CLEMSON UNIVERSITY | EPIC
CELLULAR BIOLOGY OF EUKARYOTIC PATHOGENS CONFERENCE

CBEP X Conference Schedule

October 12-14, 2022
Kresge Hall, Outdoor Lab, Clemson University

WEDNESDAY OCT. 12

Starting at 3pm — Arrival and check-in at Outdoor Lab

Dinner on your own (recommendations follow). *Outdoor dining available
- Kite Hill Brewing Co.* (excellent bar & food, live music)
- Clemson Wine Bar* (excellent bar & food)
- Blue Heron (Casual dining in cute Pendleton Square)
- Sole on the Green (Casual dining overlooking the lake)*
- Pixie & Bill's (Casual dining - old Clemson establishment)
- Mellow Mushroom Pizza*
- El Jimador*

THURSDAY OCT. 13

7:30 - 9:00 am — ***Breakfast***

Bagels w/cream cheese, yogurt w/toppings, fresh fruit

Welcome & Session I

Chair: Sourabh Dhingra, Dept. of Biological Sciences, Clemson University

9:00 - 9:15 am — Welcome and Introductory Remarks

Meredith Morris, Dept. of Genetics & Biochemistry and EPIC Member, Eukaryotic Pathogens Innovations Center (EPIC)

Kerry Smith, Dept. of Genetics & Biochemistry, Clemson University Director, Eukaryotic Pathogens Innovations Center (EPIC)

Cynthia Young, Dean of the College of Science, Clemson University

9:20 – 10:05 am — Plenary Talk

Dr. Jarrod Fortwendel, Associate Professor, Dept. of Clinical Pharmacy & Translational Science, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN

"Fungal kinase-driven fitness in the host lung niche."

10:05 - 10:20 am — **Ritu Devkota**, Clemson University

"Functional Characterization of lncRNA afu-254 in Stress Response Pathogenesis of Aspergillus fumigatus"

10:20 - 10:35 am **Stephani Martinez-Barrera**, Clemson University

"Elucidating a Novel Role for Septins During High Temperature Stress Response in Cryptococcus neoformans "

10:35 - 10:50 am **Eun Jung Thak**, Duke University

"Extended O-glycan is Critical for Cell Wall Integrity Signaling and Virulence in Cryptococcus neoformans "

11:00 am - Noon ***Professional Development Session***

Join a topic table (Undergrad, Grad Student, Postdoc/Tech, Professor) and discuss career issues and share best practices and advice.

Noon - 1:00 pm ***Lunch***

Turkey and ham sandwich tray with lettuce, tomato, onion, and cheese; Crispy garden salad with dressings; assorted chips; cookies

Session II

Chair: Dan Whitehead, Dept. of Chemistry, Clemson University

1:00 - 1:45 pm Plenary Talk

Dr. Robert Cichewicz, Regents' Professor and INPART Director, Dept. of Chemistry and Biochemistry, The University of Oklahoma, Norman, OK

"Fungi and their Biomolecules: Probing Continental-Scale Metabolite Diversity to Enhance Lead Discovery."

1:50 - 2:05 pm **Brock Miller**, Clemson University

"Expansion of the Diazacyclobutene Motif for Antiparasitic Evaluation "

2:10 - 2:25 pm **Jillian Millanes**, Clemson University

"Killing the Killer: Naegleria fowleri and enolase inhibitors "

2:30 - 3:00 pm Lightning Talks

Jin Cho, Clemson University

"Biochemical and kinetic analysis of phosphofructokinase in the eukaryotic human pathogen Entamoeba histolytica"

Morgan McCraw, Georgia State University

"Crithidia fasciculata and the impact of ethanol on a regulated cell death pathway"

Emily Knight, Clemson University

"A novel peroxin in Trypanosoma brucei is involved in the maintenance of mitochondrial morphology"

Amy Greene, Albright College

"Developing a Multi-Institutional, Cross-Disciplinary Parasitology Course Undergraduate Research Experience"

Madeline F. Malfara, Villanova

"Spatial regulation of the multifunctional dynamin-1-like protein in Trypanosoma brucei"

Poster Sessions

3:15 - 4:00 pm Poster Session A (Even-Numbered)

4:00 – 4:45 pm Poster Session B (Odd-Numbered)

