/LreB/FphA/VeA, which activates the accumulation of the *brlA* and *fluffy* genes (Ruger-Herreros et al. 2011).

f) Developmental Balancers

Balance between asexual and sexual development is regulated by various factors. First, certain **sexual developmental activators** negatively control *brlA* expression and asexual development. For instance, NsdC and NsdD were initially identified as sexual activators with DNA-binding domains (Han et al. 2001; Kim et al. 2009; Lee et al. 2014). The deletion of one of them results in the absence of sexual fruiting bodies, whereas the overexpression of these genes causes enhanced formation of Hűlle cells (Han et al. 2001; Kim et al. 2009; Lee et al. 2014). Further studies have revealed that NsdC and NsdD repress *brlA* expression and conidiophore formation (Kim et al. 2009; Lee et al. 2014). Genetic analyses indicate that NsdD functions downstream of FlbE/B/D/C and upstream of *brlA*. Second, **Psi factors** (precocious sexual inducers), derived from oleic, linoleic, and linolenic acids, control the balance between asexual and sexual development (Bayram and Braus 2012; Dyer and O'Gorman 2012). In *A. nidulans*, the three oxylipin biosynthetic genes *ppoA*, *ppoB*, and *ppoC* (psi factor producing oxygenase) are present (Brodhun and Feussner 2011; Tsitsigiannis and Keller 2007; Tsitsigiannis et al. 2004, 2005). The deletion of *ppoA* or *ppoB* causes increased conidial production, suggesting that PpoA and PpoB negatively affect asexual development (Tsitsigiannis et al. 2004, 2005). However, the deletion of *ppoC* leads to decreased asexual sporulation, suggesting that PpoC positively influences conidiation, and has antagonistic activity to PpoA and PpoB (Tsitsigiannis et al. 2004, 2005). Third, OsaA, an ortholog of Wor1 in *Candida albicans*, has been identified by a gain-of-function genetic screen and is an orchestrator of sexual and asexual development (Ni and Yu

2007). The deletion of *osaA* causes enhanced sexual fruiting with reduced conidiation, suggesting that OsaA acts as a repressor of sexual development and indirectly affects asexual development in a positive way.

g) Other Transcription Factors

A diverse range of other TFs have been shown to influence asexual development in *A. nidulans* (Fig. 1.2). RgdA (retarded growth and development) is a putative APSES TF, which is required for proper conidial production and *brlA* expression. The *rgdA* deletion mutant exhibits a reduced number of conidia, and conidiophores of this mutant show irregular shaped phialides. In addition, the deletion of *rgdA* results in decreased mRNA accumulation of *brlA* and *abaA*, suggesting that RgdA may act as an upstream regulator of central regulatory genes (Lee et al. 2013). MtfA with a C_2H_2 zinc-finger domain is termed as a master TF regulating secondary metabolism and differentiation (Ramamoorthy et al. 2013). The absence of *mtfA* results in a drastic reduction of conidiophore formation, conidial production, and expression of *brlA*. The zinc-finger TF SltA mainly plays a role in cation homeostasis (Shantappa et al. 2013). Conidia production and *brlA* expression were reduced in the *sltA* deletion mutant, suggesting that *sltA* is required for normal conidiation. RlmA containing a MADS-box domain is a major MpkA-dependent TF, which regulates cell wall integrity signaling (Fujioka et al. 2007; Kovacs et al. 2013). The deletion of *rlmA* results in an increased number of conidiospores and *brlA* accumulation in surface cultures. In addition, the *rlmA* deletion mutant produces conidiophore in liquid submerged culture, where WT strains do not. Kovács et al. proposed that *sfgA*, *flbB*, and *flbE*, upstream regulators of *brlA*, are putative target genes of RlmA, indicating that RlmA indirectly regulates *brlA* expression (Kovacs et al. 2013). Two basic leucine zipper (bZIP) transcription factors, NapA and ZipA, affect asexual and sexual development. The overexpression of *napA* or *zipA* causes increased conidial production but decreased sexual spore production (Yin et al. 2013). AreB is a putative GATA zinc-finger TF containing a leucine zipper motif at N-terminal region and acts as a negative regulator of nitrogen catabolism (Conlon et al. 2001). The *areB* deletion mutant exhibits reduced growth and conidiation, suggesting that AreB is crucial for normal growth and conidiation (Wong et al. 2009).

