Oxygen and Invasive Pulmonary Aspergillosis

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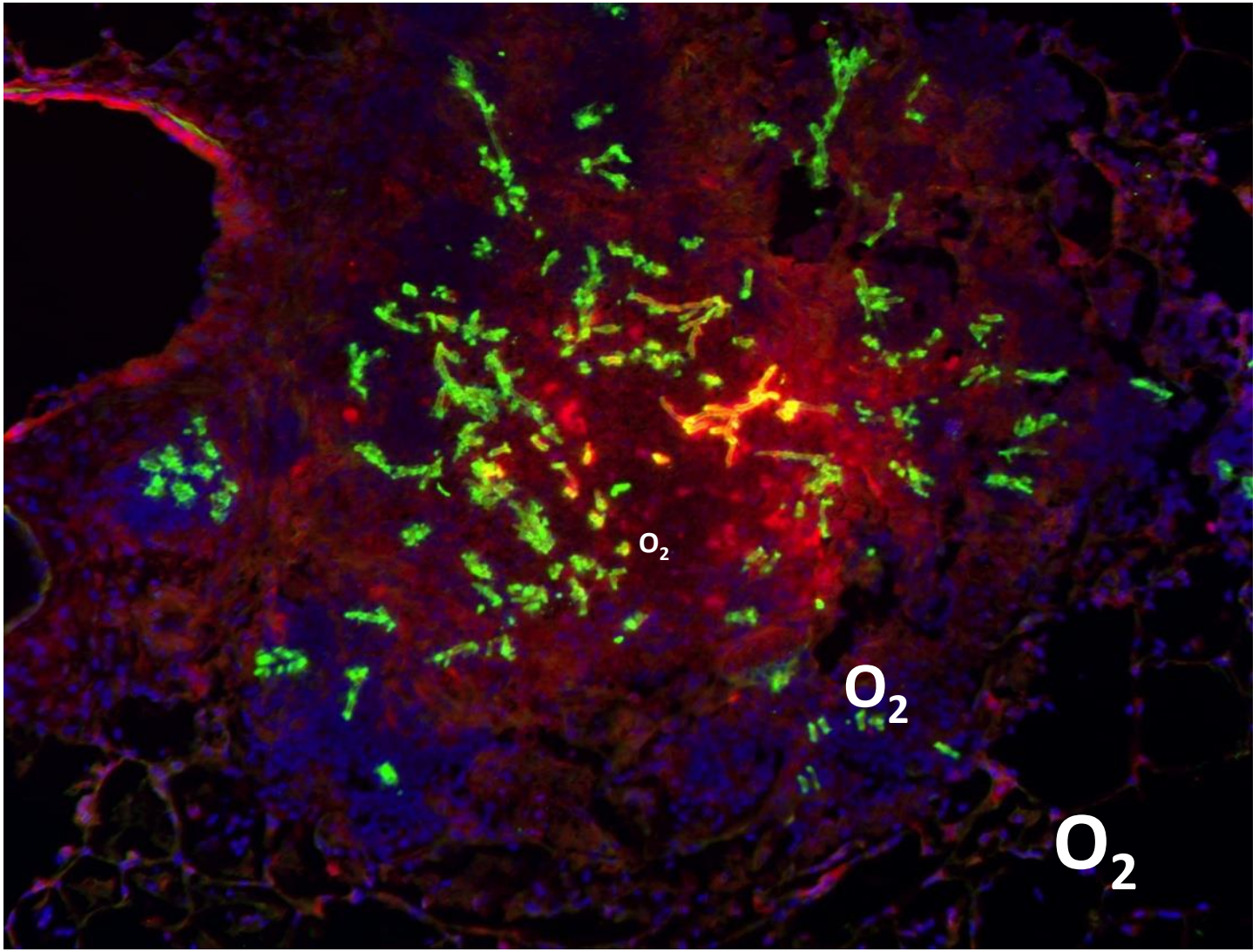
Geisel School of Medicine at Dartmouth

ICAAC 2014, Washington DC Sept. 8th 2014

Hypoxia at site of infection occurs during invasive pulmonary aspergillosis



Grahl et al. 2011 PLoS Pathogens

Oxygen requirement for organisms

- Electron acceptor in the generation of chemical energy
- Critical for biosynthesis of sterols, fatty acids, NAD, and porphyrin (heme)
- Many human fungal pathogens, including *A. fumigatus,* are generally considered obligate aerobes
- In healthy tissues in the human body, normal O_2 levels range 2.5 – 9%
- In the healthy human lungs, alveolar O_2 level is around 14%.
- O_2 levels of ≤5% are considered hypoxic (tumors and wounds)
  - ***Ultimately, hypoxia occurs when oxygen demand is not met by oxygen availability***
- Within the lung, the co-occurrence of microbial infection and hypoxia is often associated with poor clinical outcomes

Grahl et al. 2012 Euk. Cell.

Oxygen and Antifungal Drugs: Some Questions

- Are drugs metabolized by the host/fungus differently under oxygen stress?
  - At the infection site, but also what about systemically in cases of hypoxemia?

- Does hypoxia influence transport of drugs to site of infection/intracellularly to the pathogen?

- Is binding of drugs to target molecules altered in hypoxia?

- Can hypoxia promote drug resistance through enhancement of target expression? Efflux pumps? Mutation?

Perhaps Some Hints From Cancer Therapies?

Effect of Hypoxia	Result	Mechanism
Distance from Vasculature	Resistance	Compromised Drug Exposure
Acidification	Resistance	Decreased Drug Uptake
Genomic Instability	Resistance	Mutation in Drug Targets/Effectors
Lack of Oxidation	Resistance	Failure to Damage DNA
Apoptosis Inhibition	Resistance	Vary

Most of these have not been directly explored in the context of Fungal Infections

Current *in vitro antifungal drug screening points to a potential role for hypoxia in altering MICs/MFCs*

Journal of Antimicrobial Chemotherapy (2004) **53**, 743–749
DOI: 10.1093/jac/dkh153
Advance Access publication 24 March 2004

JAC

Effect of hypoxic conditions on *in vitro* susceptibility testing of amphotericin B, itraconazole and micafungin against *Aspergillus* and *Candida*

Peter A. Warn[1]*, A. Sharp[1], J. Guinea[1] and David W. Denning[1,2]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2008, p. 1873–1875 Vol. 52, No. 5
0066-4804/08/$08.00+0 doi:10.1128/AAC.01572-07

Susceptibility Testing of Anidulafungin and Voriconazole Alone and in Combination against Conidia and Hyphae of *Aspergillus* spp. under Hypoxic Conditions[▽]

Susanne Perkhofer,* Daniel Jost, Manfred P. Dierich, and Cornelia Lass-Flörl

Medical University of Innsbruck, Department of Hygiene, Microbiology and Social Medicine, Division of Hygiene and Medical Microbiology, Innsbruck, Austria

Dying Achilles at Achilleion, Corfu Greece, Ernst Herter 1884

“Beyond the Virulence Factor”

Working Hypothesis:

Defining infection microenvironment conditions can lead to improvements in diagnosis and treatment of IFIs

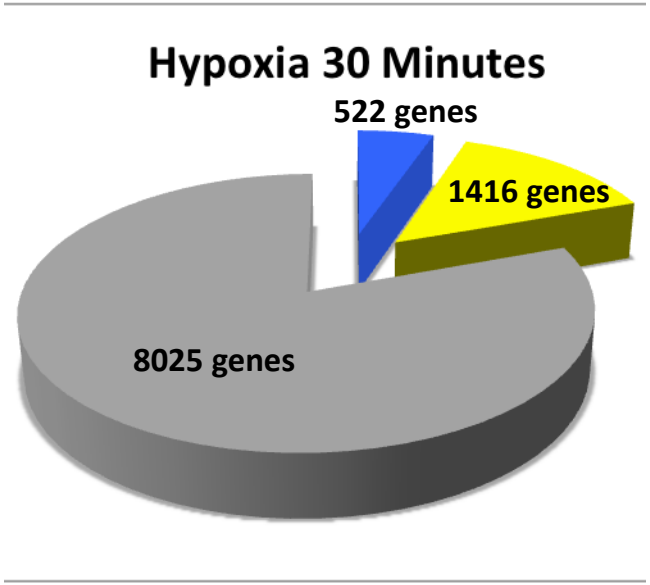
New Drugs that target Fungal Specific Pathways Required for in vivo growth

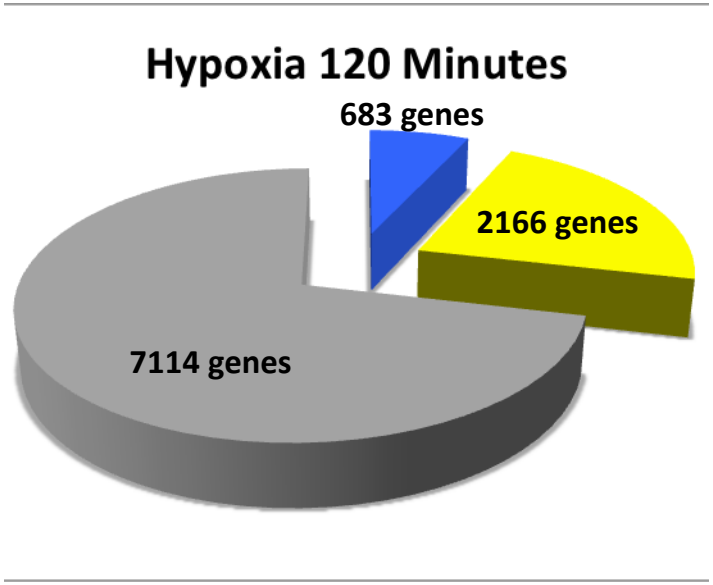
New interventions that manipulate the infection microenvironmen: alter fungal metabolism and improve efficacy of existing drugs