4:45 - 5:00 pm ***Group Photo*** *(on the back stairs)*

5:00 - 6:00 pm ***Dinner***

Marinated chicken breast; baked potatoes with sour cream, butter, and cheese; green beans; vegetable soup; yeast rolls; chocolate cake

Session III

Chair: Kerry Smith, Dept. of Genetics & Biochemistry, Clemson University

6:00 - 6:45 pm Plenary Talk

Dr. Christina Cuomo, Senior Group Leader and Institute Scientist, Fungal Genomics Group, Broad Institute, Cambridge, MA

"Population genomics and the evolution of virulence traits in Cryptococcus neoformans."

6:50 - 7:05 pm **Perry Kezh**, Clemson University

"The role of acetate utilization during primary infection by the fungal pathogen Cryptococcus neoformans"

7:10 - 7:25 pm **Blyssalyn Bieber and Faith St. Clair**, Villanova University

"Expanding the Genetic Toolkit of Crithidia bombi"

7:30 - 7:45 pm **Fidel Soto-Gonzalez**, Meharry Medical College

"Hey Tim, this is a TRAP. How BioID found TbTRAP1 neighboring TbTim17 in Trypanosoma brucei "

7:45 – 11:00 pm ***Social Time & Bonfire***

FRIDAY OCT. 14

7:30 - 8:30 am ***Breakfast***

Baked French toast casserole, yogurt with toppings, fresh cut fruit

Session IV

Chair: Emily Rosowski, Dept. of Biological Sciences, Clemson University

8:30 - 9:15 am Plenary Talk

Dr. Suzanne Noble, Associate Professor, Dept. of Microbiology and Immunology, The University of California-San Francisco, San Francisco, CA

"Candida albicans is a fungal pathobiont of mammalian hosts."

9:20 - 9:35 am **Erin Glass**, Clemson University

"Clec and Sclra as Possible Dectin-1 Homologues in Zebrafish"

9:40 - 9:55 am **Nava Poudyal**, Clemson University

"Investigating Variant Surface Glycoprotein Dynamics under Inhibition of Fatty Acid Synthesis "

10:00 - 10:15 am **Daniel Call**, Brigham Young University

"Trypanosoma brucei Bloodstream Form Regulates Glycosomal pH Different from Procyclic Form "

10:15 - 10:30 am ***Coffee Break & Survey***

Session V

Chair: Jim Morris, Dept. of Genetics & Biochemistry, Clemson University

10:30 - 11:15 am Plenary Talk

Dr. Michael Povelones, Associate Professor, University of Pennsylvania Department of Pathobiology, School of Veterinary Medicine, Philadelphia PA

"Identification of potential mosquito immune modulators in the excretory/secretory products of Dirofilaria immitis."

11:20 - 11:35 am **Melanie Key**, Clemson University
"Host glycolytic metabolites serve as a vital fuel source for energy metabolism and intracellular survival of Toxoplasma gondii"

11:40 - 11:55 am **Vanessa Enriquez**, University of Florida
"Cryptococcus neoformans GXM alters microglial cell migration and morphology in vivo"

12:00-12:15 am **Mikayla M. Mangrum**, University of Tennessee
"Uridine auxotrophy disrupts cell wall architecture but attenuates virulence by slowing growth in mice"

12:15-12:30 pm ***Awards & Closing Remarks***

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Abstract #1

Functional Characterization of lncRNA *afu-254* in Stress Response and Pathogenesis of *Aspergillus fumigatus*

Ritu Devkota, Sourabh Dhingra
Department of Biological Sciences; Eukaryotic Pathogen Innovation Center
Clemson University, Clemson, SC, 29634

Aspergillus fumigatus is a saprophytic fungus that can cause a collection of diseases in an immunocompromised population termed aspergillosis, most severe amongst them is invasive pulmonary aspergillosis (IPA). Humans and animals are exposed to asexual spores or conidia – infectious propagules of *A. fumigatus* daily. These conidia are about 2.5µm in diameter and are hydrophobic, making them easier to carry by air and reach lung alveoli. Azoles are the major classes of antifungal drugs used to treat invasive pulmonary aspergillosis. However, in recent years there has been an increase in fungal resistance to azole drugs exacerbating the problem. In addition, the fungal response to azole drugs is not entirely understood, resulting in poor disease outcomes associated with azole susceptible strains.