3. Feedback Regulators of Conidiation

Along with the formation of conidiospores, expression of the key developmental activator *brlA* is repressed, and many other spore-specific genes are induced (Adams et al. 1998). As mentioned, AbaA is required for the repression of *brlA* through the control of *brlA*β (Han and Adams 2001). However, AbaA indirectly represses *brlA* expression, because the promoter of *brlA* does not contain AbaA response element (Han and Adams 2001). Recent genetic analyses demonstrated that the VosA–VelB complex is involved in AbaA-mediated repression of *brlA* (Ni and Yu 2007; Park et al. 2012b). During the conidiophore development, the VosA and VelB proteins are completely degraded. Then, in phialides, AbaA directly binds to the promoter regions of *vosA* and *velB* and induces their expression, thereby a large amount of the VosA and VelB proteins are newly synthesized in the developing cells (Park et al. 2012b). VosA, then, interacts with VelB, and the VosA–VelB heterocomplex binds to the *brlA* promoter and represses *brlA* expression in conidia (Ahmed et al. 2013). Moreover, the VosA–VelB complex regulates spore-specific genes, suggesting that the VosA–VelB dimeric complex acts as a key functional unit regulating spore maturation (trehalose biogenesis and spore wall synthesis), viability, and attenuating conidial germination in conidiospores (Ahmed et al. 2013; Park et al. 2012b).

III. Asexual Sporulation in *Penicillium marneffei*

A. Morphology of Asexual Structure

Penicillium marneffei is an emerging fungal pathogen and a dimorphic fungus, which