Identification of immune factors that can be targeted to augment immunity or reduce immunopathogenesis in the context of immune suppression

Hypoxia induces significant changes in the transcriptome of *Aspergillus fumigatus*

Genes with < 4 fold change
Genes with Increased mRNA Abundance ≥ 4 fold
Genes with decreased mRNA Abundance ≥ 4 fold



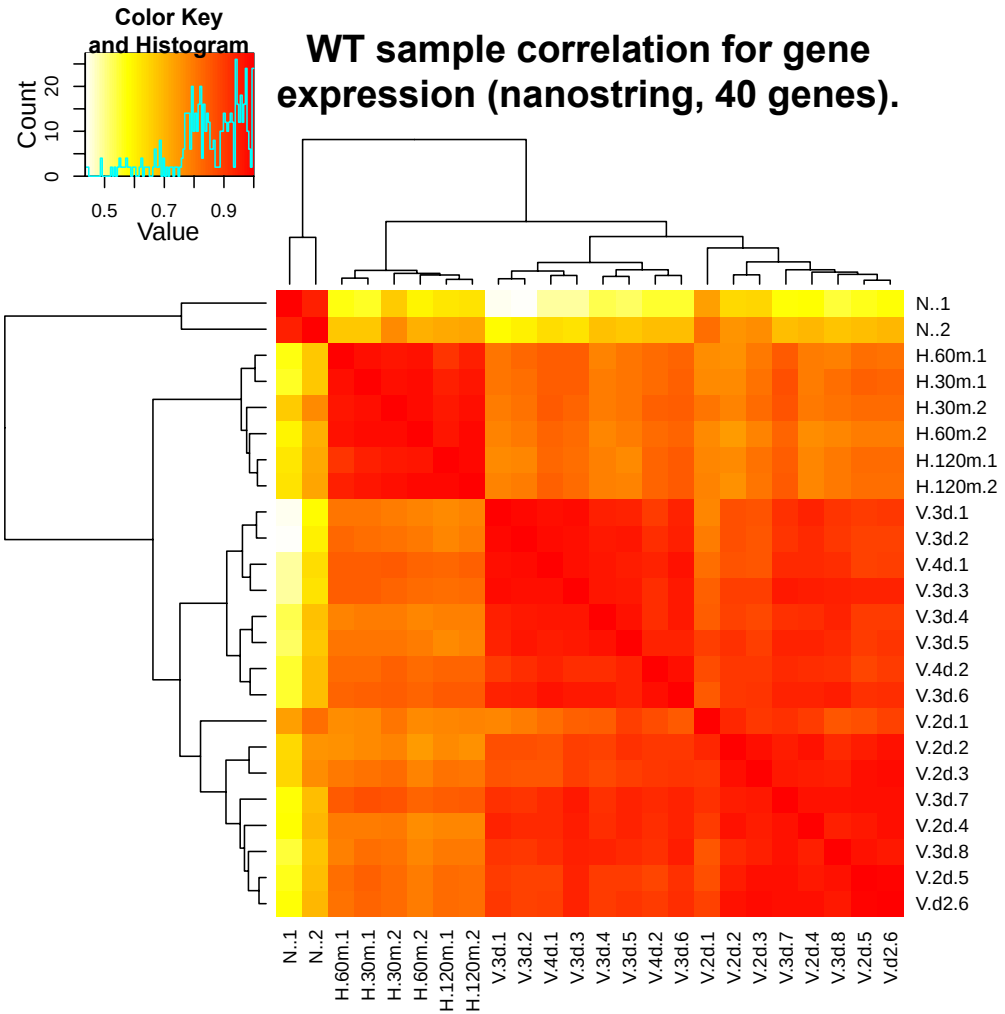


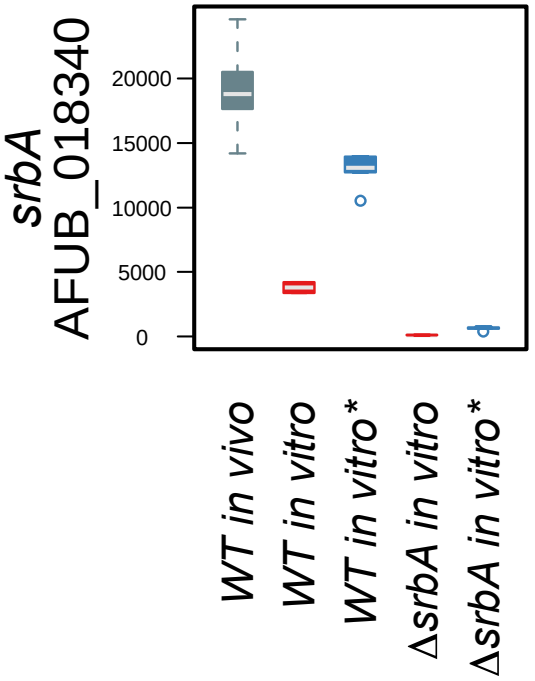
Hypoxia alters transcript levels for ~30% of the genome in 30 minutes!

Collaborators: Dr. William Nierman, Dr. Liliana Losada et al. JCVI

Losada et al. 2014 *Mycopathologia*

In vivo transcript levels in host lung tissue correlate with *in vitro* hypoxia conditions





Nanostring nCounter Gene Expression Data

Dr. Bridget Barker and Dr. Chuck Carey, in collaboration with Dr. Aaron Mitchell and Dr. Wenjie Xu Carnegie Mellon

Defining the Aspergillus fumigatus hypoxia transcriptome and Proteome

1. Shake Flask Cultures 2. Chemostat Cultures
   A. Microarray and B. RNA-SEQ C. Proteomics

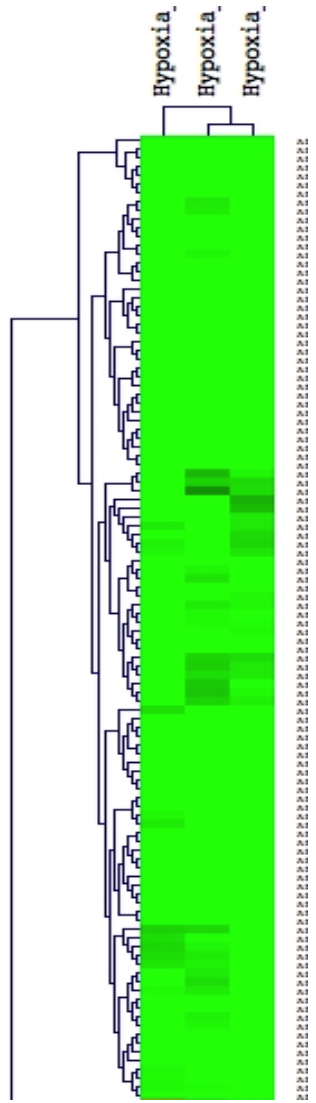
<u>Hypoxia Increases Transcripts/Proteins For</u>:

1. **Ergosterol Biosynthesis**
2. Amino Acid Metabolism
3. Respiration – early time points !?
4. **Metal Uptake**
5. **Various Transporters**
6. **Cell Wall biosynthesis**
7. Fermentation Pathways
8. **Transcriptional Regulators**

~15% of Genome affected

<u>Hypoxia Reduces Transcripts/Proteins For</u>:

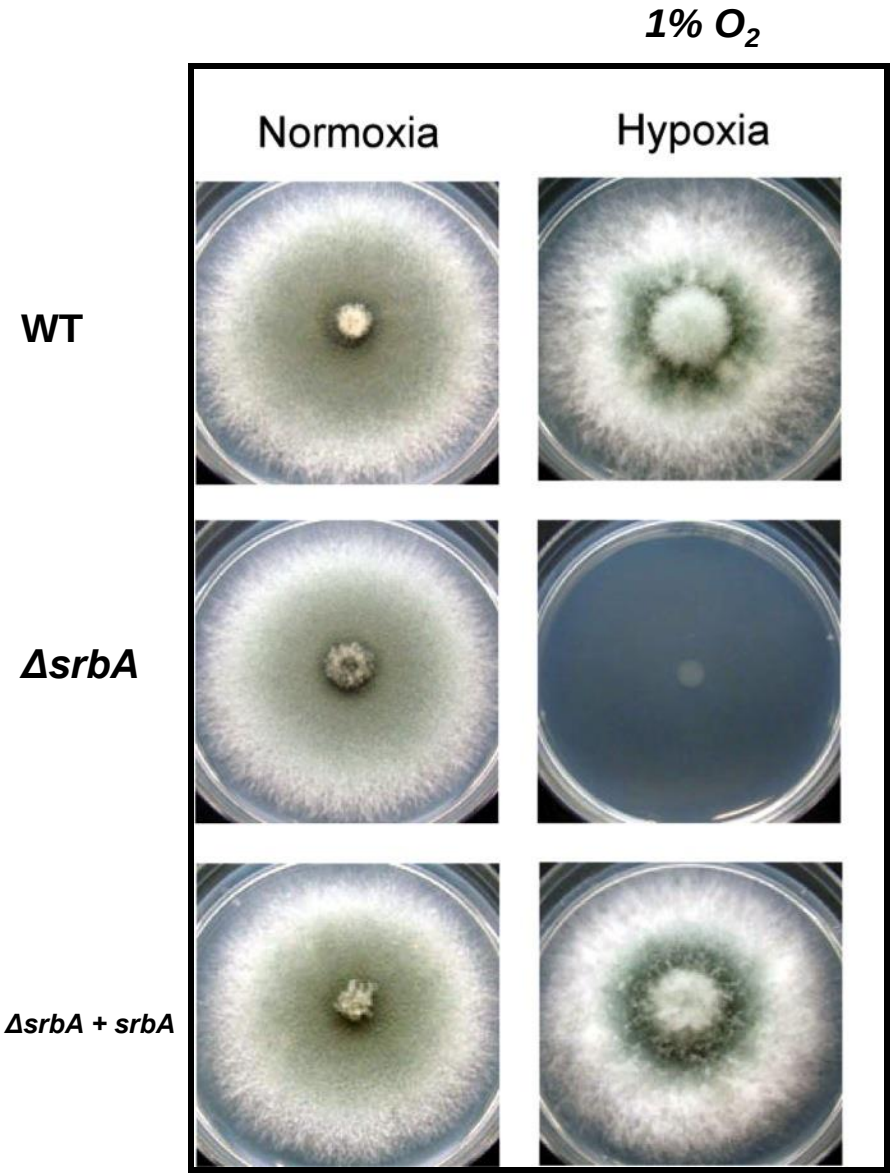
1. Ribosome Proteins
2. Nucleic Acid biosynthesis
3. Cell Wall biosynthesis
4. Transcriptional Regulators
5. Various Transporters

