Regulation of Secondary Metabolism and Development in *Aspergillus flavus:* From Genes to Metabolites

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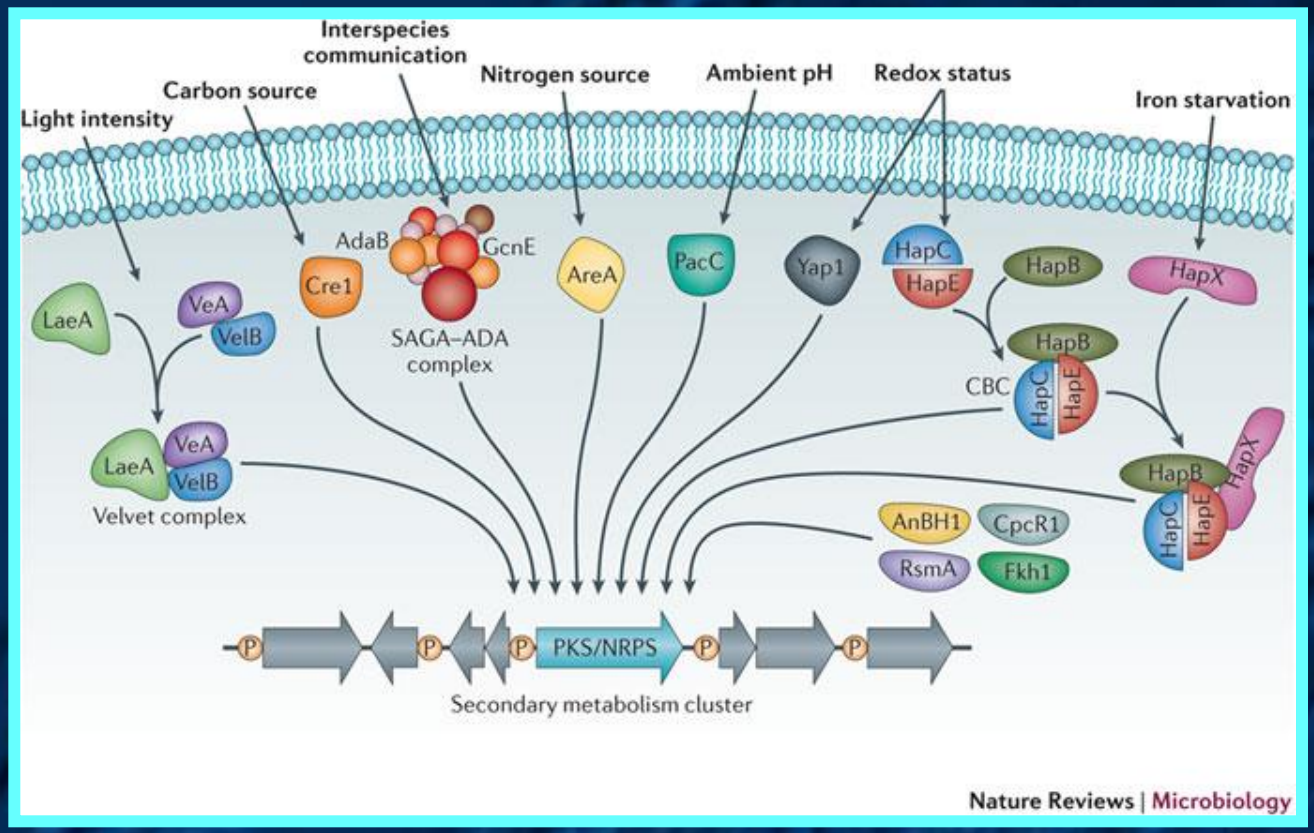
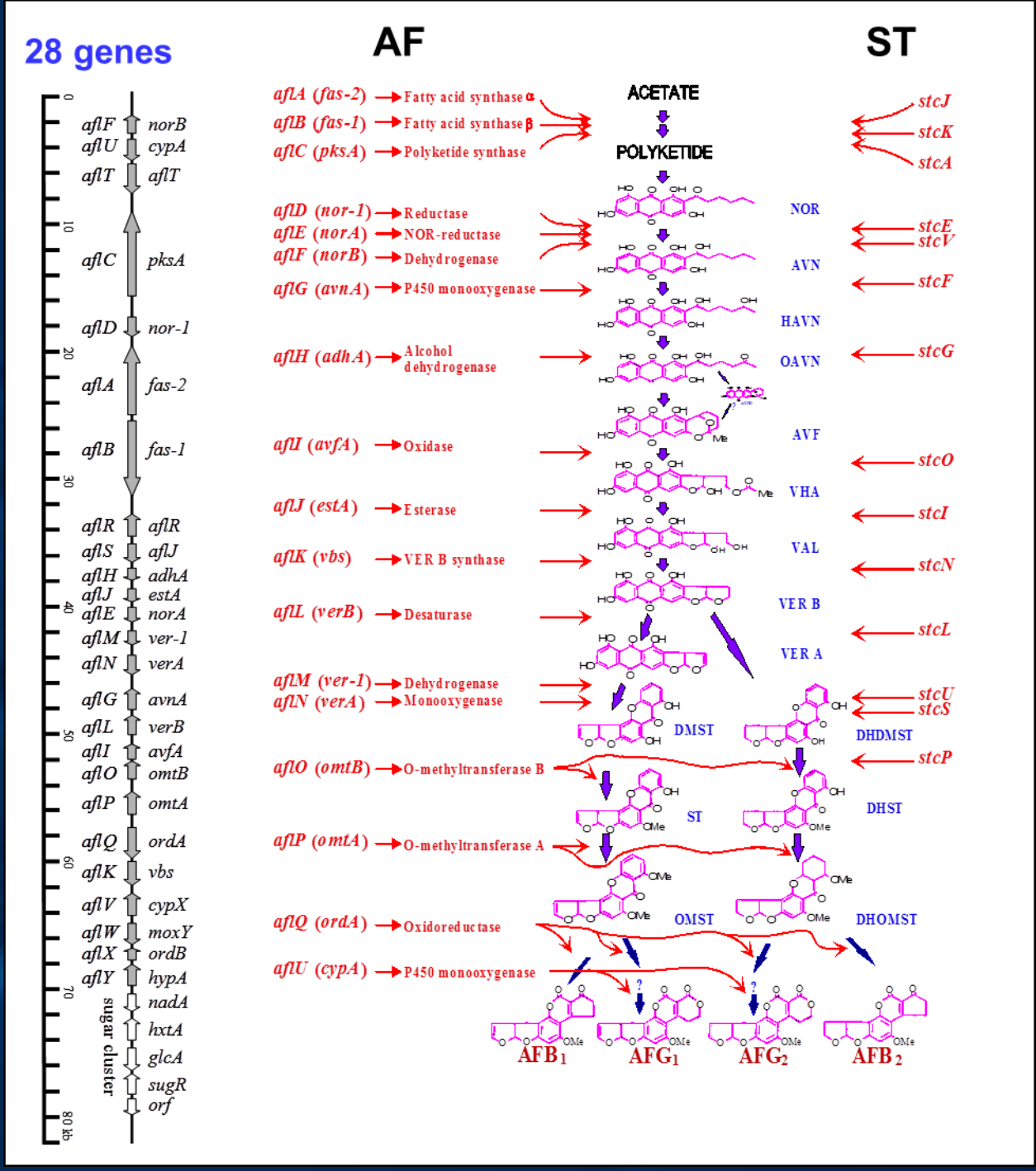
Complex "Molecular Switches" Controlling Secondary Metabolism