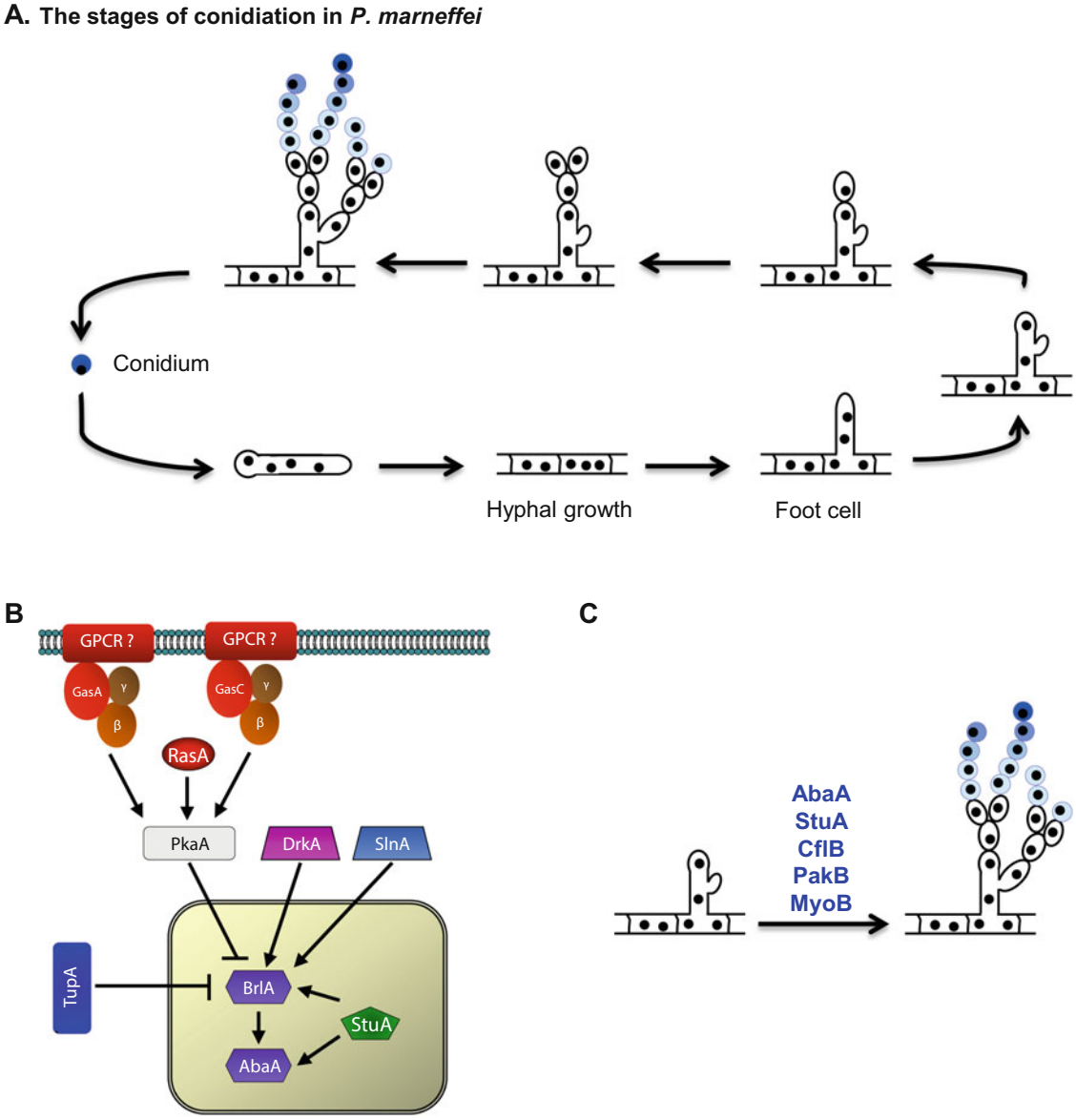

Fig. 1.3 Stages and regulation of conidiation in *P. marneffei*. (**a**) The stages of conidiation in *P. marneffei*. (**b**) Simplified model for the genetic regulation of conidiation in *P. marneffei*. (**c**) Genes involved in the conidiophore morphogenesis

grows in the yeast form at 37 °C and the filamentous form at 25 °C. In the filamentous phase, *P. marneffei* undergoes the complex asexual development and forms conidiophores (Andrianopoulos 2002; Pasricha et al. 2013). In response to inducing environmental signals, hyphal growth is arrested and the fungus starts to form asexual structures. Multinucleate stalk cells, formed from foot cells, are subsequently septated and form the subapical branches called rama. Two sterigmata, metulae and phialides, are formed from the apical cells. Finally, phialides produce conidia, resulting in the formation of brush-like structures called penicillin (Fig. 1.3a) (Andrianopoulos 2002; Roncal and Ugalde 2003; Vanittanakom et al. 2006).

B. Regulators of Asexual Development

The role of two central regulatory genes, *brlA* and *abaA*, is conserved in *A. nidulans* and *P. marneffei* (Borneman et al. 2000). The *brlA* deletion mutant in *P. marneffei* produces only conidiophore stalks but not conidia (Borneman et al. 2000; Boyce and Andrianopoulos 2013). AbaA in *P. marneffei* plays a similar role in asexual development (Borneman et al. 2000). The *abaA* deletion mutant can produce conidiophore stalks, rama and metulae, but not phialides and conidia. In addition, ectopic overexpression of *abaA* results in the formation of aberrant apical and multinucleate hyphal cells. These results indicate that AbaA is required for controlling cell cycle events during conidiation (Borneman et al. 2000).