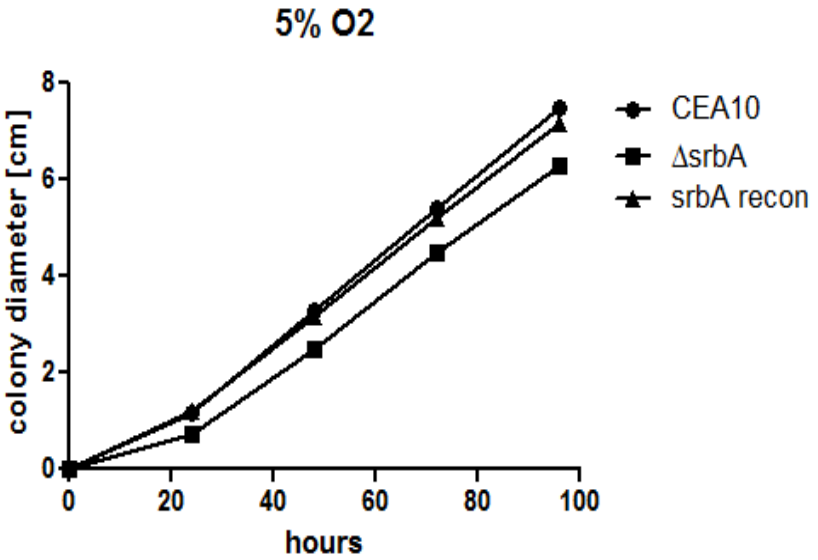


Bridget Barker, Post-Doc

Barker et al. 2012 *BMC Genomics, Losada et al. 2014 Mycopathologia*

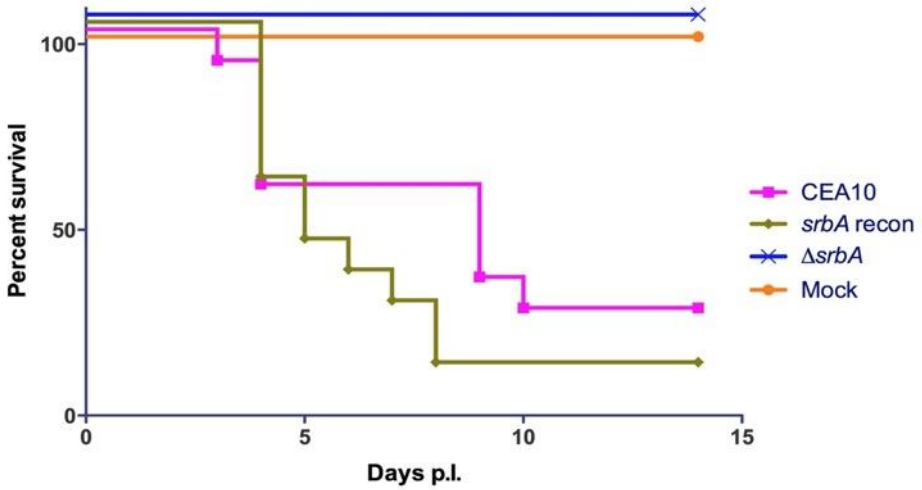
A. fumigatus SrbA is required for growth in hypoxia





Willger et al. 2008, *PLoS Pathogens*

The transcription factor SrbA is Required for *A. fumigatus* Pathogenicity



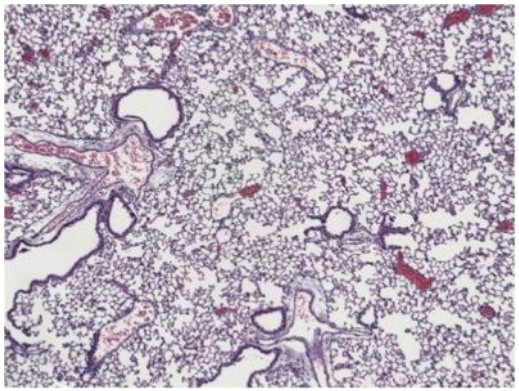
Log Rank P = 0.0002

*<u>Severe virulence attenuation</u> in 3 different immunological settings:

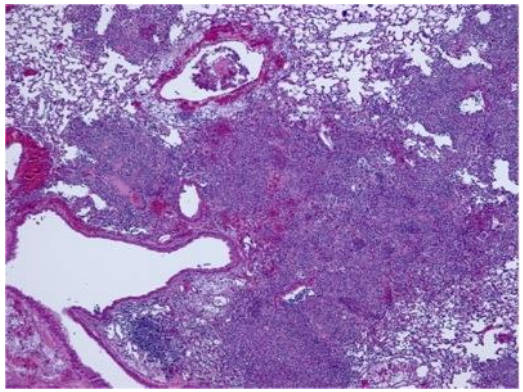
(1) Leukopenic

(2) Corticosteroid

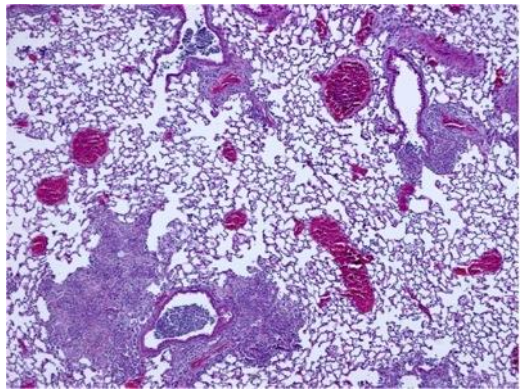
(3) X-CGD



PBS Mock



Wild-Type



Δ*srbA*

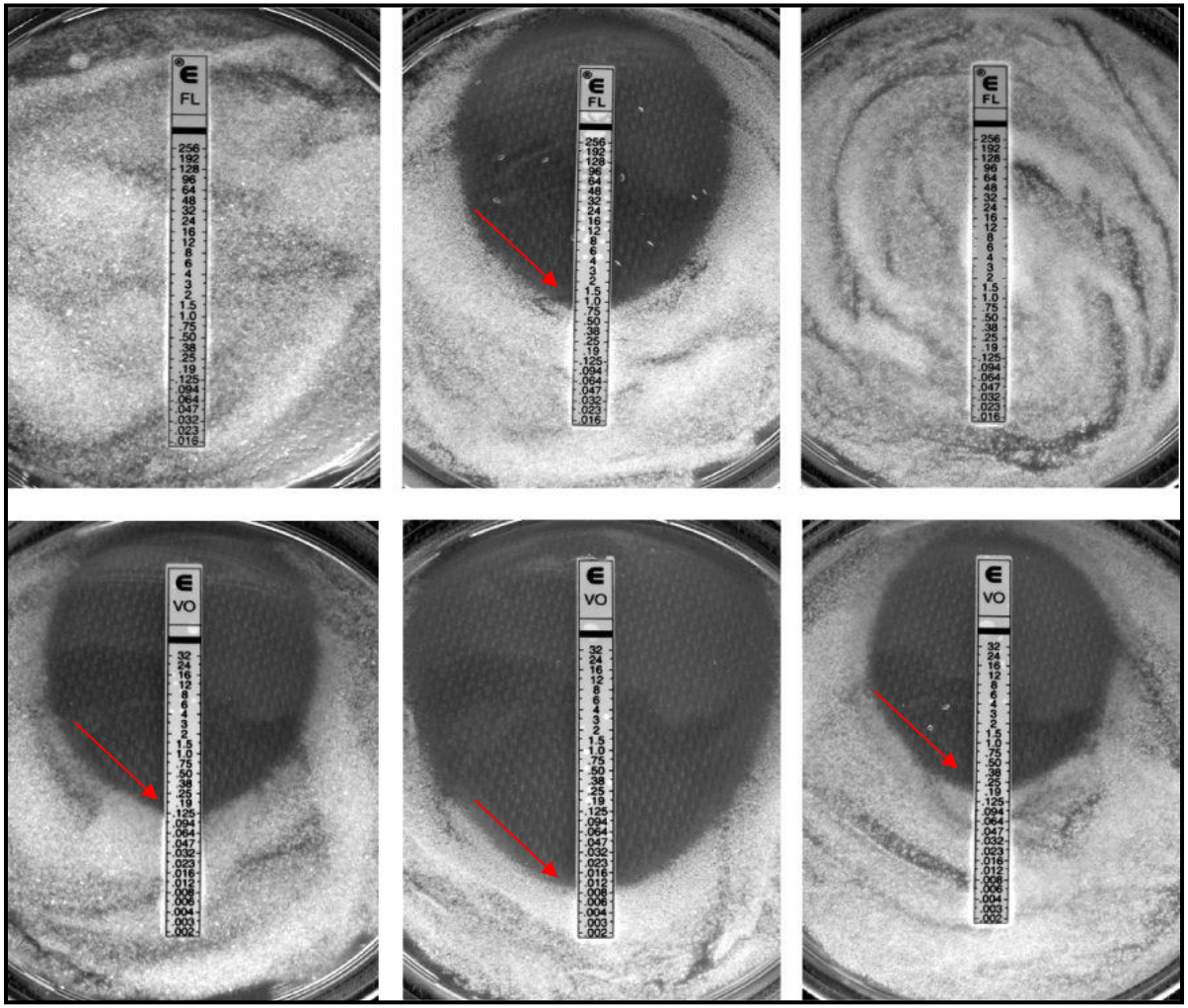
Willger et al. 2008, *PLoS Pathogens*

SrbA is Required for Fluconazole and Triazole Drug Responses

CEA10 | ***ΔsrbA*** | ***ΔsrbA + srbA***

Fluconazole

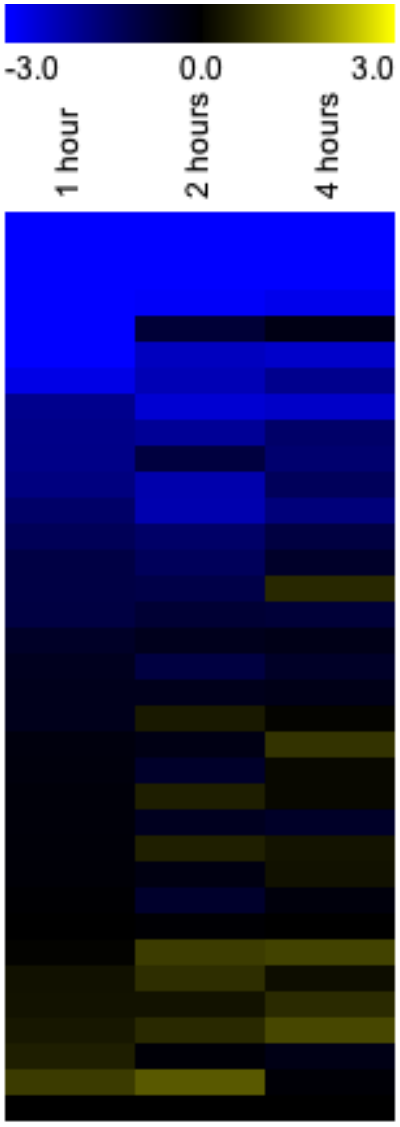
Voriconazole



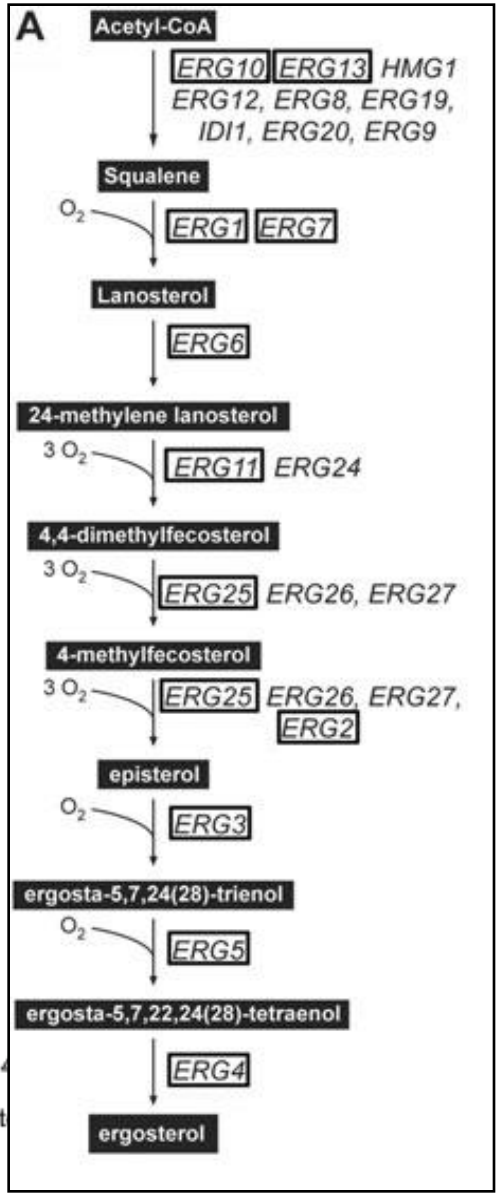
**Azoles Inhibit Sterol Biosynthesis

Willger et al. 2008, *PLoS Pathogens*

Ergosterol Biosynthesis Related mRNA abundance is regulated by SrbA

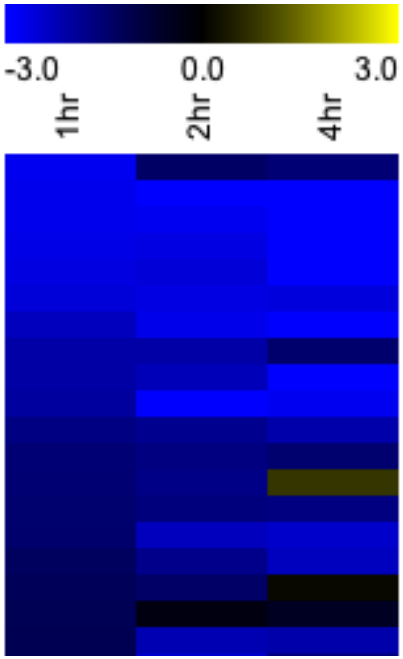


Afu1g03150 , erg24A, c-14 sterol reductase
Afu2g00320 , erg3B, sterol delta 5,6-desaturase
Afu8g02440 , erg25A, C-4 methyl sterol oxidase, (1.-.-.-)
Afu3g00810 , hyd1, cholestenol delta-isomerase, (5.3.3.5)
Afu8g07210 , erg13A, hydroxymethylglutaryl-CoA synthase, (2.3.3.10)
Afu6g05140 , erg3A, sterol delta 5,6-desaturase, (1.3.3.-)
Afu7g03740 , erg11B, 14-alpha sterol demethylase Cyp51B, (1.14.13.-)
Afu1g05720 , erg24B, c-14 sterol reductase
Afu5g07780 , erg1, squalene monooxygenase
Afu4g04820 , erg25B, C-4 methyl sterol oxidase, (1.-.-.-)
Afu1g03950 , erg5, cytochrome P450 sterol C-22 desaturase, (1.14.14.-)
Afu4g03630 , erg6, sterol 24-c-methyltransferase, (2.1.1.41)
Afu5g02450 , erg20, farnesyl-pyrophosphate synthetase
Afu4g11500 , erg27, 3-ketosteroid reductase
Afu7g01220 , erg9, farnesyl-diphosphate farnesyltransferase, (2.5.1.21)
Afu4g07130 , mvd1, diphosphomevalonate decarboxylase, (4.1.1.33)
Afu5g10680 , erg8, phosphomevalonate kinase
Afu5g04080 , erg7A, oxidosqualene:lanosterol cyclase
Afu1g07140 , erg4B, c-24(28) sterol reductase
Afu4g12040 , erg7B, lanosterol synthase
Afu3g10660 , erg13B, hydroxymethylglutaryl-CoA synthase, (2.3.3.10)
Afu8g04000 , erg10B, acetyl-CoA acetyltransferase, (2.3.1.9)
Afu1g11230 , hmg2, HMG-CoA reductase
Afu2g15030 , erg26, C-3 sterol dehydrogenase/C-4 decarboxylase
Afu4g14770 , erg7C, squalene-hopene-cyclase, (5.4.99.7)
Afu6g11160 , idi1, isopentenyl-diphosphate delta-isomerase, (5.3.3.2)
Afu5g14350 , erg4A, c-24(28) sterol reductase
Afu4g07780 , erg12, mevalonate kinase, (2.7.1.36)
Afu6g14200 , erg10A, acetyl-CoA-acetyltransferase, (2.3.1.9)
Afu2g03700 , hmg1, HMG-CoA reductase
Afu8g01070 , erg3C, sterol desaturase
Afu4g09190 , smt1, S-adenosyl-methionine-sterol-C- methyltransferas, (2.1.1.
Afu1g04720 , erg2, C-8 sterol isomerase
Afu2g17400 , erg26, C-3 sterol dehydrogenase/C-4 decarboxylase family prot
Afu4g06890 , erg 11A* 14-alpha sterol demethylase Cyp51A, (1.14.13.-)



Blatzer, Barker et al.
PLoS Genetics 2011

SrbA Regulates Iron Uptake Associated Transcripts



Afu2g07680 , sidA, L-ornithine N5-oxygenase, (1.13.12.-)
Afu1g00510 , dld1, D-lactate dehydrogenase (cytochrome), (1.1.2.4)
Afu1g00500 , FMN dependent dehydrogenase, (1.-.-.-)
Afu4g03940 , fre7, ferric-chelate reductase, (1.16.1.7)
Afu6g04920 , AciA/Fdh, NAD-dependent formate dehydrogenase, (1.2.1.2)
Afu5g03790 , fet3, ferrooxidoreductase, (1.10.3.2)
Afu7g06060 , sit1, siderochrome-iron transporter
Afu3g00800 , oxidoreductase, 2OG-Fe(II) oxygenase family
Afu3g02270 , cat1, mycelial catalase, (1.11.1.6)
Afu3g14880 , oxidoreductase, 2OG-Fe(II) oxygenase family
Afu6g08440 , hem14, protoporphyrinogen oxidase, (1.3.3.4)
Afu3g03440 , mirD, MFS siderophore iron transporter
Afu8g02750 , cgrA, rRNA processing protein
Afu2g00750 , oxidoreductase, 2OG-Fe(II) oxygenase family
Afu6g10720 , alpha-ketoglutarate-dependent taurine dioxygenase, (1.14.11.17)
Afu1g07480 , hem13, coproporphyrinogen III oxidase, (1.3.3.3)
Afu6g03050 , oleate delta-12 desaturase, (1.14.99.-)
Afu8g01670 , cat2, bifunctional catalase-peroxidase, (1.11.1.6)
Afu5g03800 , ftrA, high-affinity iron transporter

****Erg11/Cyp51A and Erg5, SrbA Targets, are HEME Dependent**

****Erg25 and Erg3, SrbA Targets, are Fe^{2+} Dependent**

Blatzer, Barker et al. *PLoS Genetics* 2011

What are the direct targets of SrbA in Hypoxia?