It is becoming increasingly clear that lncRNA-mediated regulation is vital in stress response; however, their roles in fungi are lacking. Here, we have identified a lncRNA, *afu-254*, which acts as a positive regulator of azole drug response in *A. fumigatus*. We are testing the hypothesis that lncRNA *afu-254* is a global stress response regulator. Thus, we aim to characterize the role(s) of *afu-254*, including antifungal drug response and pathogenesis. Successful completion of these aims will aid in filling the knowledge gap in fungal stress, especially azole response.

Abstract #2

Elucidating a Novel Role for Septins During High Temperature Stress Response in *Cryptococcus neoformans*

Stephani Martinez and Lukasz Kozubowski
Department of Genetics and Biochemistry, Eukaryotic Pathogens Innovation Center, Clemson University, Clemson SC, USA

The pathogenic yeast *Cryptococcus neoformans* needs to adapt to changes in temperature upon entering its human host. *C. neoformans* strains lacking septin proteins Cdc3 or Cdc12 are viable at 25°C but fail to proliferate at 37°C and are avirulent in the heterologous host infection model. Septins are a family of conserved filament-forming GTP-ases that bind to phosphoinositides and assemble as higher order complexes at the cell cortex to support cytokinesis and morphogenesis in fungal and animal cells. The exact contribution of septins to growth of *C. neoformans* at high temperature remains unclear and a similar putative stress-related function has not been investigated in any other organism. Current model assumes that *C. neoformans* septins contribute to growth at 37°C by localizing to the mother-bud neck and supporting cytokinesis and/or final cell separation. However, our recent findings suggest a novel role for septins in high temperature response. We find that upon temperature change to 37°C, septins Cdc12, Cdc10, and Cdc11 accumulate at the plasma membrane (PM) as puncta. The localization of Cdc10 to the PM is dependent on Cdc12 suggesting that septins associate with the PM as a complex. Mutants lacking septin Cdc3 or Cdc12 exhibit an increased internalization of propidium iodide and are hypersensitive to drugs that perturb PM lipid composition suggesting that absence of septin Cdc3 or Cdc12 causes an aberrant bio-physical state of the PM that increases its permeability. Our study points to a novel function of septin proteins in the regulation of PM homeostasis that may be critical for high temperature stress response.

Abstract #3

Extended *O*-glycan is Critical for Cell Wall Integrity Signaling and Virulence in *Cryptococcus neoformans*

Eun Jung Thak,[a] Ye Ji Son,[a] Dong-Jik Lee,[a] Hyunah Kim,[a] Jung Ho Kim,[a] Su-Bin Lee,[a] Yu-Byeong Jang,[b] Yong-Sun Bahn,[b] Connie B. Nichols,[c] J. Andrew Alspaugh,[c,#] Hyun Ah Kang[a,#]

[a]Department of Life Science, Chung-Ang University, Seoul, Republic of Korea
[b]Department of Biotechnology, College of Life Science of Biotechnology, Yonsei University, Seoul, Republic of Korea
[c]Department of Medicine, Duke University School of Medicine, Durham, USA

The human pathogenic yeast *Cryptococcus neoformans* assembles on its proteins two types of *O*-glycans with and without xylose. In this study, the *CAP6* gene, encoding an α1,3-mannosyltransferase responsible for the second mannose addition to the minor *O*-glycans with xylose, was identified and functionally analyzed. The *CAP6* deletion in the *ktr3Δ* strain, in which the α1,2-mannose addition at the second position of the major *O*-glycans is blocked, resulted in the shift of all peaks of *O*-glycans to the single mannose M1 peak in HPLC analysis. Two cell surface sensor proteins, Wml (WSC/Mid2-Like) 1p and Wml2p, were shown to be an independent substrate of Cap6p-mediated minor or Ktr3p-meidated major *O*-mannosylation, respectively. The phosphorylation of Mpk1p, induced by tunicamycin, was greatly decreased in the *ktr3Δcap6Δ* as well as in the *wml1Δ wml2Δ*, along with the decreased expression of target genes in Mpk1p-mediated cell wall integrity (CWI) signaling, indicating an essential role of the extended *O*-glycans in CWI signaling. As reflecting its defective growth under several stress conditions, including high-temperature, ER and cell-wall stresses, the *ktr3Δ cap6Δ* strain showed fully attenuated virulence in a mouse model of cryptococcosis. Moreover, *ktr3Δcap6Δ* showed decreased proliferation within macrophages and blood brain barrier (BBB) transcytosis ability. Altogether, the results demonstrate the *O*-glycan extension in the Golgi plays critical roles in various processes, such as cell wall integrity signaling, stress resistance, and interaction with host cells, essential for pathogenicity of *C. neoformans*. The delineation of the roles of protein glycosylation in fungal pathogenesis will not only provide a deep insight into the glycan-based fungal infection mechanism but also aid in the development of novel antifungal agents.