As in *A. nidulans*, the central developmental pathway in *P. marneffei* is controlled by various regulatory inputs (Fig. 1.3b). First, **G protein signal pathways** are involved in the activation of conidiation. GasA, the homologue of FadA in *A. nidulans*, negatively regulates asexual development in *P. marneffei* (Zuber et al. 2002). The *gasA* deletion and dominant interfering $gasA^{G203R}$ mutant strains show elevated and precocious asexual development and *brlA* expression, whereas the dominant activating *gasA* mutant ($gasA^{G42R}$) fails to display conidiation and *brlA* expression in developmental cultures, suggesting a conserved cellular response to this particular G protein signal transduction. GasC, another G-protein alpha subunit, also plays a crucial role in germination and negatively controlling asexual development (Zuber et al. 2003). The small GTPase RasA acts downstream of heterotrimeric G proteins and negatively regulates the onset of conidiation (Boyce et al. 2005). The dominant-negative allele $RasA^{D125A}$ causes precocious initiation of conidiation, whereas the dominant positive $RasA^{G19V}$ allele causes reduced conidiation. The GasA-, GasC-, and RasA-mediated regulation of asexual development occurs via activation of the PKA signaling pathway (Boyce and Andrianopoulos 2007). Second, **TupA**, an ortholog of Tup1p in *S. cerevisiae*, acts as a **repressor of asexual development** (Todd et al. 2003). The deletion of *tupA* leads to premature conidiation and *brlA* expression, suggesting that TupA is required for proper control of the *brlA*-dependent conidiation. Third, the two-component histidine kinases DrkA and SlnA also play a crucial role in *brlA*-dependent asexual development (Boyce et al. 2011). The deletion of *slnA* or *drkA* results in delayed and reduced conidiation. The *drkA* deletion mutant also exhibits decreased expression of *brlA* mRNA. Interestingly, mRNA levels of *drkA* are increased in the Δ*brlA* mutant but reduced in the Δ*abaA* mutant, suggesting a potential feedback regulatory circuit (Boyce and Andrianopoulos 2013; Boyce et al. 2011).

Proper morphogenesis of conidiophore in *P. marneffei* is governed by multiple genes (Fig. 1.3c). StuA, a member of the APSES protein, is required for the formation of metula and phialide during conidiation (Borneman et al. 2002). The *stuA* deletion mutant fails to produce sterigmata cells, but can elaborate spores directly from the tips of stalks. The *cflB* gene, which encodes a Rho GTPase, is required for the polarized growth and cell division in both hyphal growth and conidiogenesis (Boyce et al. 2003, 2005). The *cflB* deletion mutant produces abnormal conidiophores with swollen and malformed cells. The dominant-negative $cflB^{D123A}$ mutant displays aberrant conidiophores with a single, terminal, and multinucleate conidium (Boyce et al. 2003, 2005). RfxA, an RFX (regulatory factor X) protein, is important for the cell division and checkpoint regulation with morphogenesis. Decreased expression of *rfxA* leads to the production of abnormal conidiophores containing multiple nuclei (Bugeja et al. 2010). MyoB is a type II myosin protein required for chitin deposition at the sites of cell division. The *myoB* deletion strain produces defective conidiophores, which lack clearly defined cell types due to malformed septa and faulty nuclear division (Canovas et al. 2011).

IV. Asexual Sporulation in *Fusarium graminearum*

A. Morphology of Asexual Structure

Fusarium graminearum (teleomorph *Gibberella zeae*) is a major plant pathogen that causes