Nature Reviews Microbiology 11, 21-32 (January 2013)

- SM is tightly regulated by environmental cues- C and N source, pH, light , stress
- GPCR ⟶ signal transduction relay systems ⟶ TF ⟶ SM cluster gene expression
- Characterization of components of some of these signaling networks by use of techniques like mutagenesis, protein-protein interaction, genomics, transcriptomics

Aflatoxin and Sterigmatocystin Biosynthetic Pathways



Regulation of Aflatoxin Biosynthesis and Fungal Development

Pathway-Specific Regulators

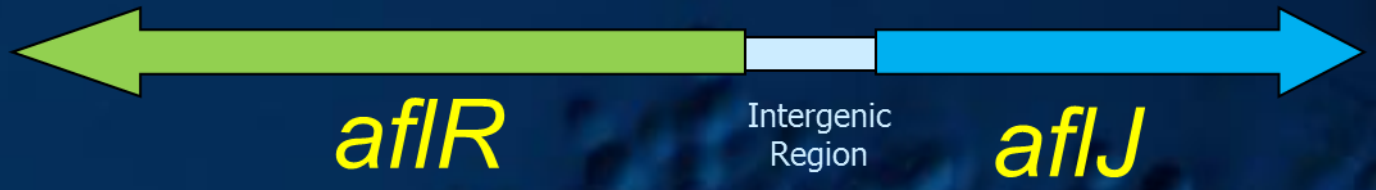
AflR and AflJ

Globally-Acting Transcriptional Factors

NsdC and Hbx1

Global Regulators- Velvet Complex

VeA-LaeA-VelB



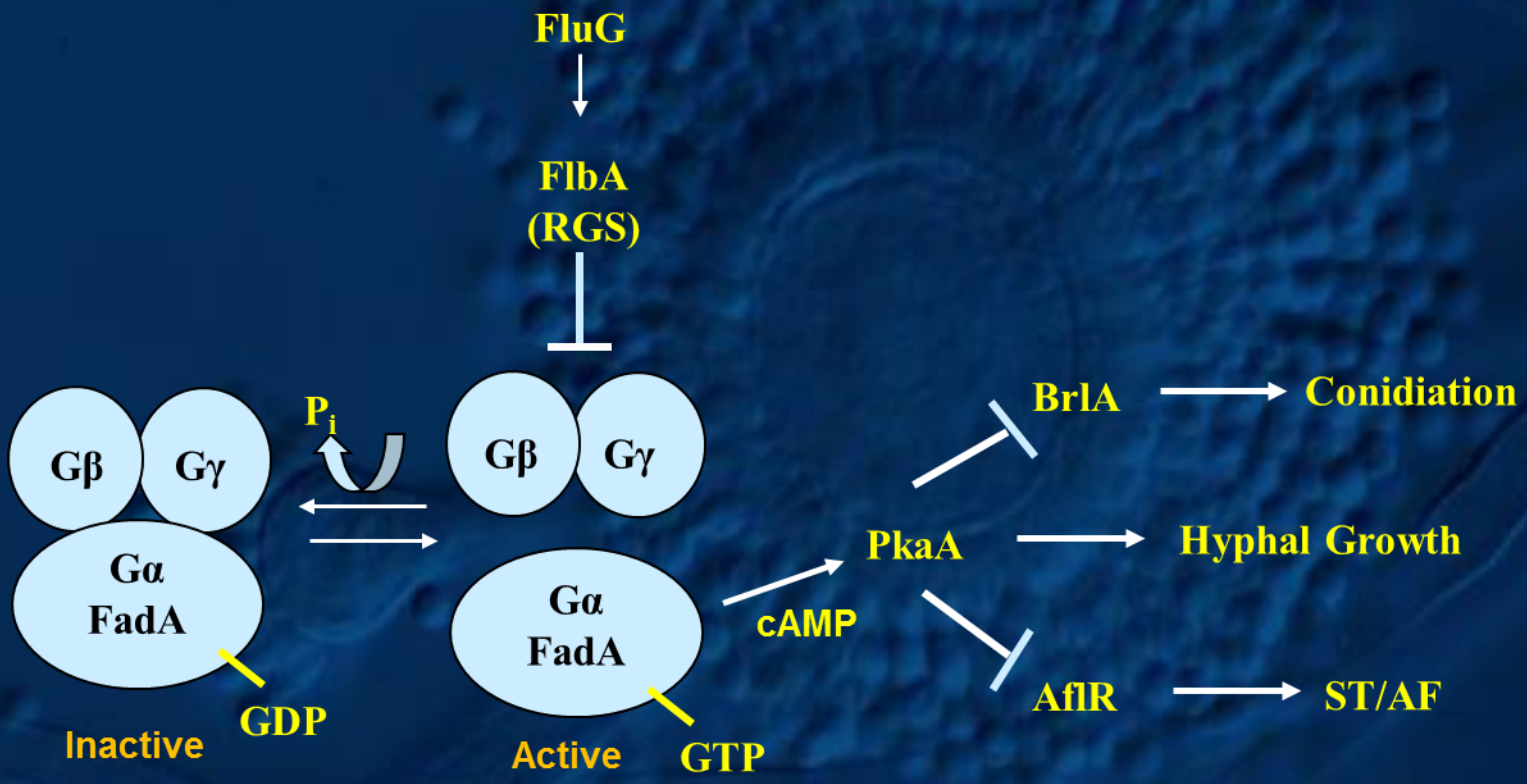
aflR (Payne et al., 1993; Chang et al., 1993)

- AflR is a Gal4-type, Zn-finger transcription factor that is required for expression of all AF biosynthetic genes.
- AflR activates transcription by binding to a conserved 11 bp palindromic sequence found in just about all AF pathway gene promoters and also ST pathway genes in A. nidulans.
- AflR deletion mutants do not produce AF and do not express AF pathway genes and overexpression of AflR results in increased expression of AF pathway genes and AF production.

aflJ (aflS) (Meyers et al., 1998)

- Divergently transcribed from *aflR*
- Functional copy needed for WT levels of AF
- Shown to bind to C-terminal region of AflR (Chang et al., 2003)
- Involved in modulating the expression of AF pathway genes perhaps via interaction with AflR.

