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# Conserved and Distinct Developmental Regulatory Mechanisms in Two *Aspergillus* Species

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Reproduction of fungi results in the formation of enormous numbers of spores. Asexual sporulation (conidiation) is a highly successful and effective reproductive mechanism for a diverse group of fungi because vast numbers of spores (called conidia for higher fungi) can be produced from a single colony through repetitive cycles of mitosis.

The filamentous Ascomycete *Aspergillus nidulans* is an excellent model system for studying multicellular development and secondary metabolism. Recent studies showed that activities of two antagonistic regulatory pathways control growth, conidiation and the mycotoxin sterigmatocystin (ST) production. Activation of signaling by a heterotrimeric G protein composed of FadA (Gα), SfaD-GpgA (Gβγ) causes enhancement of vegetative (hyphal) growth, which represses both development and ST biosynthesis. FlbA is an RGS protein that rapidly turns off this FadA-mediated growth signaling. At least partial inhibition of growth signaling is required in order for development and ST production to occur. Commencement of conidiation requires the activities of FluG and other developmental genes, *flbB, flbC, flbD, flbE* and *brlA*. FluG is thought to have dual functions, activating conidiation and positively modulating FlbA activity. BrlA is a key $C_2H_2$-type transcription factor required for conidiophore (conidia-bearing structure) development.

Loss of *fluG* function results in the blockage in both conidiation and production of ST, and it has been proposed that FluG is responsible for the production of an extracellular diffusible conidiation factor. However, the molecular mechanisms underlying the developmental switch have remained elusive. Employing suppressor analyses, we found that the FluG-mediated conidiation in *A. nidulans* occurs via de-repression. Among four suppressors, the *sfgA* gene encoding a novel protein with the Gal4-type $Zn(II)_2Cys_6$ binuclear cluster DNA binding motif at the N-terminus has been identified. Deletion and 31 other loss-of-function *sfgA* mutations bypassed the need for *fluG* in conidiation and production of ST. Moreover, both Δ*sfgA* and Δ*sfgA* Δ*fluG* mutations resulted in identical phenotypes in growth,

conidiation and ST production, indicating that the primary role of FluG is to remove repressive effects imposed by SfgA. In accordance with the proposed regulatory role of SfgA, overexpression of *sfgA* inhibited conidiation, and delayed/reduced expression of conidiation- and ST-specific genes. Genetic analyses demonstrated that SfgA functions downstream of FluG but upstream of transcriptional activators (FlbD, FlbC, FlbB and BrlA) necessary for normal conidiation. Moreover, we identified a downstream regulator of conidiation necessary for the viability of spores (thus called *vosA*). The *vosA* gene is required for proper down-regulation of *brlA*, which appears to be crucial for spore maturation in *A. nidulans*.

Based on the useful framework of developmental regulation in *A. nidulans*, we further investigated the mechanisms controlling growth and sporulation in the opportunistic human pathogen *Aspergillus fumigatus*. Both loss of function Af-*flbA* and the dominant activating $GpaA^{Q204L}$ (Gα, the FadA homolog) mutations result in reduced levels of conidiation with enhanced vegetative growth, indicating that Gα and RGS play a predominant role in balancing hyphal proliferation and spore formation. As GpaA is the primary target of Af-FlbA, the dominant interfering $GpaA^{G203R}$ mutation suppressed the sporulation defect caused by loss of function Af-*flbA*. These results corroborate the hypothesis that functions of G proteins and their regulators are conserved in aspergilli.

We then examined the functions of homologs of the two major developmental activators FluG and BrlA in *A. fumigatus*. Interestingly, while deletion of Af-*brlA* completely eliminated formation of conidia, deletion of Af-*fluG* did not cause severe alterations in *A. fumigatus* sporulation. These results indicate that whereas two aspergilli may share a common downstream developmental activator(s), the upstream mechanisms activating *brlA* may be distinct. Expression analyses of *gpaA*, Af-*flbA*, Af-*fluG*, Af-*brlA* and Af-*wetA* throughout the lifecycle of *A. fumigatus* revealed that, while transcripts of the three upstream controllers are constitutively expressed, the latter two downstream developmental activators are specifically expressed during conidiation. Summaries of our findings are: 1) *A. fumigatus* and *A. nidulans* share conserved G protein and RGS-mediated growth signaling; 2) *A. fumigatus* may have distinct and persistent upstream (*fluG*-level) regulatory mechanisms for activation of conidiation; and 3) BrlA a key transcription factor necessary for conidiophore development in *A. nidulans* is also essential for conidiation in *A. fumigatus*.