Why is this question important?

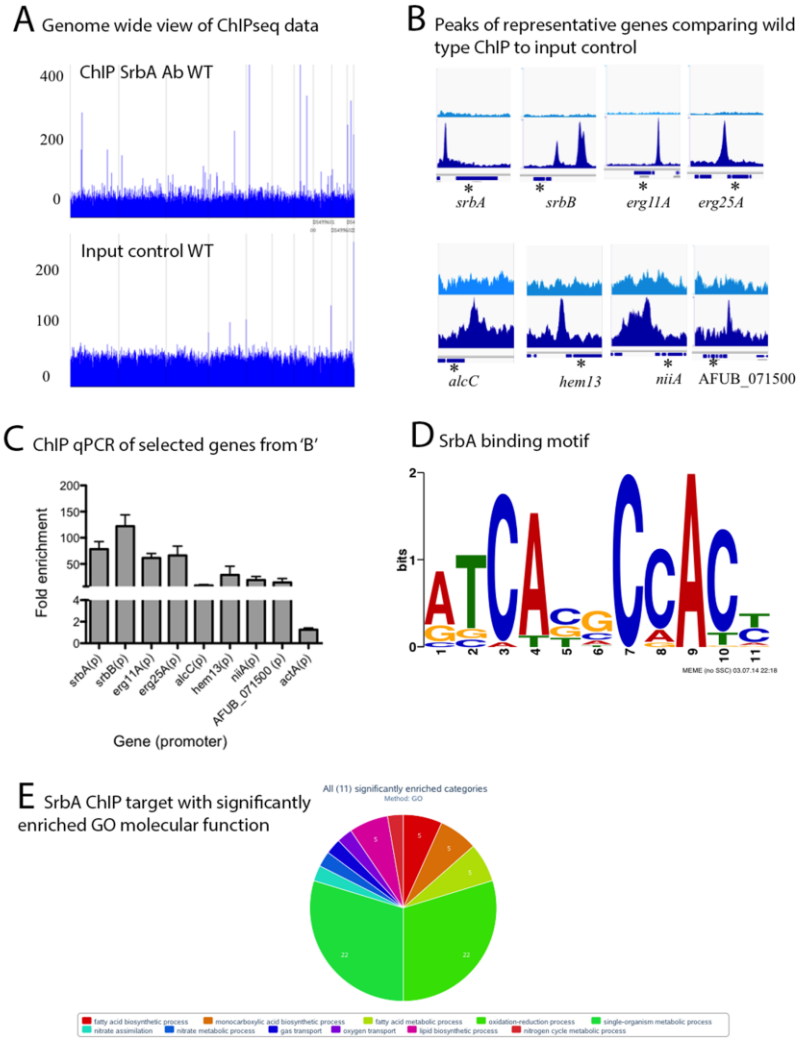
(1) Identify Mechanism which could lead to new drug targets

(2) Better understanding of how A. fumigatus adapts to hypoxia – what is essential?

(3) Potential to identify genes with novel biological functions.

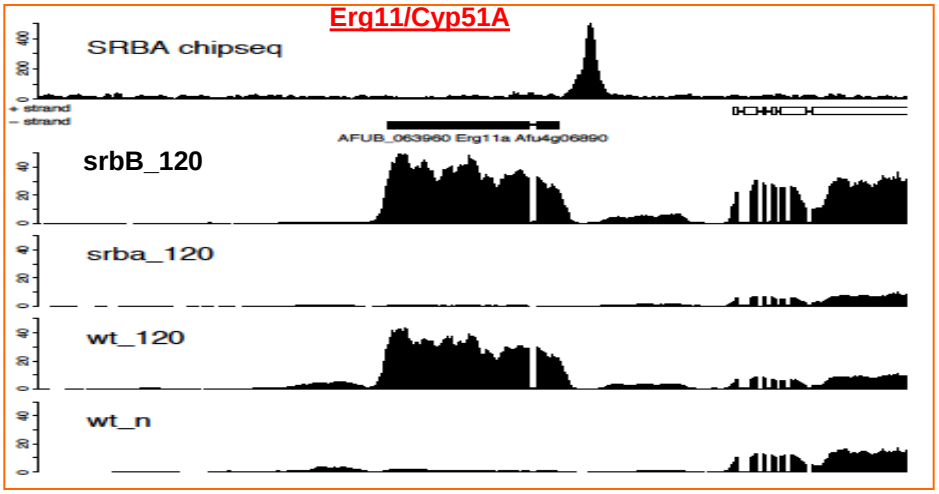
Approach:

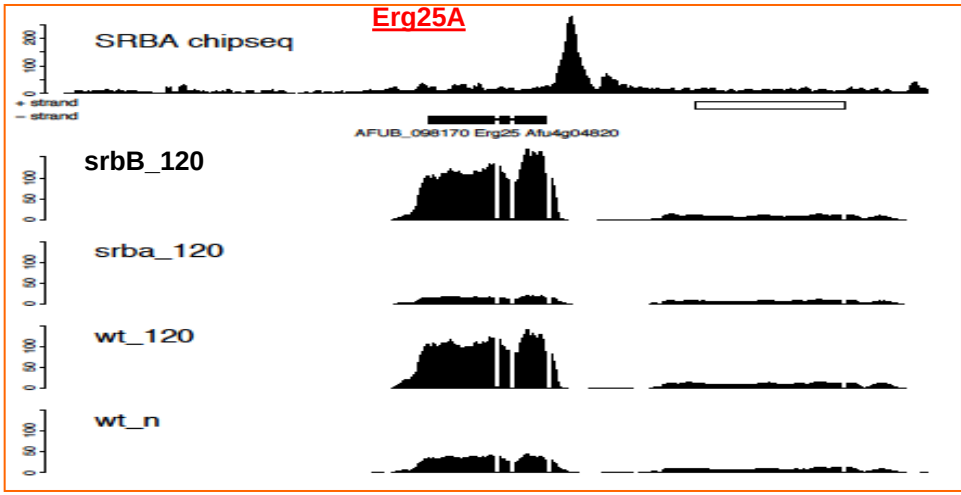
***ChIP-Seq at 4 hours hypoxia – 110 peaks, 97 genes, FDR 0.05*

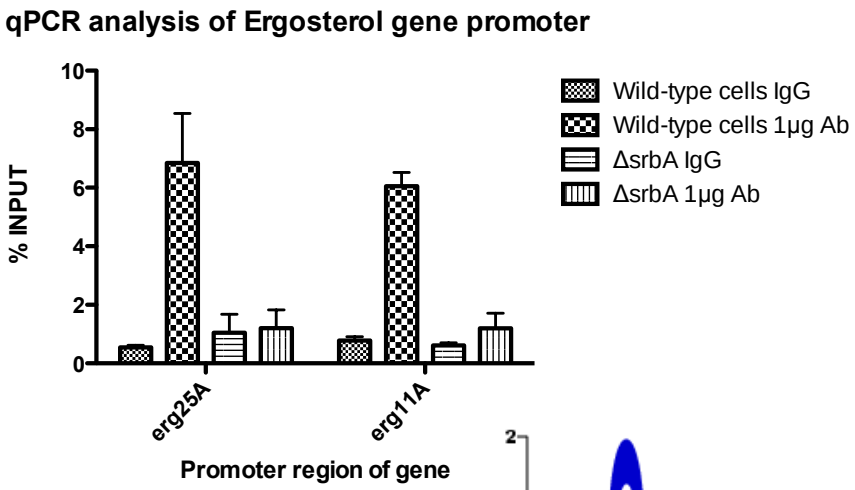


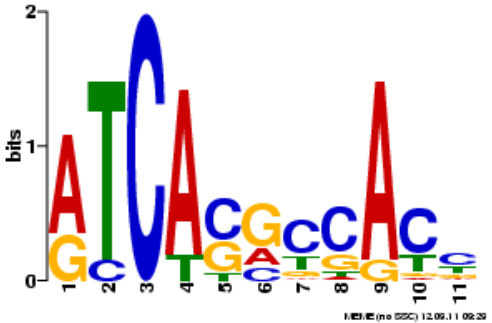
Dr. Chuck Carey, MSU Bioinformatics, In Revision
Dr. Bridget Barker, former Cramer Lab Post-Doc, now at TGen

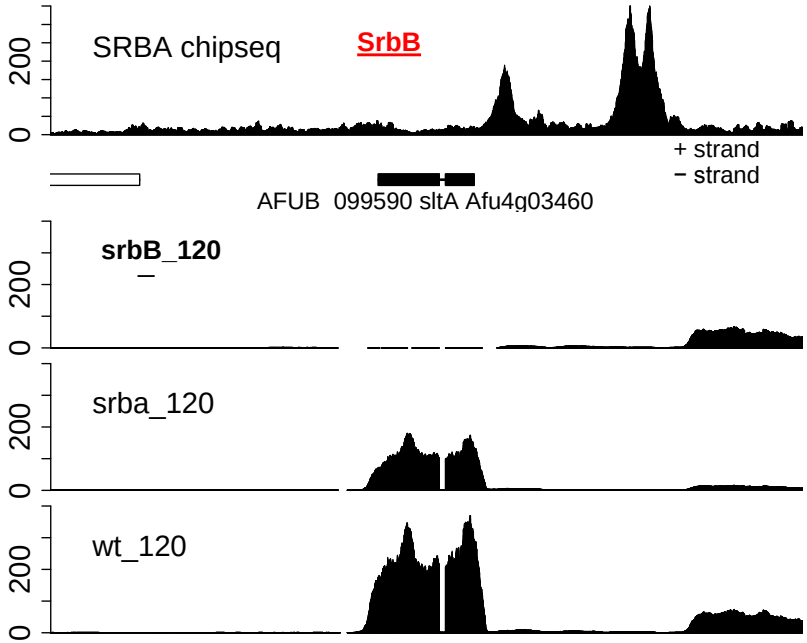
Erg gene transcription regulation by SrbA is DIRECT