Relationship of Aflatoxin Biosynthesis and Fungal Development



G-Protein/cAMP/Protein kinase A Signaling Pathway (Hicks et al., 1997)

Regulation of Aflatoxin Biosynthesis and Fungal Development

Pathway-Specific Regulators- AflR and AflJ

Globally-Acting Transcription Factors

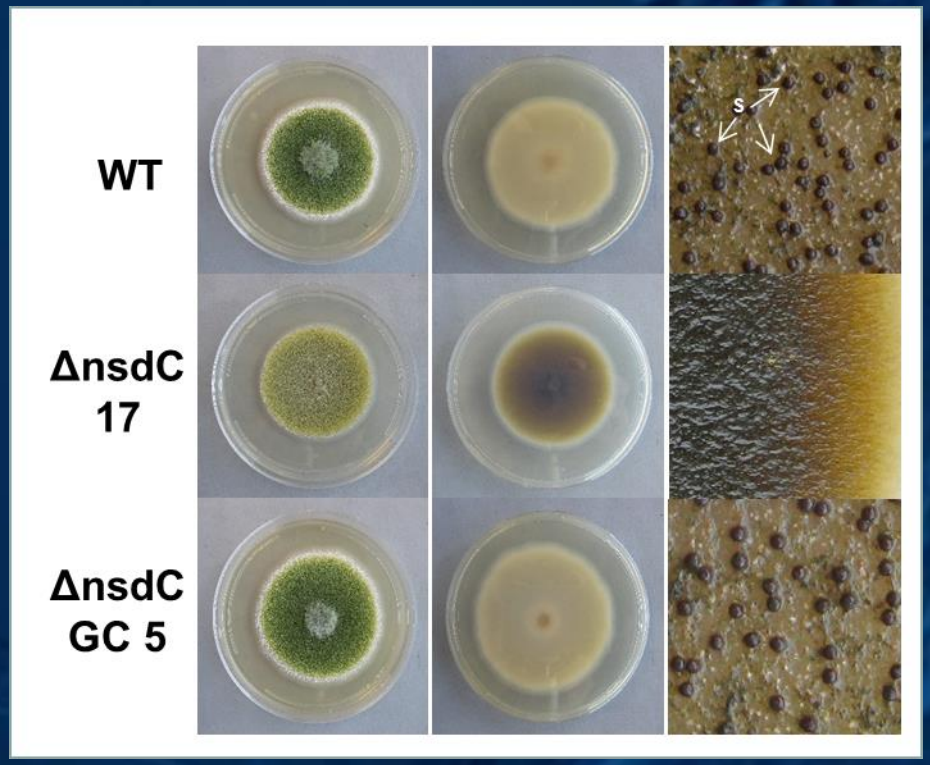
NsdC and Hbx1

Global Regulators- Velvet Complex

VeA-LaeA-VelB

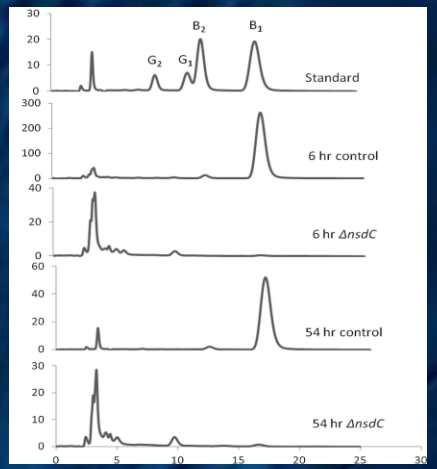
Functional Analysis of the *A. flavus nsdC* Transcription Factor

Development

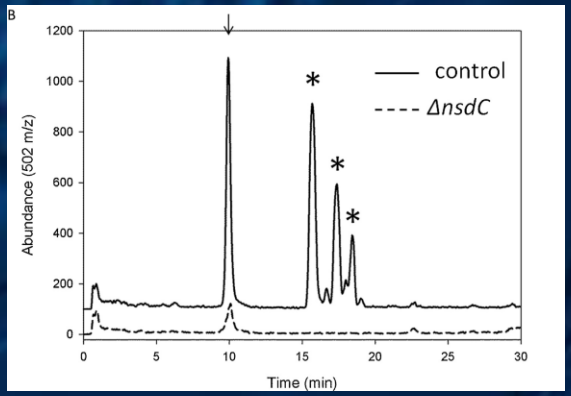


Cary et al., Eukaryotic Cell 2012

Aflatoxin production



Aflatrem production



Gilbert et al., Microbiol Res 2016

Regulation of Aflatoxin Biosynthesis and Fungal Development (2)

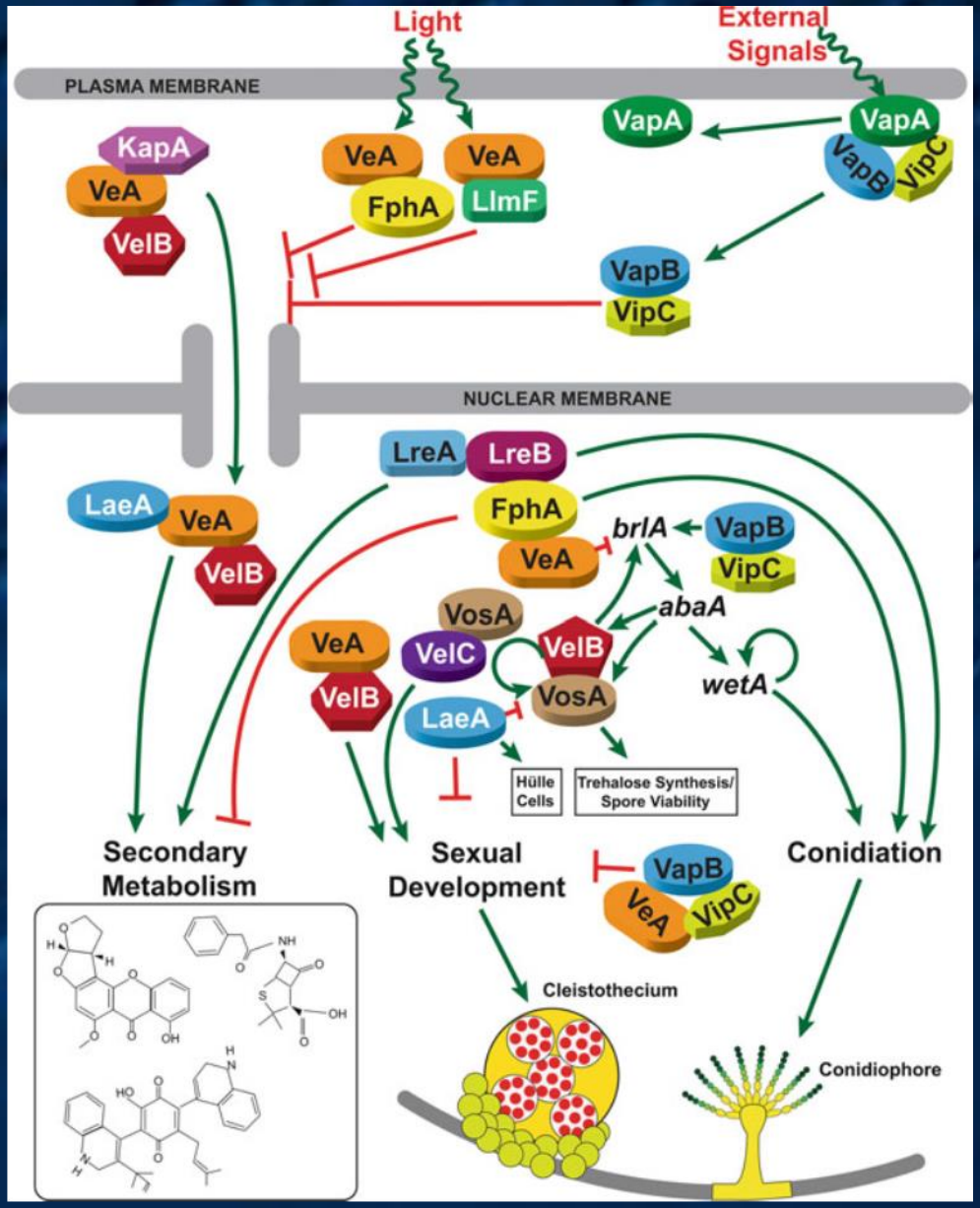
Pathway-Specific Regulators- AflR and AflJ

