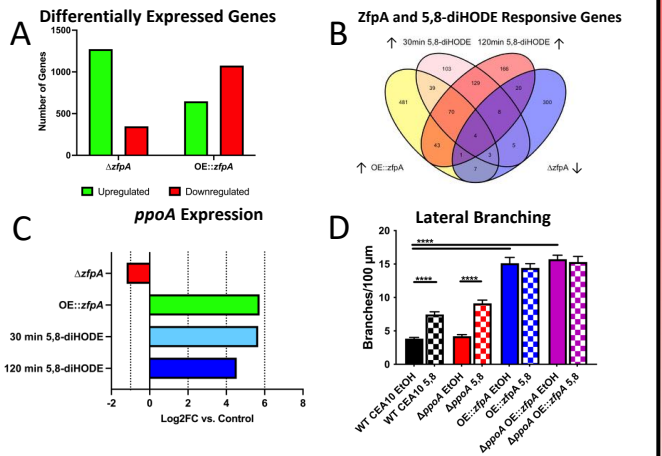
THE TRANSCRIPTION FACTOR ZFPA IS A GLOBAL REGULATOR ESSENTIAL FOR PROPER HYPHAL DEVELOPMENT IN *ASPERGILLUS FUMIGATUS*

Dante Calise[1,2], Jin Woo Bok[1], Nancy Keller[1,3]
[1] Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, USA
[2]Microbiology Doctoral Training Program, University of Wisconsin-Madison, Madison, WI, USA
[3]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

Purpose

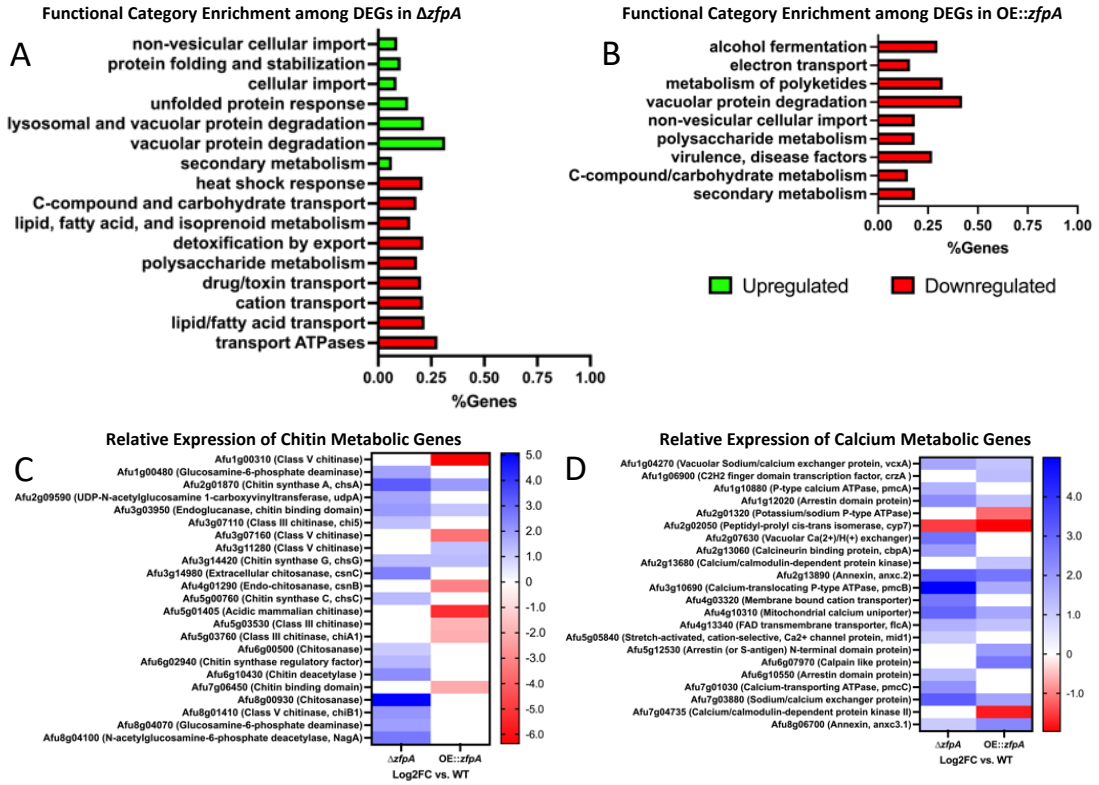
A key developmental process in *A. fumigatus* is the balance between polar growth and lateral branching, both of which are necessary for colony expansion within host substrates. Recently our lab found the transcription factor ZfpA to be essential in the hyperbranching response of *A. fumigatus* to the signaling oxylipin 5,8-diHODE which is produced by the fungal enzyme PpoA[1]. *zfpA* deletion generates a hypobranching phenotype whereas its overexpression yields a hyperbranching phenotype[1]. In order to begin to understand the regulatory role of ZfpA in hyphal development of *A. fumigatus*, RNA-seq analysis was conducted to identify potential regulatory pathways of ZfpA.