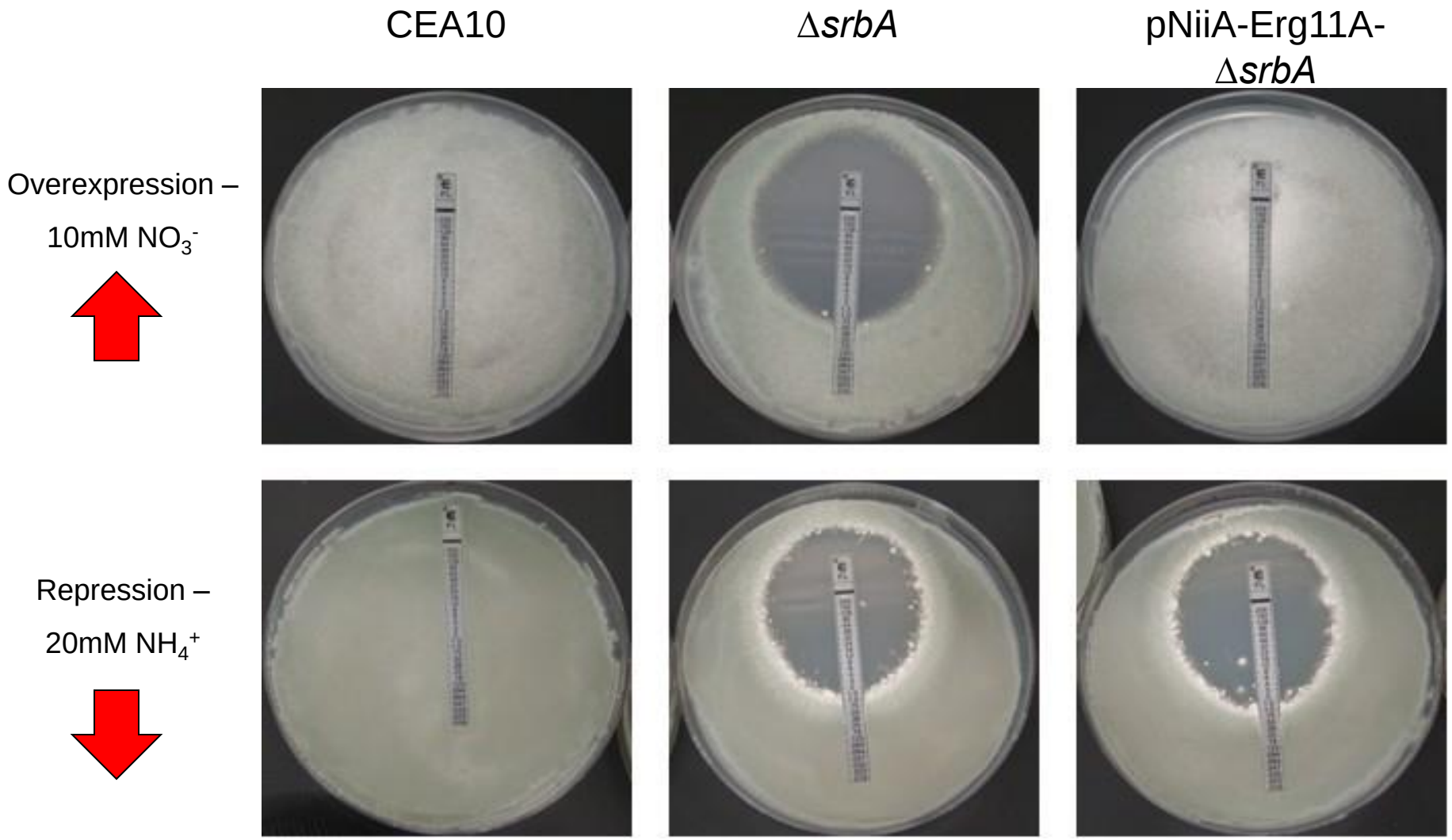






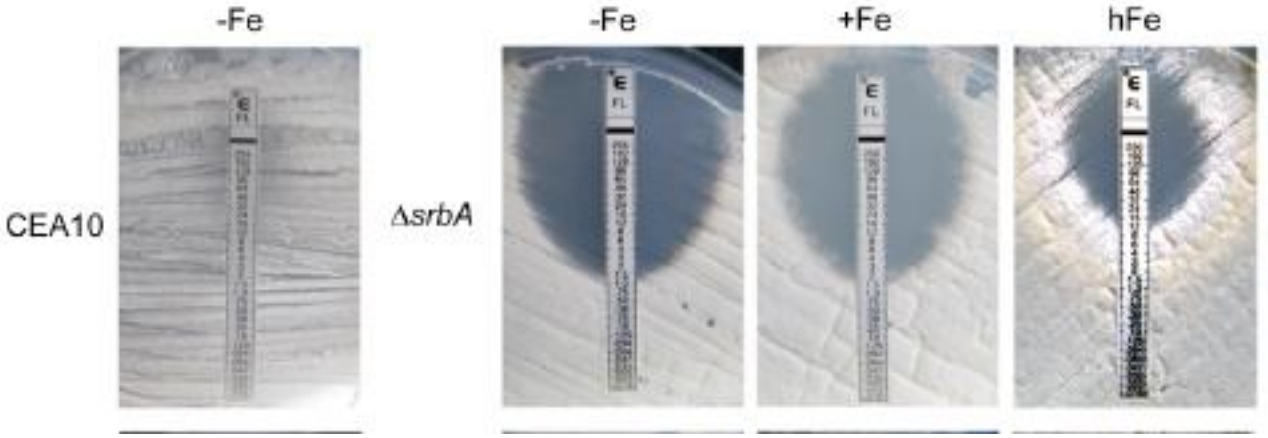
Barker et al. In Preparation

Erg11/Cyp51AA Conditional Expression in Δ*srbA* background Rescues Fluconazole Resistance



Blosser, S.J. *and Cramer R.A. 2012 Antimicrobial Agents and Chemotherapy*

SrbA Cells are Iron depleted and affects triazole drug sensitivity



Fluconazole E-Tests

****The infection microenvironment is complex!**

Collaborator: Dr. Hubertus Haas, Innsbruck, Austria

Blatzer, Barker et al. 2011 PLoS Genetics

Transcription Factor Alteration in fungi: an understudied resistance mechanism with *Aspergillus*?