Globally-Acting Transcription Factors
NsdC and Hbx1

Global Regulators- Velvet Complex
VeA-LaeA-VelB

The Velvet Complex

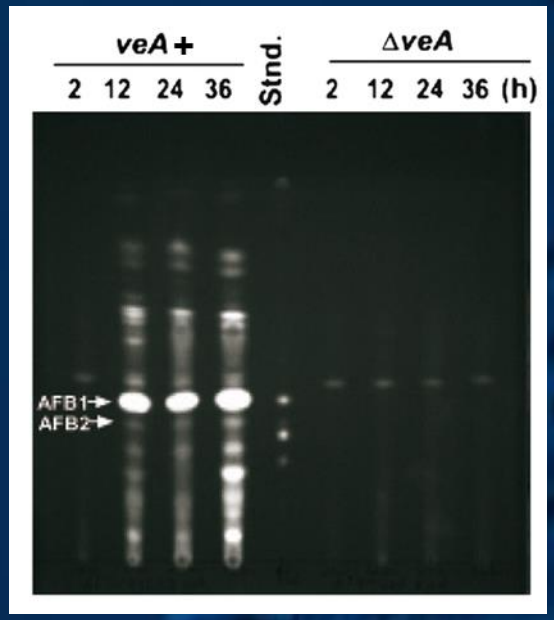
Global Regulation of Fungal Development and Secondary Metabolism