Results



ZfpA acts downstream of ppoA in the branching response to 5,8-diHODE. Total RNA was extracted from 16hr mycelial cultures of WT Af293, Δ*zfpA* and OE::*zfpA* for mRNA-sequencing. (A) Differentially expressed genes in each mutant were defined as |Log2FC| ≥ 1, p adj. ≤0.01. (B) Venn diagram of shared genes among those significantly upregulated (log2FC ≥ 1, p adj. ≤ 0.01) under ZfpA overexpression and 5,8-diHODe treatment (30 or 120min) and downregulated under *zfpA* deletion. (C) The expression of the *ppoA* gene was found to be highly upregulated in an OE::*zfpA* strain relative to WT as well as under 5,8-diHODE treatment while downregulated in a Δ*zfpA* mutant[1]. (D) Lateral branches per 100µm of WT CEA10, Δ*ppoA*, OE::*zfpA*, and a double mutant were measured at 20hrs growth under 5,8-diHODE or vehicle only treatment. **** p < 0.001

Results cont.

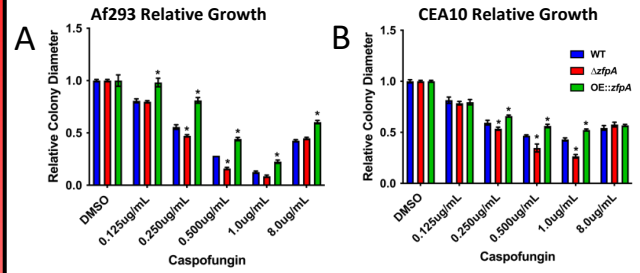


ZfpA is a transcriptional regulator of genes involved in diverse physiological processes including chitin and calcium metabolism. (A) Genes significantly up- and downregulated in the Δ*zfpA* mutant were assessed for functional category enrichment using FungiFun2[2] (FDR ≤0.05). (B) Genes significantly up- and downregulated in the OE::*zfpA* mutant were assessed for functional category enrichment using FungiFun2[2] (FDR ≤0.05). No functional categories were found to be enriched among genes upregulated in the OE::*zfpA* strain. (C) Manual analysis of DEGs in both ZfpA mutants revealed near opposite regulation of genes involved in chitin metabolism compared to the wild type. (D) Manual analysis of DEGs in the ZfpA mutants revealed disrupted regulation of genes involved in calcium metabolism in both mutants compared to the wild type.

1. Niu, M., Steffan, B. N., Fischer, G. J., Venkatesh, N., Raffa, N. L., Wettstein, M. A., Bok, J. W., Greco, C., Zhao, C., Berthier, E., Oliw, E., Beebe, D., Bromley, M., and Keller, N. P. "Fungal Oxylipins Direct Programmed Developmental Switches in Filamentous Fungi." *Nature Communications* 11, no. 1 (2020): 5158. doi:10.1038/s41467-020-18999-0

This work was funded by NIAID 1R01AI150669-01A1

Results cont.



Deletion of *zfpA* increases sensitivity to cell wall stressor caspofungin while its overexpression increases tolerance. (A) Radial growth of 1x10[4] WT Af293, Δ*zfpA*, or OE::*zfpA* conidia spotted in triplicate on glucose minimal media containing up to 8µg/mL caspofungin were measured after 4 days at 37˚C relative to growth on GMM without caspofungin. (B) Radial growth of 1x10[4] WT CEA10, Δ*zfpA*, or OE::*zfpA* conidia spotted in triplicate on glucose minimal media containing up to 8µg/mL caspofungin were measured after 4 days at 37˚C relative to growth on GMM without caspofungin. * denotes adj. p < 0.01

****See poster 81 by TJ Schoen et al. for complementary *in vivo* results****

Discussion & Conclusions

Our lab previously showed that the PpoA enzyme product 5,8-diHODE directs important physiological processes in hyphal development including the balance between apical growth and lateral branching as well as cell wall composition. The data presented here demonstrate that although 5,8-diHODE acts to induce the transcription of the *ppoA* gene via the oxylipin responsive ZfpA, this transcription factor acts downstream of oxylipin signaling in the hyperbranching response. Additionally, our data suggest ZfpA is a global regulator of many diverse processes related to but also independent of oxylipin signaling. Furthermore, our data suggest that ZfpA regulates many calcium and chitin metabolic genes, which are two processes known to be important in responding to cell wall stress such as the antifungal agent caspofungin. Indeed, susceptibility testing revealed ZfpA to be important in resistance to caspofungin both *in vitro* and *in vivo* (see poster 81). Given the findings presented here, we conclude that the transcription factor ZfpA plays a central role in the morphological development and cell wall metabolism of *A. fumigatus* hyphae.