OPEN ACCESS Freely available online

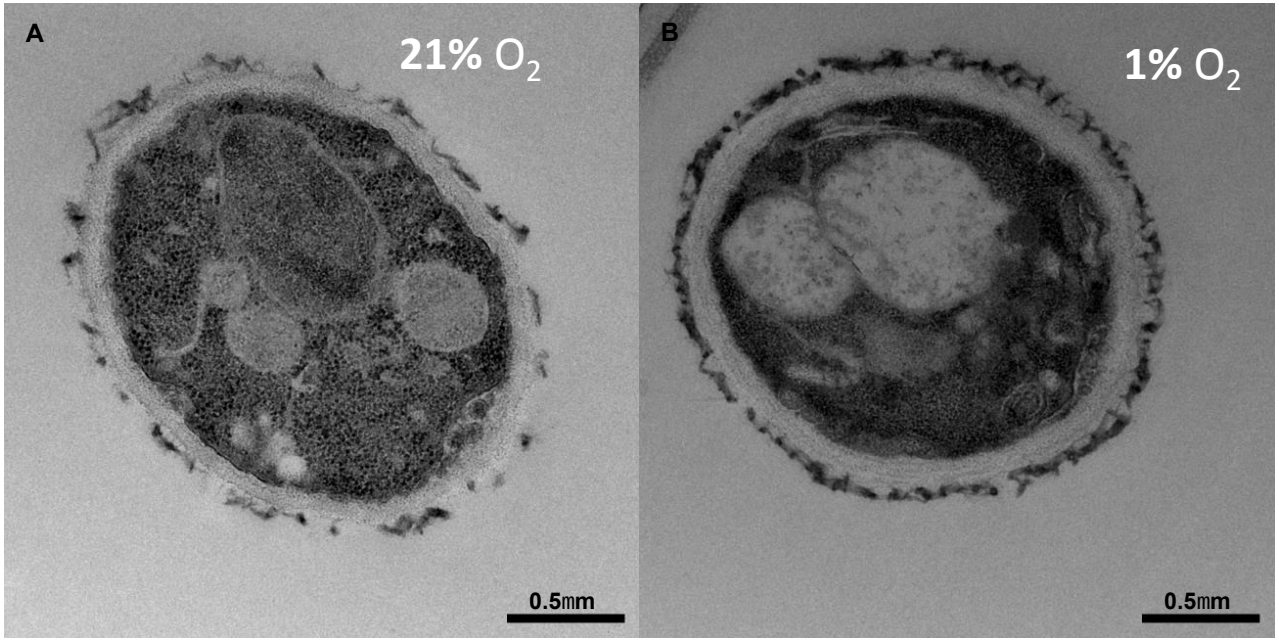
PLOS ONE

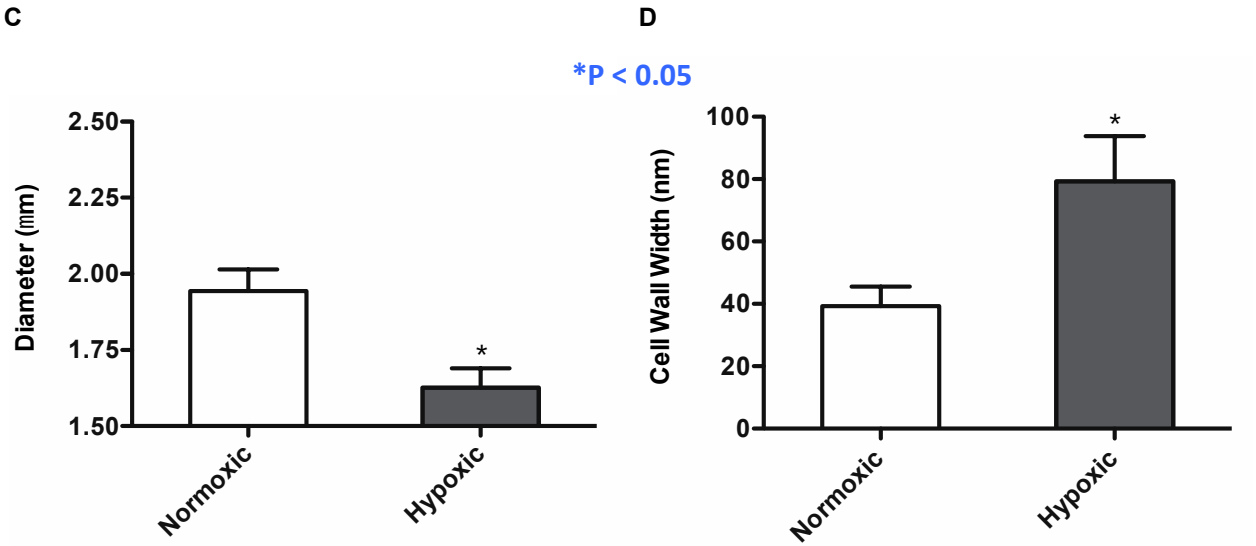
Discovery of a *hapE* Mutation That Causes Azole Resistance in *Aspergillus fumigatus* through Whole Genome Sequencing and Sexual Crossing

Simone M. T. Camps[1,2*], Bas E. Dutilh[3], Maiken C. Arendrup[4], Antonius J. M. M. Rijs[1,2], Eveline Snelders[1,2], Martijn A. Huynen[3], Paul E. Verweij[1,2], Willem J. G. Melchers[1,2]

Mehta, K. and Siddik, Z.H. 2009; Dharia, N.V., *et al.,* 2010; Schubert, S. *et al*., 2011

Hypoxia increases fungal cell wall material and alters exposure

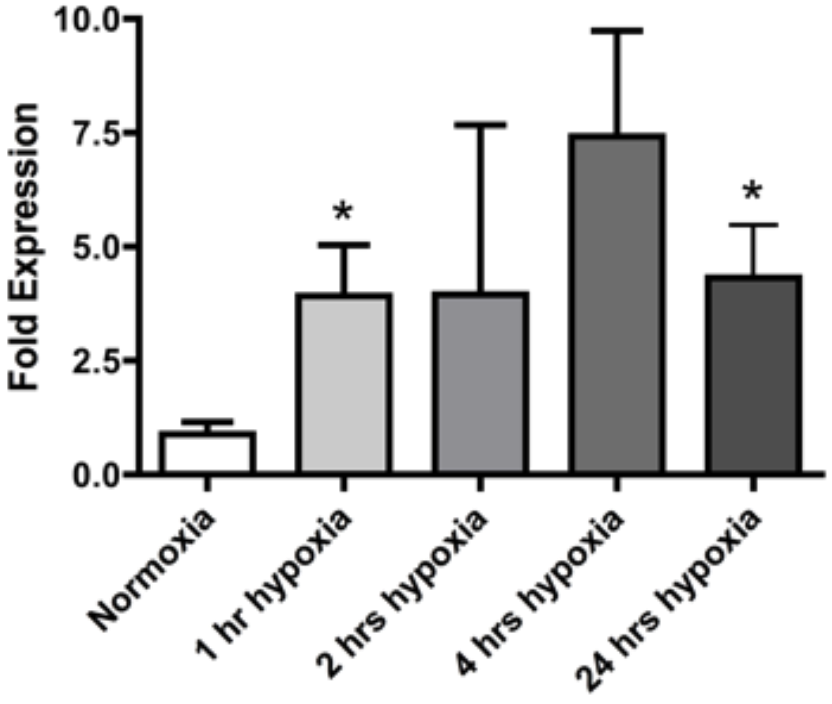




Shepardson et al. 2013, Microbes and Infection

Collaboration with Dr. Tobias Hohl, FHCRC

Hypoxia-grown Hyphae have increased Fks1 transcript levels and more soluble Dectin-1 staining



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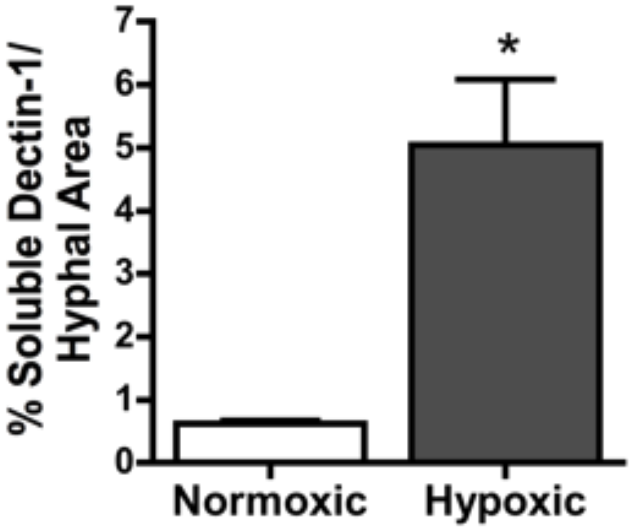

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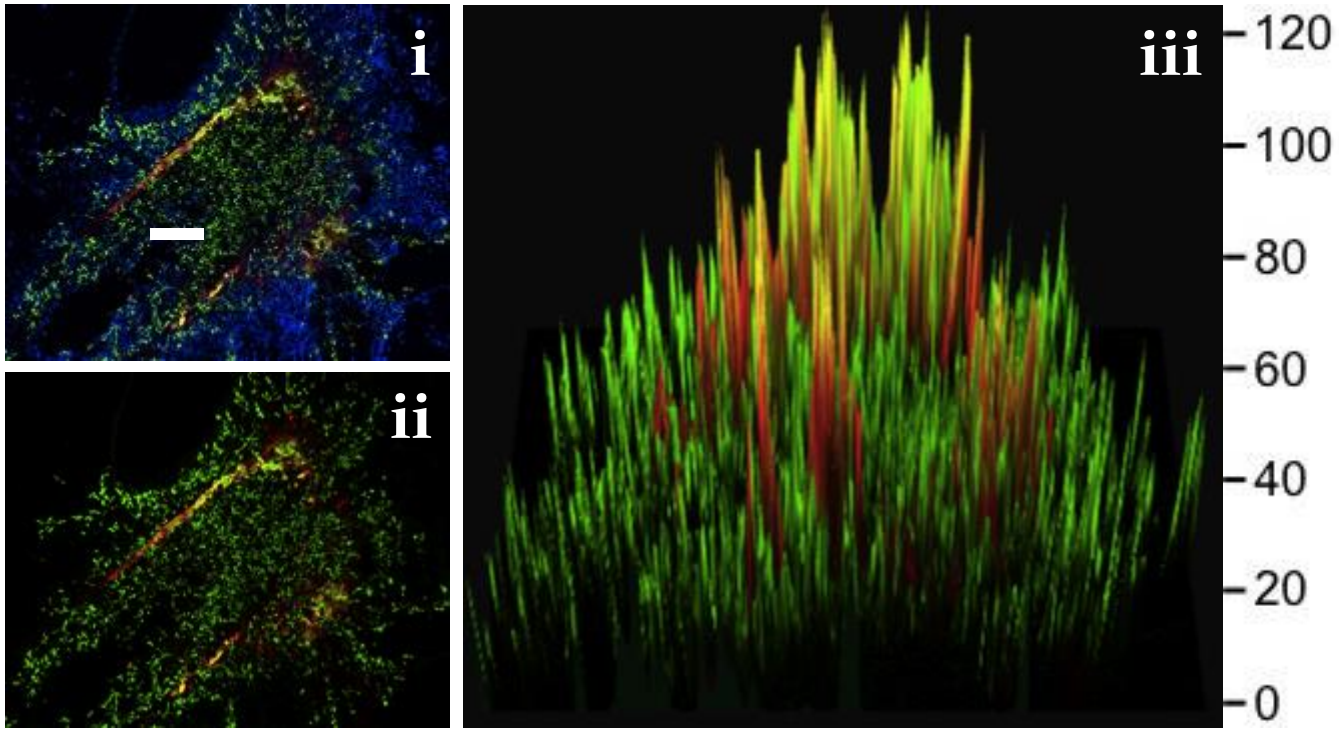
Shepardson et al. 2013 *Microbes and Infection*