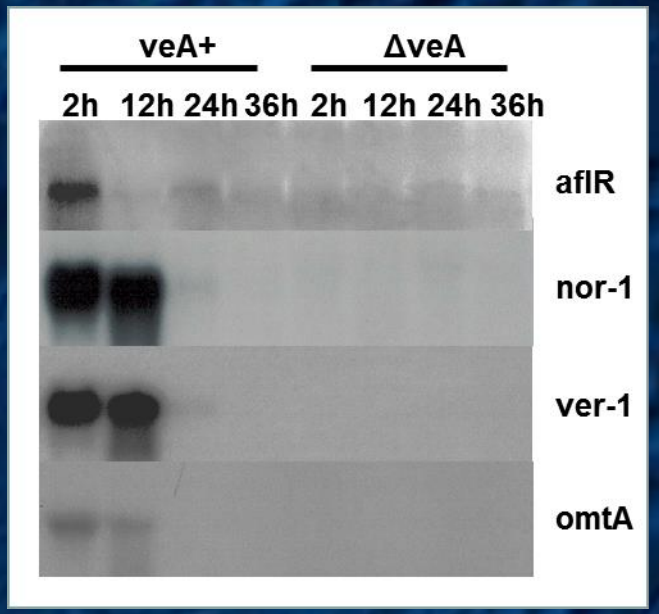
Adapted from Calvo et al., 2016

VeA is Required for Aflatoxin and Sclerotial Production

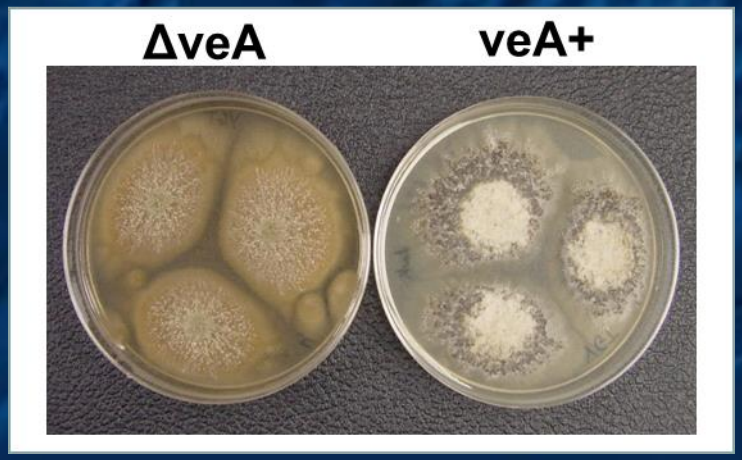
Aflatoxin



AF Gene Expression



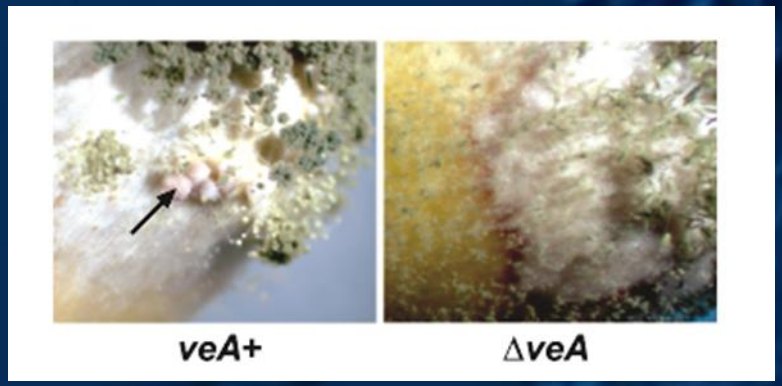
Development

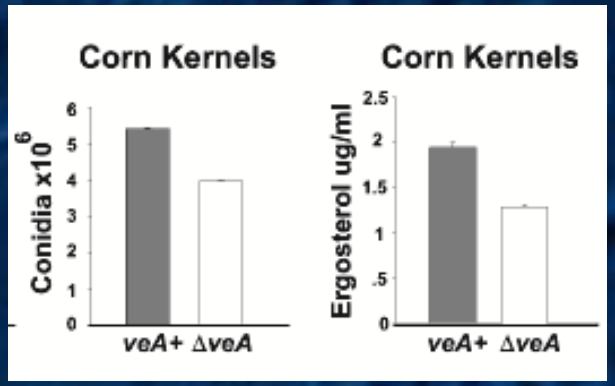


Duran et al., Appl. Micro. Biotechnol. 2007

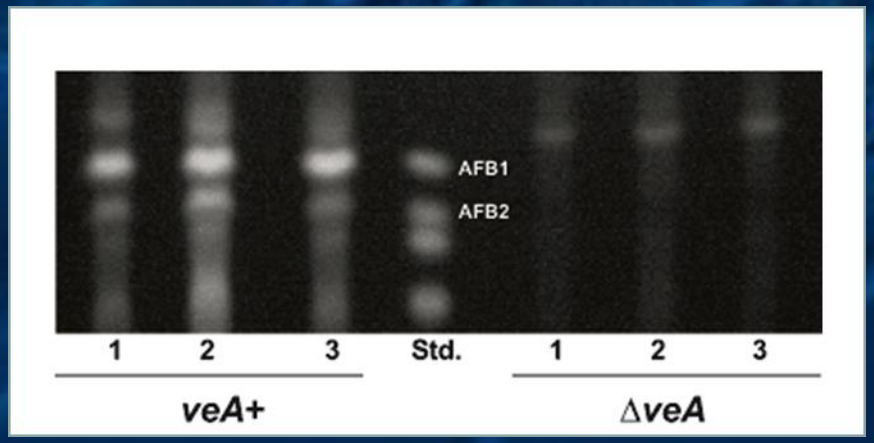
VeA is Required for Normal Virulence in *A. flavus*

Growth and Development





Aflatoxin Production



Duran et al., Open Mycology J. 2009

Identification of VeA-Regulated SM Gene Clusters using Genomics, Transcriptomics and Metabolomics

Why Study SM Clusters in *A. flavus?*

- Determine the biological function of the SM
  e.g. role in development, virulence, survival
- New insights into the regulation of SM clusters during the plant-fungus interaction. Possible virulence factors?
- Possible genetic cross-talk between SM clusters- potential to modulate aflatoxin production?
- Possible synergy of SMs with aflatoxins that may increase the toxicity of the producing strain

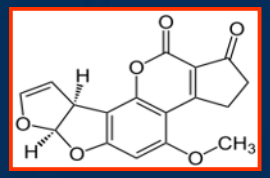
Use of Genome Sequence Data to Identify SM Gene Clusters in Aspergilli

Computational prediction of SM clusters is *Aspergillus* species.

Organism	SMURF	antiSMASH	Experimental	Total Predicted
A. nidulans	49	66	9	71
A. fumigatus	33	38	5	39
A. niger	79	70	0	81
A. oryzae	57	73	2	75
A. flavus	**55**	**61**	**10**	**62**

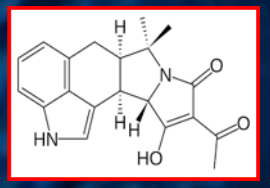
PKS/PKS-like- 28 NRPS/NRPS-like- 32 PKS-NRPS- 2 DMATS-7

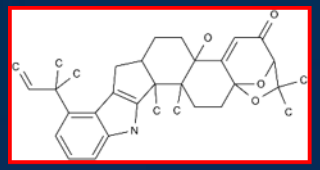
SM Cluster Metabolites Experimentally Identified in *A. flavus*