Abstract #4

Expansion of the Diazacyclobutene Motif for Antiparasitic Evaluation

Brock A. Miller, Chandima J. Narangoda, William T. Bridges, Monireh Noori, Daniel C. Whitehead

Molecules bearing four, five, and six-membered nitrogen heterocycles such as β-lactams, pyrroles, indoles, and pyridine are important motifs in pharmaceutically relevant molecules. Recently, our group has developed a two-step synthesis to access the rarely studied diazacyclobutene motif via a formal [2+2] cycloaddition between phenyl-triazolinedione and electron-rich thioalkynes. Subsequently, we have continued studying this motif with the objective of expanding the substrate scope. Namely, we have developed a telescoped oxidation/cycloaddition protocol that allows for structural variability in the triazolinedione component. We have also begun to explore the ability of other electron rich alkynes such as ynamide derivatives to engage in the cycloaddition. Preliminary biological evaluation of the scaffold suggests that the diazacyclobutene exhibit potent cytotoxicity against eukaryotic pathogens including *Trypanosoma brucei* and *Trichomonas vaginalis*. Prior to our efforts, there were no recorded biological evaluations of these molecules in any sense. This presentation will describe the synthetic efforts to expand the scope of the cycloaddition leading to stable diazacyclobutenes as well as structure-activity relationship evaluations of the motif against medically relevant parasites.

Abstract #5

Killing the Killer: *Naegleria fowleri* and enolase inhibitors

Jillian Milanes[1], Samuel Kwain[2], Elijah Harding[1], Alexander Richardson[1], Brian Dominy[2], Dan Whitehead[2], James Morris[1]
Clemson University Eukaryotic Pathogens Innovation Center
[1]Department of Genetics and Biochemistry, Clemson University, Clemson, SC
[2]Department of Chemistry, Clemson University, Clemson, SC

Glucose is essential for *Naegleria fowleri* trophozoite growth (Milanes, 2018). Recently, we have tested a series of phosphonate human enolase 2 (ENO-2) specific inhibitors against the amoebae. These compounds cure ENO-1 deleted glioblastoma in a rodent model and have very limited toxicity in non-human primates. These inhibitors were toxic to the amoebae *in vitro* with (1-hydroxy-2-oxopiperidin-3-yl) phosphonic acid (HEX) being the most potent, with an EC_{50} value of 0.21 ± 0.02 μM (~1500-fold lower than the CC_{50}). The compounds were potent inhibitors of recombinant *N. fowleri* ENO. Molecular docking simulation with HEX and HEX analogs revealed the agents bind to the NfENO active site with varying affinities (-8.6 to-6.2 kcal/mol), mirroring potency. In a pilot rodent infection assay, HEX failed to extend mean days to death when compared to vehicle treated animals. Amoebae isolated from HEX-treated rodents were transiently resistant to HEX, with reversion of sensitivity after culturing for ~2 weeks. While exploring possible changes in metabolism that could contribute to the loss of sensitivity, we found that amoebae cultured in glycerol instead of glucose were 10-fold more resistant to HEX, suggesting that the amoebae may alter their metabolism in response to HEX. Nevertheless, the amoebae remained sensitive to HEX at levels tolerated in mammals, suggesting altered dosing scheme could be developed for use of the agent against the amoebae.

