DEVELOPMENTAL REGULATION OF *ASPERGILLUS* MYCOTOXIN BIOSYNTHESIS

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Abstract

Cotton is a crop grown for fiber and seed cotton, which is used as a feed additive for livestock. Therefore, any contaminant that jeopardizes the quality of cottonseed can result in both health and economic losses. Several *Aspergillus* fungi can contaminate cottonseed with the carcinogenic mycotoxins, aflatoxin (AF) and sterigmatocystin (ST). If cottonseed contains AF levels (more commonly analyzed than ST) above the accepted national and international limits, the seed must be destroyed or decontaminated. Our longterm goal is to eliminate the ST/AF problem by first understanding how ST/AF biosynthesis is regulated in *Aspergillus* and then applying this understanding in a logical control strategy.

Because ST and AF are end products of the same metabolic pathway and we have recently shown that both ST/AF enzymatic and regulatory genes (e.g. *stcU* and *aflR* respectively) are conserved in a gene cluster in *A. flavus*, *A. parasiticus* and *A. nidulans* (Brown et al., In press; Keller et al., 1995), we are using *A. nidulans* to understand the molecular genetics of ST/AF regulation since it has been used a model system to study fungal biology for ~ 50 years. We have recently been investigating the relationship between fungal development (e.g. sporulation) and secondary metabolism (e.g. ST/AF production). We and others have found that *Aspergillus* strains impaired in asexual reproduction are likewise impaired in ST/AF production (Table 1; Bennett, 1981; Torres, 1980).

We have identified six developmental genes, *flbA*, *flbB*, *flbC*, *flbD*, *flbE*, and *fluG*, which are required for normal sporulation in *A. nidulans.* These mutants are characterized by a loss or delay in spore formation. These mutants grow as a "fluffy" mass of undifferentiated mycelium or have delayed and reduced spore formation. Mutations in *flbA* and *fluG* result in no to barely detectable ST production (Table 1).

We have also determined that *fluG*$^-$ and *fluA*$^-$ mutants do not transcribe *stcU* (e.g. a gene encoding a ketoreductase needed for both ST and AF biosynthesis) in comparison to the wildtype strain, FGSC26, which expresses *stcU* between 24 and 36 h of fungal growth. In contrast, overexpression of *flbA*, but not *fluG*, results in early expression of *stcU*. This was determined by fusing the *flbA* coding region to the threonine inducing *alcA* promoter, *alcA*[p] (e.g. *alcA* encodes alcohol dehydrogenase).

Fungal cultures (wildtype and *alcA*[p]::*flbA*)were grown in glucose media for 12 hours and then transfered to threonine media. The cultures were then allowed to grow for 0, 6, 12, 18 and 24 hours. Total RNA was isolated from mycelia of each culture at each different time point. The *stcU* message was seen between 6 and 12 hours in *alcA*[p]:*flbA* and between 24 and 36 hours in wildtype.

These results clearly show a genetic link between *Aspergillus* development and the ability to produce ST. *flbA* shows homology to a protein in yeast thought to be involved in G protein mediated signalling pathways and it is possible that *flbA* has a similar role in directing sporulation and ST biosynthesis. Our future goals are to further clarify this linkage in *A. nidulans* and to identify *flbA* homologs in the AF producing species, *A. flavus* and *A. parasiticus*. Pursuit of these projects should provide exciting avenues for future research that promises to have a major impact in the cotton industry.

References

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Table 1. Sterigmatocystin production by *Aspergillus nidulans* mutants grown on oatmeal.

Isolate	Morphology	HPLC[a]
A. nidulans FGSC26	Wild-type sporulation	155-1000 mg
A. nidulans fluG$^-$	Fluffy (some spores)	5 mg
A. nidulans flbA$^-$	Fluffy (no spores)	Below detectable limit

[a] HPLC = high pressure liquid chromatography; ST was quantitated in μg/ml.

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