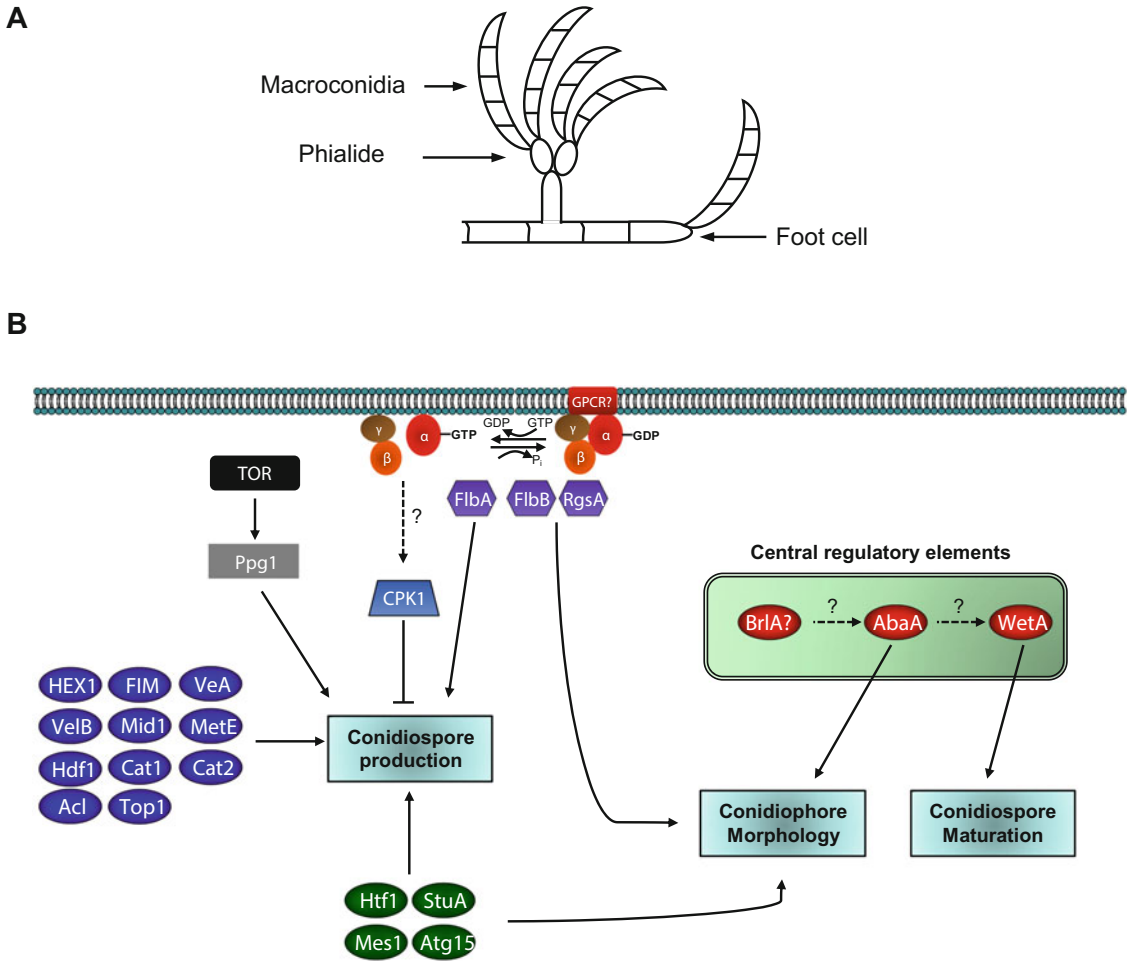

Fig. 1.4 Morphology and regulation of conidiation in *F. graminearum*. (**a**) Morphology of the asexual developmental structure in *F. graminearum*. (**b**) A simplified model for the genetic regulation of conidiation in *F. graminearum*

Fusarium head blight (FHB) in cereal crops, such as wheat, rice, and oats (Fernando et al. 1997; Goswami and Kistler 2004; Parry et al. 1995). This pathogenic fungus produces both asexual (conidia) and sexual (ascospore) spores, which can be major propagules causing FHB (Markell and Francl 2003). Different from *A. nidulans* and *P. marneffei*, *F. graminearum* directly produces phialides from the hyphae and continuously generates macroconidia or microconidia from phialides through a basipetal division (Leslie and Summerell 2006; Zheng et al. 2012) (Fig. 1.4a). Macroconidia are moderately curved containing multiple septa, whereas microconidia are formed on the simple conidiophores (Harris 2005; Leslie and Summerell 2006).

B. Regulators of Asexual Development

A number of regulators are associated with the asexual development in *F. graminearum* (Fig. 1.4b). First, *abaA* and *wetA*, the orthologs of those central regulators in *A. nidulans*, are required for conidiogenesis (Son et al. 2013a, 2014). The Δ*abaA* mutant strains produce abnormally shape phialides. The overexpression of *abaA* causes a reduction in the number of conidia and the formation of abacus-like phialides. Genomic studies have revealed that AbaA regulates several genes required for conidiation, suggesting a pivotal role of AbaA in asexual sporulation (Son et al. 2013a). WetA in *F. graminearum* is essential for conidiogenesis

and maturation of conidia (Son et al. 2014). The deletion of *wetA* results in decreased number of conidia and the formation of abnormal conidiospores with longer and fewer septa. Conidia of the *wetA* deletion mutant are sensitive to various stresses and exhibit reduced long-term viability. In addition, the *wetA* deletion mutant contains numerous autophagic bodies in the conidium. These results indicate that WetA plays a crucial role in conidial dormancy by suppressing microcycle conidiation (Son et al. 2014).