aflatoxins
PKS-FAS

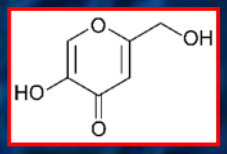
aflatrem
DMATS-GGPS

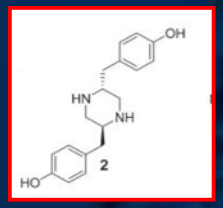




cyclopiazonic acid
PKS-NRPS

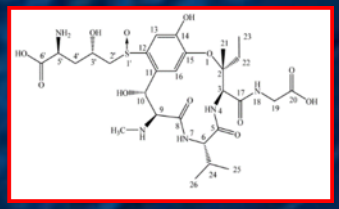
kojic acid
oxred

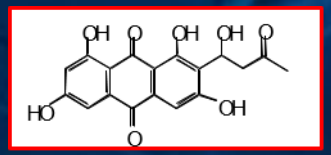




diketopiperazines
NRPS

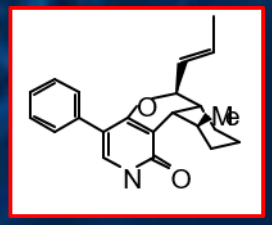
ustiloxin B
RiPS

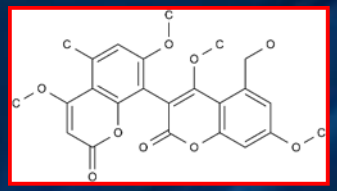




asparasone
PKS

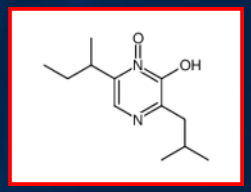
leporin
PKS-NRPS



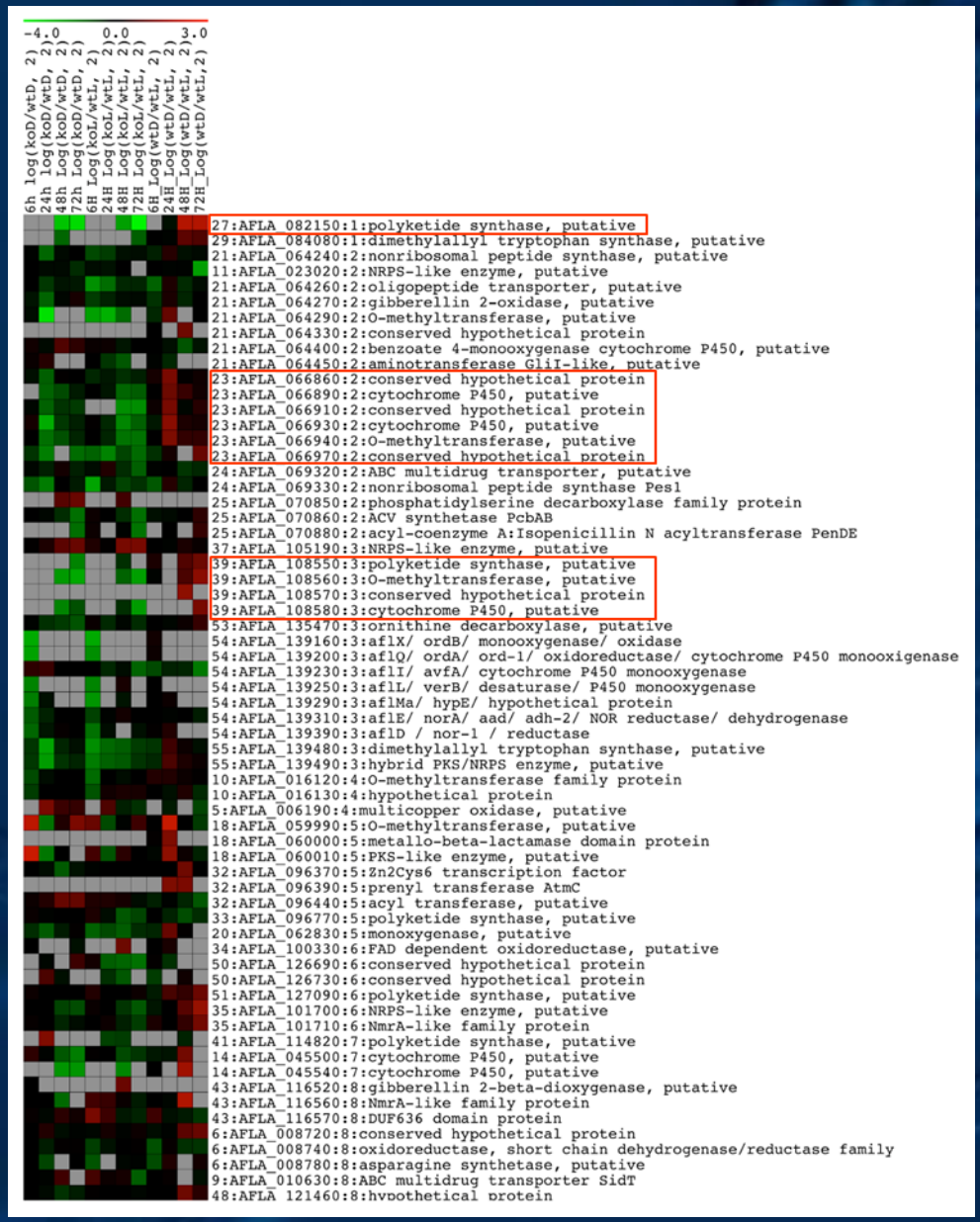


aflavarin
PKS

aspergillic acid
NRPS



Transcriptomics to Identify VeA-Regulated *A. flavus* SM Cluster Genes



Cary et al., Eukaryotic Cell, 2015

Comparative Metabolomics to Identify VeA-dependent SM Cluster Metabolites in *A. flavus*

