Genetic and Genomic Studies of the Velvet Regulated Genes Associated with Spore Formation in Aspergillus

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Abstract
The filamentous fungus Aspergillus nidulans is an excellent model organism used in genetic research and in studying the gene regulatory mechanisms of fungal development. This allows researchers to make educated guesses about the research they may perform in many other filamentous fungi, and indeed humans. A. nidulans, like many others in the Aspergillus species, mostly reproduces asexually through the process of conidiation. In conidiation A. nidulans produces conidia: asexual spores that contain all the genetic material necessary to create a new fungal colony. The vosA gene (viability of spores) of A. nidulans is crucial to the development and viability of conidia. To understand the genetic mechanisms of conidiation, we performed RNA sequencing and Northern blot analyses of conidia from wild type (WT) and vosA deletion (ΔvosA) strains. These data indicated that the ANST09 (VadB) and AN8643 (VadA) genes had differential levels of expression. Both of these genes' predicted products show potential as transcription factors and could play a crucial role in controlling conidiation in A. nidulans. Using DJ-PCR and fungal transformation techniques we created mutant strains of A. nidulans without vadA and with the vadA gene under an inducible strong promoter causing an overexpression of the gene. By evaluating RNA expression and phenotype of these mutants we will be able to characterize the function of VadB and VadA and be able to more fully understand Aspergillus nidulans, filamentous fungi, and eukaryotic cells as a whole.

INTRODUCTION & METHODS

Life cycle of conidia: Asexual Spore

Aspergillus nidulans can reproduce asexually through the process called conidiation. This process serves as an excellent model system for understanding the mechanisms controlling fungal development. From the foot cell grows the stalk then from the stalk grows the vesicle. After growth, these structures fuse to become a single unit. From each vesicle is produced around 120 metulae. These metulae then produce around 120 phialides. These phialides are very similar to the metulae, excepting their ability to produce a chain of conidia. At the end of the chain are the oldest and most mature conidia, while closest to the phialides lay the immature conidia.

RNA-Sequencing methods

RNA-sequencing is a technology that uses the capabilities of Next Generation Sequencing (NGS) to reveal a snapshot of RNA population and quantity from a genome at a given moment in time. This sequencing of the RNA transcripts in a cell provides the ability to look at alternative gene spliced transcripts, post-transcriptional modifications, mutations and changes in gene expression at the genome level.

Model for regulation of conidiation in Aspergillus nidulans

The velvet regulators such as VelB (VELBetB) and VosA (Viability Of Spores) play major roles in sporegenesis, or the creation of spores such as conidia. These two regulators, VelB and VosA, are transcription factors, governing the expression of thousands of other genes within the developing cells.

The velvet regulators having conserved velvet domain

VelB

VosA

Phenotypes of velB and vosA deletion mutants

WT

ΔvelB

ΔvosA

RNA Sequencing data

VosA and WT gene regulation

Up-regulated by ΔvosA

Down-regulated by ΔvosA

Top genes analyzed

15

7

32% Up regulated

68% Down regulated

GO term
Function
Meaning
Genes

Molecular function
Many predicted functions, includes: D-alanyl carrier activity, morphogen activity, receptor regulator activity, and channel regulator activity

AN10045, AN11045, AN11337, AN12192, AN2042, AN6862, AN8175, AN8643, AN9299, AN10040, AN0723, AN10039, AN3042, AN6862

Transporter activity
Alloster movement of macromolecules, small molecules, and ions across a cell membrane

AN10826, AN3345

Hydrolase activity
Catalysis of certain bonds, ex. C-O, C-N, C-C, phosphoric anhydride bonds

AN10826, AN2395

Transferase activity
Catalysis of the transfer of a group like a methyl group, glycoly group, acetyl group, phosphorus-containing from one compound to another compound

AN7914, ccE

Peptidase activity
Catalysis of the hydrolysis of a peptide bond. A peptide bond is a covalent bond formed when the carbon atom from the carbonyl group of one amino acid shares electrons with the nitrogen atom from the amino group of a second amino acid.

AN296

Oxidoreductase activity
Catalysis of a redox reaction, a reversible chemical reaction where the oxidation state of an atom or atom within a molecule is changed. One substrate acts as a hydrogen or electron donor and becomes oxidized, while the other acts as hydrogen or electron acceptor and becomes reduced.

AN7914

No function found

Cannot be mapped to a GO term

cell, sunA

DNA gene cloning for mut strains

Conclusions
• Expression of VosA in A. nidulans is dependent on VosA and VelB during spore formation.
• Overexpression and complementation strains will be created using specific vector systems
• VadA is conserved across many Aspergillus species
• Twenty two genes of further interest have been selected from AN8643
• Fifteen genes by ΔvosA are up regulated and seven genes are down regulated by ΔvosA

Characterization of AN8643 (VadA)

Conservation across different Aspergillus species