Abstract #6

Uridine auxotrophy disrupts cell wall architecture but attenuates virulence by slowing growth in mice

Mikayla M. Mangrum, Andrew S. Wagner, and Todd B. Reynolds

The cell wall of *Candida albicans* is composed of the highly immunogenic epitope β-1,3-glucan as well as the polymers β-1,6-glucan and chitin. However, these epitopes are covered, or “masked”, by an outer layer of glycosylated proteins, which impairs immune detection and hampers fungal clearance. Mutations or drugs that increase the exposure of β-1,3-glucan in the cell wall of *C. albicans* decrease fungal burden and virulence in a murine model of systemic infection, suggesting this could be a new therapeutic strategy. UDP-glucose and UDP-*N*-acetylglucosamine are important for cell wall construction as they are the precursors for β-1,3-glucan and chitin respectively. Both products are derived from uridine, and previous studies have demonstrated attenuated virulence of uridine auxotrophs in mouse infections, which has been attributed to insufficient uridine levels to maintain growth in the host. We have discovered that uridine deprivation in the uridine auxotrophic mutant *ura3ΔΔ* disrupts cell wall architecture by increasing surface mannans and exposing β-1,3-glucan and chitin, and this can be rescued with uridine supplementation into the media. This may be related to effects on UDP-sugar levels. Here we used a murine model of systemic infection which showed that mice infected with a *ura3ΔΔ* mutant exhibit increased survival and reduced kidney fungal burden compared to mice infected with wildtype *C. albicans*. Suppression of the immune response with cyclophosphamide did not decrease survival nor increase kidney colonization of *ura3ΔΔ* infected mice, indicating the attenuation in virulence of uridine auxotrophs can be attributed to decreased fitness in the host but not increased exposure of β-1,3-glucan. Moreover, a *ura3ΔΔ* mutant is unable to grow on *ex vivo* kidney agar which also demonstrates its inability to colonize the kidneys due to poor growth. Thus, although uridine auxotrophy elicits changes to cell wall architecture that increase the exposure of immunogenic epitopes, this exposure does not fully explain the attenuation in virulence observed in uridine auxotrophs.

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Abstract #7

Biochemical and kinetic analysis of phosphofructokinase in the eukaryotic human pathogen *Entamoeba histolytica*

Jin Cho and Cheryl Ingram-Smith
Department of Genetics and Biochemistry and Eukaryotic Pathogens Innovation Center, Clemson University

Entamoeba histolytica is a water- and food-borne intestinal parasite that causes amoebiasis and liver abscess and causes symptomatic disease in 100 million people each year leading to ~100,000 deaths. This amitochondriate parasite lacks many metabolic pathways including the tricarboxylic acid cycle and oxidative phosphorylation, and cannot synthesize purines, pyrimidines, or most amino acids. As a result, *E. histolytica* is presumed to reply on its modified pyrophosphate (PP_i)-dependent glycolytic pathway for ATP production during growth on glucose. This pathway relies on a PP_i-dependent rather than ATP-dependent phosphofructokinase (PFK) and thus has a net production of three ATP per glucose. However, the *E. histolytica* genome encodes four putative PFKs, one of which is PP_i-dependent and the other three of which are ATP-dependent. We have produced and purified the three recombinant ATP-PFKs (designated as PFK1-3) to analyze their enzymatic activities and regulation. Various ligands such as AMP that have been shown to regulate PFKs in other organisms have been tested to analyze their effects on *E. histolytica* PFK activities. Specifically, we have found that phosphoenolpyruvate (PEP) is a potent inhibitor and CoA is a potent activator, differentiating PFK2 from the canonical PFK. Results from the IC_{50} experiments showed that PEP, PP_i, and citrate all bind at different allosteric sites. In addition, progress curves for both substrates ATP and fructose 6-phosphate (F6P) in the presence of these inhibitors showed differences on the effect of PFK substrate binding. The gene encoding PPi-dependent PFK is highly expressed during standard trophozoite growth. RNAseq studies in the reptile pathogen *Entamoeba histolytica* demonstrated that the gene encoding the PP_i-dependent PFK is highly expressed during trophozoite growth whereas one of its two genes encoding putative ATP-dependent PFKs is strongly upregulated during excystation. The differences in enzymatic activity and regulation as well as transcriptional regulation suggest that the four PFKs play different metabolic roles.