Old School

NGS

+

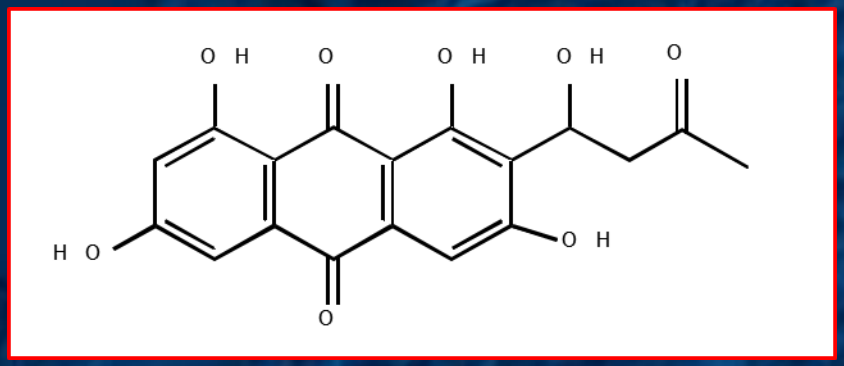
LC-MS

New School

Cluster 27

Asparasone A

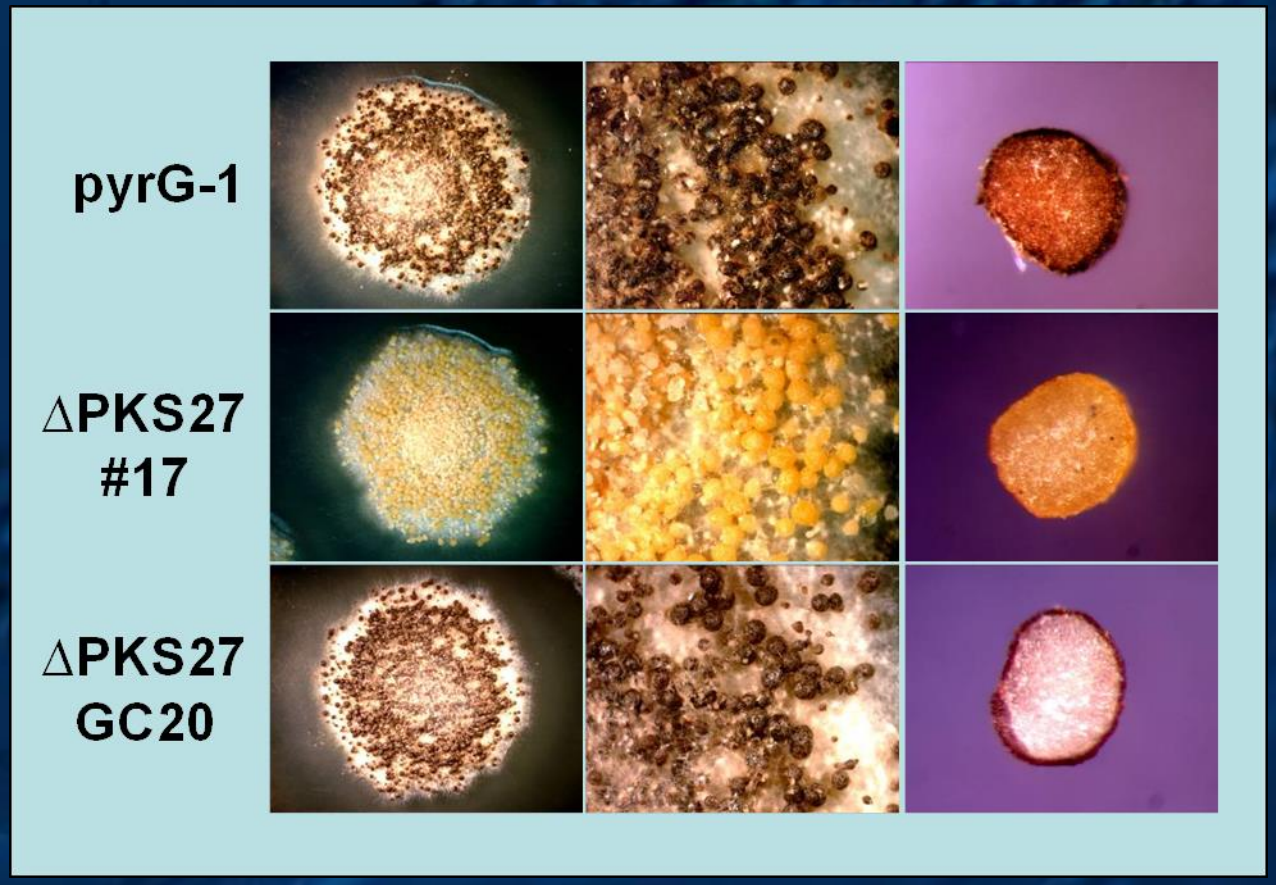
Sobolev et al.,J. Nat Prod, 1997



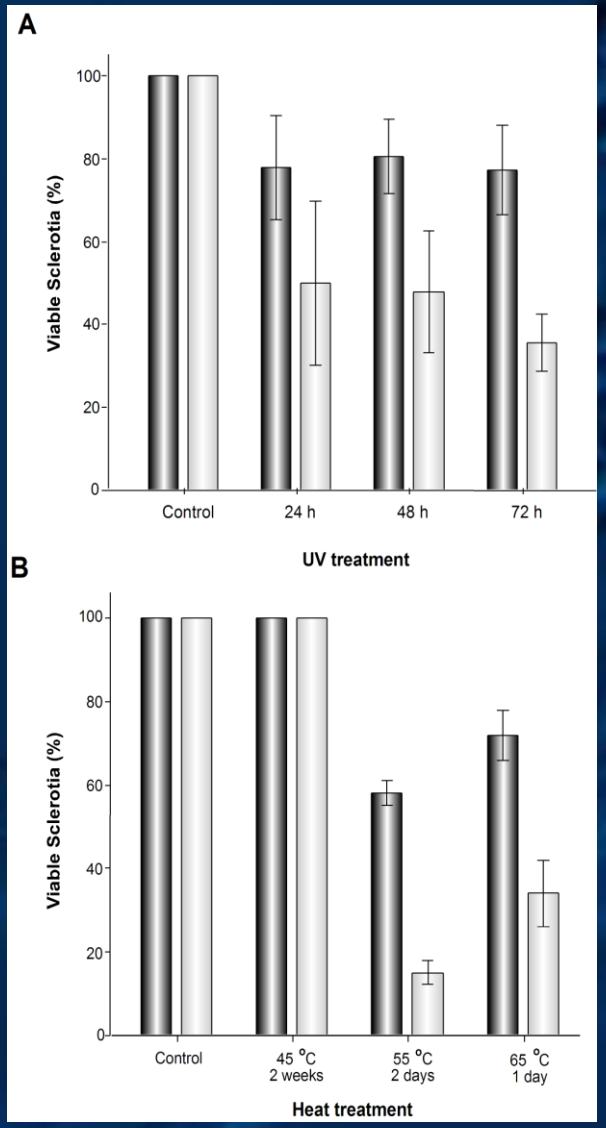
PKS-derived

Cary et al., FG&B 2014

Asparsone A is a Sclerotial Pigment



Asparasone A Functions in Resistance of Sclerotia to Abiotic and Biotic Stress



dark bars- control

white bars- pks mutant

Insect antifeedant

Table 2. Amounts of *A. flavus* sclerotia consumed after 4 d in no-choice and choice assays by *C. freemani*

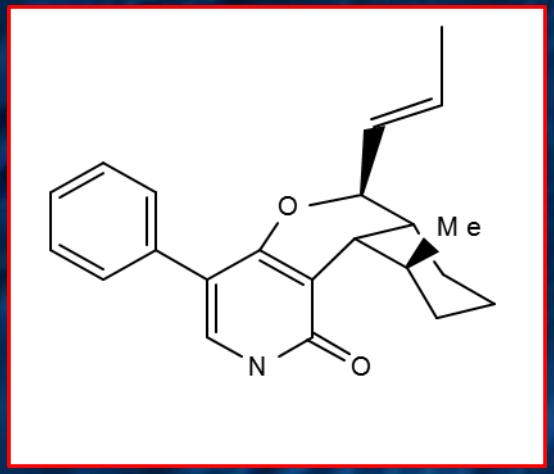
mg of sclerotia consumed	Control	Mutant
No-choice	6.3 ± 0.8 a	15.0 ± 0.9 b
Choice	12.3 ± 0.4 a	21.9 ± 0.7 b

Values are means ± standard errors for at least 7 replicates. Values on rows followed by different letters are significantly different by analysis of variance.