Three RGS proteins are required for conidia morphology or production in *F. graminearum* (Park et al. 2012a). The deletion of *flbA* results in a significant reduction of conidia production. The *flbB* deletion mutant produces thinner and short conidia with few septa, whereas conidia of the *rgsA* deletion mutant show wider and longer conidia. The cAMP-PKA pathway plays a crucial role in growth and differentiation in *F. graminearum*. The deletion of *cpk1* encoding the main catalytic subunit of PKA, or the adenylate cyclase encoding gene *fac1* causes growth defects, indicating that the cAMP-PKA pathway is required for proper hyphal growth. In addition, the *cpk1* mutant produces phialides and conidia earlier than WT strain and the deletion of *cpk1* causes elevated mRNA expression of genes related to conidiation, suggesting that *cpk1* is negatively associated with conidiation (Hu et al. 2014). The target of rapamycin (TOR) signaling pathway also plays an important role in vegetative growth and differentiation in *F. graminearum* (Yu et al. 2014). Rapamycin has an inhibitory effect on fungal growth and asexual development. Moreover, the deletion of *ppg1* encoding a component of TOR signaling pathway causes decreased conidiophore production and impaired septum formation.

Several other proteins are required for proper production of asexual spores in *F. graminearum*. Both the deletion and overexpression of *hex1* encoding a hexagonal peroxisome protein result in the reduced production of conidia, suggesting that appropriate expression of HEX1 is important for controlling conidiogenesis (Son et al. 2013b). The actin bundling protein, Fim, plays a vital role in various cellular processes, and the *fim* deletion mutant exhibits reduced conidiation (Zheng et al. 2014). The *velvet* genes, *veA* and *velB*, act as repressors of conidia production (Jiang et al. 2011, 2012; Lee et al. 2012). Both the *veA* and *velB* deletion mutants show increased conidial production, and their conidia contain a large number of bulky lipid droplets. Additional proteins involved in conidiation include Mid1 (Stretch-activated ion channel) (Cavinder et al. 2011), MetE (Homoserine *O*-acetyltransferase) (Han et al. 2004b), HDF1 (Histone Deacetylase) (Li et al. 2011), CATs (Carnitine AcetylTransferases; CAT1 and CAT2) (Son et al. 2012), Acl (ATP Citrate Lyase) (Son et al. 2011), and Top1 (Topoisomerase I) (Baldwin et al. 2010).

A number of other regulators function in the production and morphogenesis of conidia. For instance, the homeobox TF Htf1 is required for phialidegenesis, conidiogenesis, and macroconidia basal cell division (Zheng et al. 2012). The deletion of *htf1* results in reduced conidia production and abolished macroconidia development, and this function is conserved in other *Fusarium* species. StuA, an APSES protein, acts as a master regulator controlling diverse processes in *F. graminearum*. The Δ*stuA* mutant fails to form conidiophores or phialides and produces aberrant macroconidia directly from the hyphae. The autophagy-related lipase Atg15 is also important for conidia formation and morphogenesis (Nguyen et al. 2011). The deletion of *atg15* leads to a reduced number of conidia and production of aberrantly shaped conidia. Mes1, a homologue of MesA in *A. nidulans* required for the formation of stable polarity axes, is also necessary for conidiogenesis in *F. graminearum*. The deletion of *mes1* leads to a reduction of asexual production and causes production of abnormal macroconidia (Rittenour and Harris 2008).

V. Conclusions

In this chapter, we have summarized current molecular biology of asexual sporulation in the three important filamentous fungal species. As discussed, several regulatory and signaling elements are conserved in three major fungal genera, and they play vital roles in various aspects of conidial production and morphogenesis of conidiophore. Further studies aimed at revealing the detailed molecular mechanisms of

asexual sporulation in diverse fungal species will illuminate the common and distinct regulators and signaling cascades governing growth and development in fungi.

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