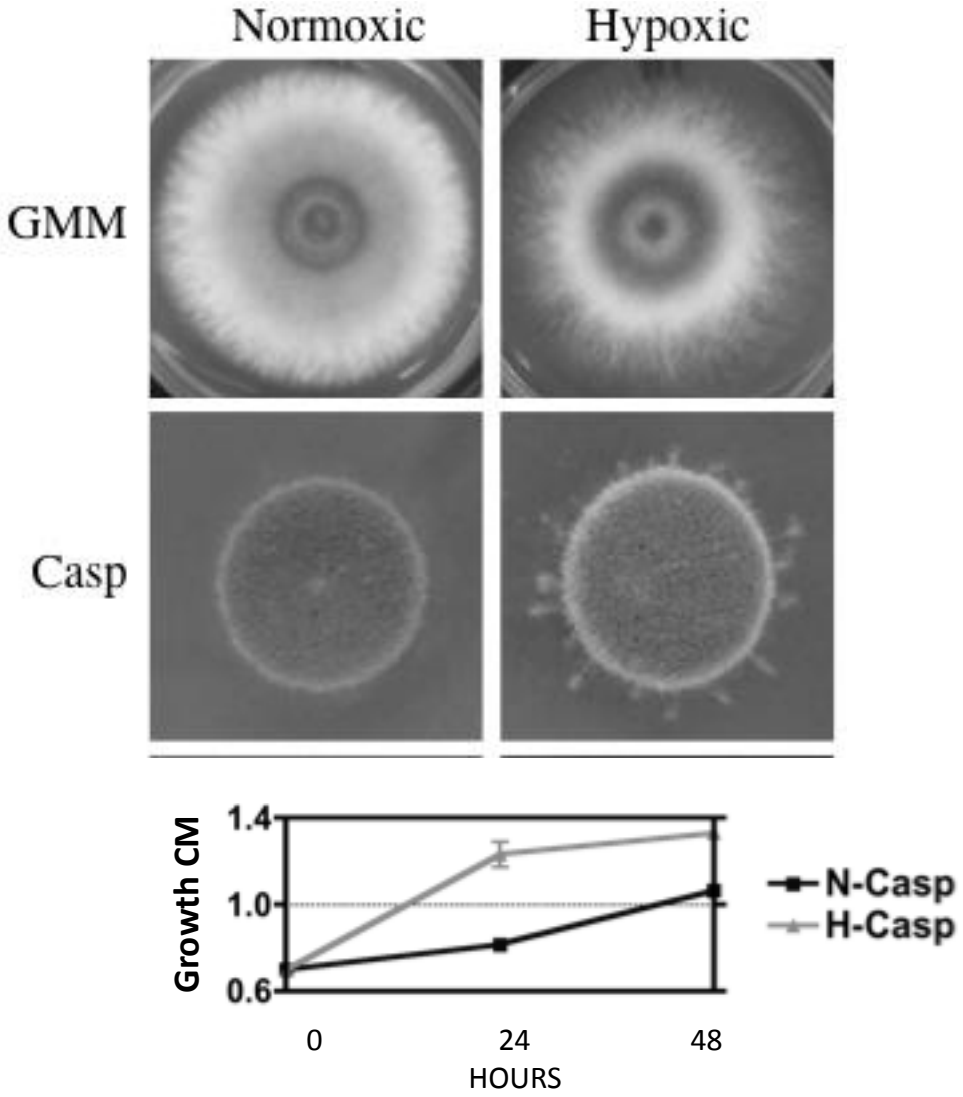
In vivo hypoxia increases Beta Glucan Exposure



Corticosteroid Murine Model of Invasive Pulmonary Aspergillosis

Shepardson et al. 2013 *Microbes and Infection*

Hypoxic Colonies are more resistant to Caspofungin



Can the infection site microenvironments be manipulated to alter fungal and host metabolism to reduce damage?

Hypoxia

Immuno-Pathogenesis

Fungal Pathogenesis

Host DAMAGE

+Hyperbaric Oxygen

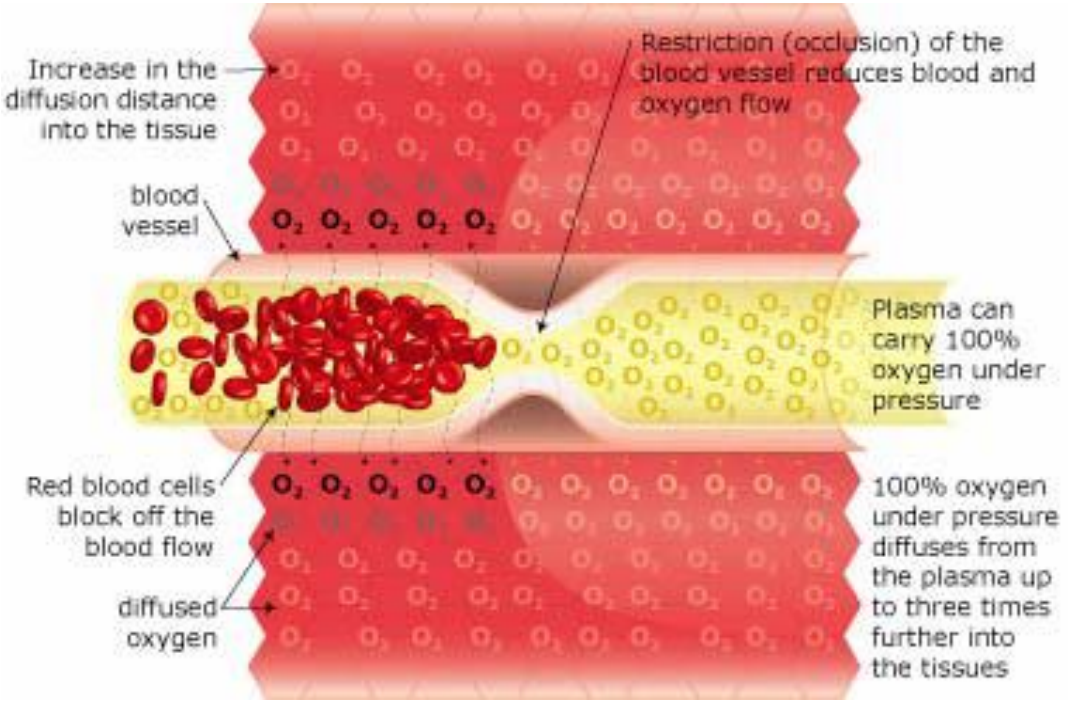
Improved Invasive Fungal Infection Outcomes?

***Expand the therapeutic window?**
***Fungicidal activity?**
***Immunomodulatory?**
***Increase Drug Activity/Penetration?**

Collaboration Dr. Jay Buckey, Dartmouth Hitchcock Hyperbaric Medicine

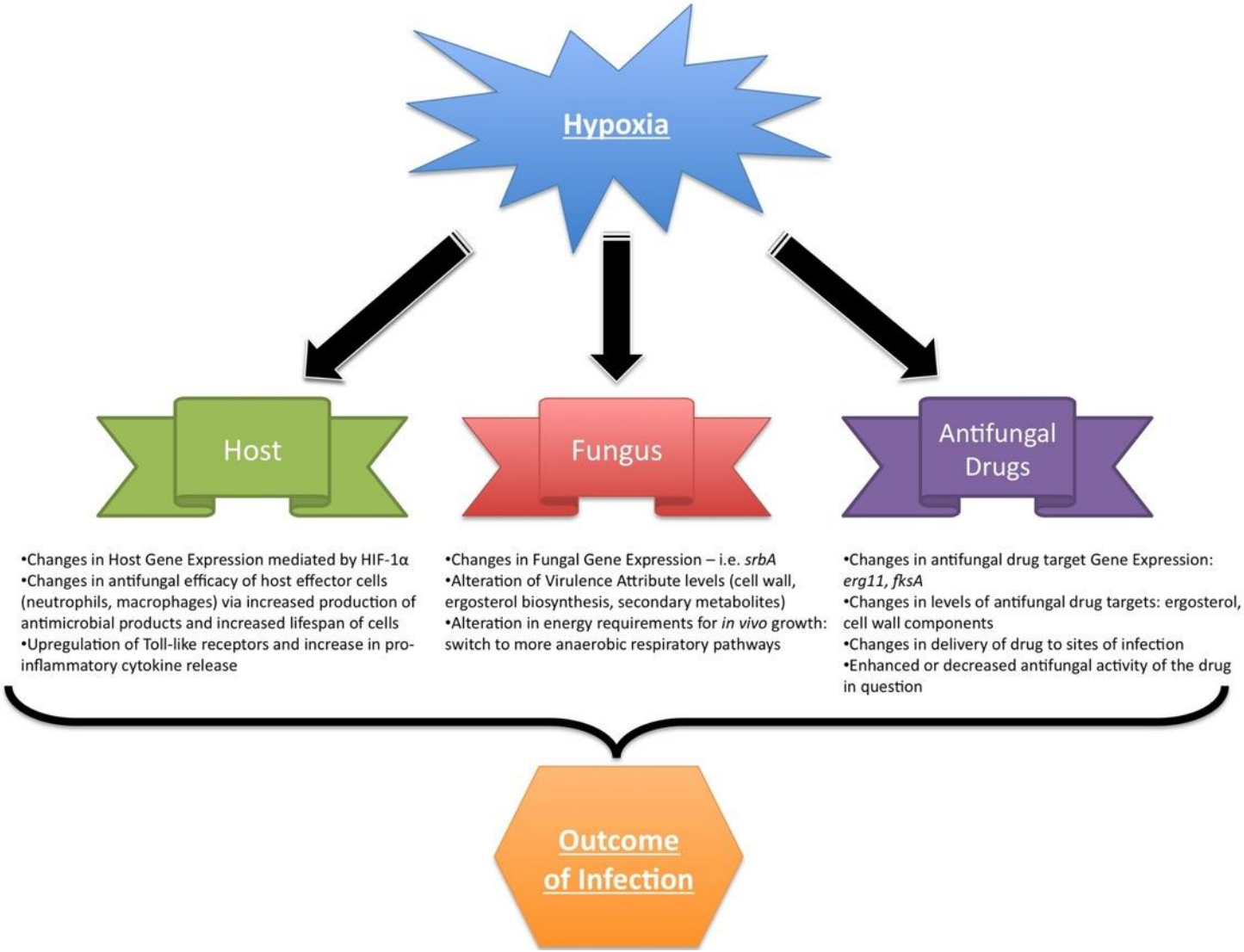
HBOT Physiology

- Dissolved oxygen diffuses in RBC impassible areas
- Increases blood flow even in absence of functional Hb
- Increases angiogenesis



Source: http://www.hbot4u.com/hyperbarics.html

Hypothesis:
In vivo hypoxia has significant implications for IPA Pathophysiology



Wezensky and Cramer 2010 Med Mycol.

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