Cluster 23

Leporins

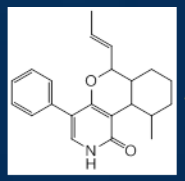
TePaske et al., Tetrahedron Lett, 1991



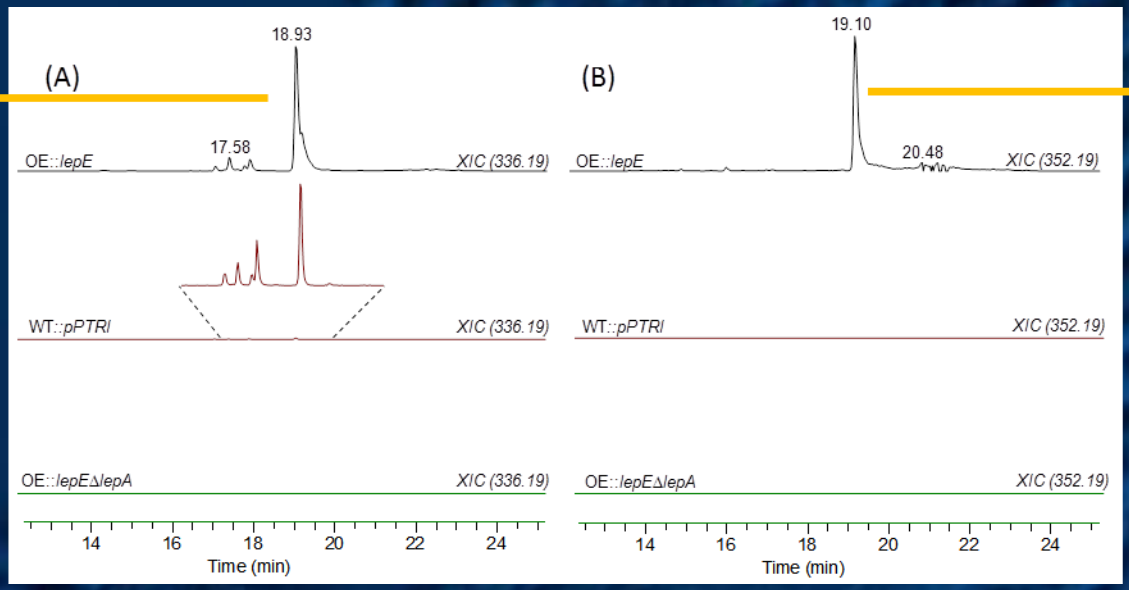
PKS-NRPS derived

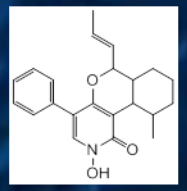
Cary et al., FG&B 2015

Leporins Constitute a Family of 2-Pyridones That Can Form Dimers and Trimers



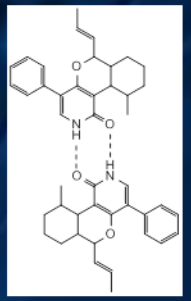
leporin C



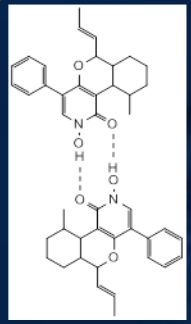


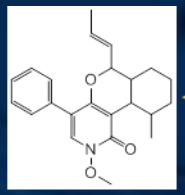
leporin B

leporin C dimer

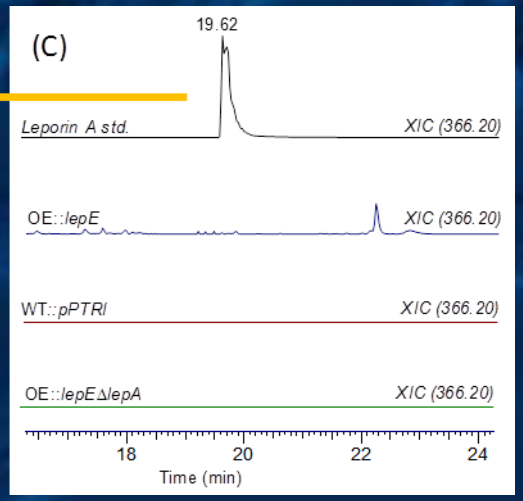


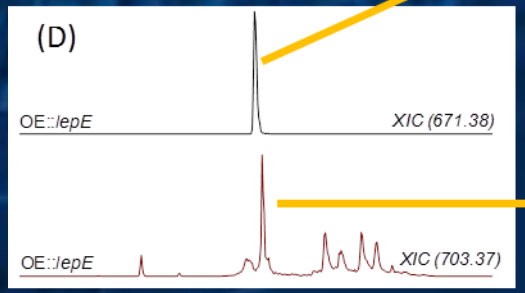
leporin B dimer



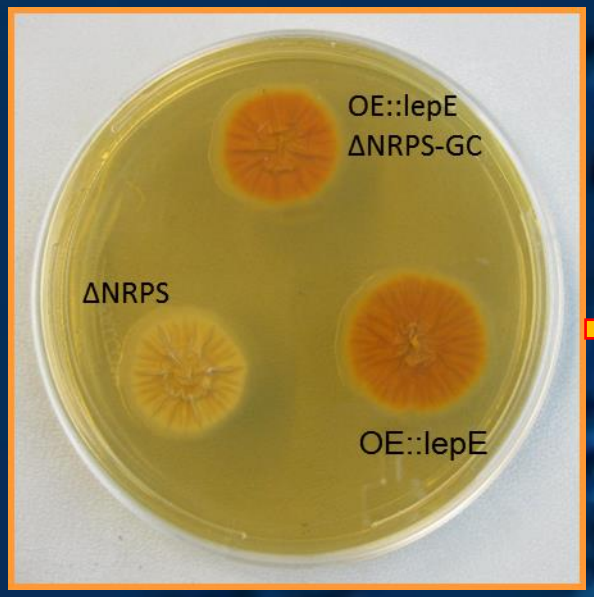


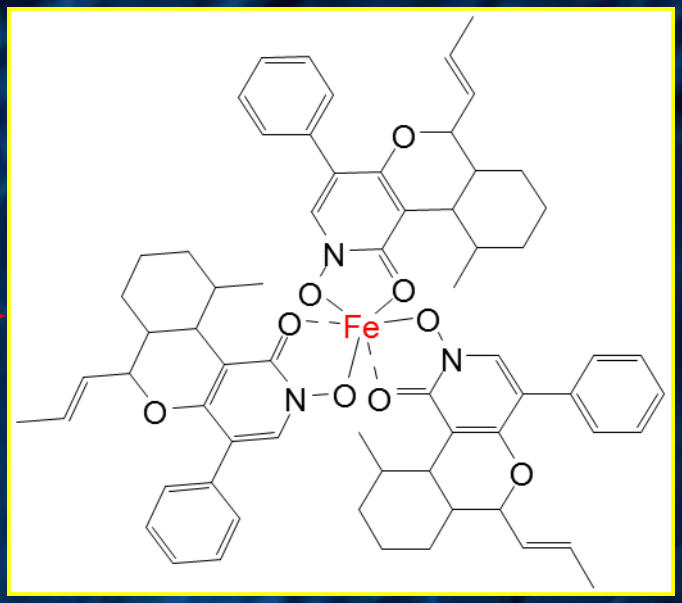
leporin A





Leporin B trimer- Fe Complex





- Responsible for orange-red pigmentation
- 1st description of a 2-pyridone forming an iron-chelate
- Possible role of leporin B in iron homeostasis and/or response to oxidative stress due to excess free iron?

Summary and Future Directions

- **Both AflR and AflJ have been identified as aflatoxin pathway-specific regulatory proteins required for aflatoxin production.**
- **We have identified a number of globally-acting regulatory genes that control both fungal secondary metabolism and development in *A. flavus* (nsdC, veA, velB, hbx1).**
  - **Perform RNA-Seq analysis of the *A. flavus*-corn interaction to identify additional regulatory genes and gene networks involved in *A. flavus* virulence/toxin production and corn resistance.**
  - **Better elucidate the mechanisms by which the NsdC and Hbx1 globally-acting transcription factors regulate *A. flavus* development and secondary metabolism by identifying interacting genes and proteins using ChIP-Seq and TAP-Tag/Y2H protein interaction methods, respectively.**
  - **Use some of these transcription factor genes in the development of RNAi-based binary vectors for host-induced gene silencing of *A. flavus* genes to reduce fungal growth and aflatoxin contamination in crops.**
- **We have identified the aflatoxin gene cluster as well as a number of other previously uncharacterized clusters and their metabolites (asparasone, leporins, aspergillic acid, etc.)**
  - **Continue to identify metabolites produced by uncharacterized *A. flavus* secondary metabolic gene clusters and define their biological activities.**

USDA, SRRC

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HAPPY MARDI GRAS

How will Studying Regulation of Secondary Metabolism and Development in *A. flavus* Aid in Mitigation of Aflatoxin Contamination?

- Gaining knowledge of how different environmental and nutritional conditions modulate expression of key regulatory genes and signaling pathways controlling *A. flavus* secondary metabolism and development.

- Information gained from RNA-Seq studies of the *A. flavus*-crop interaction will be used identify gene networks critical for virulence and toxin production as well as identifying crop genes/proteins involved in resistance to *A. flavus* infection and aflatoxin contamination.

- Fungal genes identified as key global regulators required for *A. flavus* growth, toxin production and virulence can be used as targets for inactivation using RNAi-based host-induced gene silencing intervention strategies.