Abstract #8

Spatial regulation of the multifunctional dynamin-1-like protein in *Trypanosoma brucei*

Madeline F. Malfara[1], Alexa Pereira[1], Frank Donio[1] & Megan L. Povelones[1]
[1]Department of Biology, Villanova University, Villanova, PA 19085, USA

Trypanosoma brucei undergoes morphological changes to adapt to host environments, including remodeling of their mitochondrial network. In yeast and mammals, membrane remodeling events involve both classical dynamins and dynamin-related proteins (DRPs), with DRPs mediating organelle division. In contrast, *T. brucei* have only one multifunctional dynamin-like protein (TbDLP) with roles in endocytosis and mitochondrial fission. Currently there are no known interacting proteins for TbDLP, and uncovering its molecular mechanism remains complicated by its involvement in multiple cellular pathways. Using tandem affinity purification in the related kinetoplastid *Crithidia fasciculata*, we have identified 30 putative DLP interacting proteins, almost a third of which have been found associated with glycosomes, glycolytic organelles related to peroxisomes. To explore the role of TbDLP in glycosome structure, we localized a glycosomal marker in procyclic form *T. brucei* during TbDLP RNAi and overexpression. While control and TbDLP knockdown cells showed a granular pattern, overexpression of TbDLP caused an increase in cytoplasmic signal, indicating a defect in glycosomal import. Intriguingly, overexpression of TbDLP also alters its cellular localization, changing from cytoplasmic puncta to accumulations in the periflagellar pocket region. When we probed *T. brucei* lysates for endogenous TbDLP by western blot, we found two bands of similar size, possibly representing modified forms. While both bands decrease during RNAi, overexpression of TbDLP increases only the lower band. We favor a hypothesis whereby overexpression of TbDLP alters its post-translational modification and therefore its subcellular localization, providing a possible mechanism for the spatial regulation of TbDLP function.

Abstract #9

Crithidia fasciculata and the impact of ethanol on a regulated cell death pathway.

Morgan McCraw and Paul Ulrich

Crithidia fasciculata is a trypanosomatid, mosquito parasite. Given the early divergence of trypanosomatids in eukaryotic evolution and the absence of genes central to “classical” cell death pathways, investigating induction of and mechanisms of regulated cell death in *C. fasciculata* will shed light on early evolution of these processes. There are limited data on inducing stress in *C. fasciculata* with the use of ethanol besides causing necrosis with 70% ethanol. Exposure of *C. fasciculata* to low concentrations of ethanol (<20%) resulted in a subpopulation of annexin V+/propidium iodide- cells distinct from a pattern characteristic of necrosis (PI+/AV+). Propidium iodine is used to detect necrotic cells and the annexin v is used to identify the cells that have flipped their membranes during the apoptosis process. To further elucidate the mechanism of inducing this cell death-like phenotype, alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH) were inhibited to demonstrate the increase in stressed cells with the addition of ethanol to observe the mechanisms that cause a regulated cell death. Low concentrations of ethanol (9%) induce exposure of phosphatidylserine to the external surface of the cell in a subset (2%) of cells. ADH is responsible for the production of ethanol at the end of the metabolic pathway and breaking down ethanol. ALDH is used for converting acetaldehyde to acetate. I hypothesized that the ALDH inhibitor disulfiram shows annexin positive and propidium iodine negative cells demonstrating a regulated cell death pathway. With the inhibitor disulfiram and the low concentration of ethanol of (9%) there is an increase in cells experiencing a regulated cell death pathway. Fomepizole an ADH inhibitor can cause an increased amount of regulated cell death with the addition of low concentrations of ethanol (9%). By inducing reliable ways of causing regulated cell death in *C. fasciculata* that is missing the genes that can lead to more discoveries about the early evolution process of the “classical” cell death pathway.

Abstract #10

A novel Peroxin in *Trypanosoma brucei* is involved in the maintenance of mitochondrial morphology.

Emily Knight, Andrew Gianos, Logan Crowe, Meredith Morris
Department of Genetics and Biochemistry, Clemson, SC

Cells compartmentalize metabolic pathways within organelles that must communicate and interact with each other. In eukaryotic cells, peroxisomes and mitochondria play important roles in cellular metabolism and communicate through several mechanisms including physical contact points, intraorganellar diffusion of metabolites or vesicular traffic. *Trypanosoma brucei*, the causative agent of Human African trypanosomiasis, has specialized peroxisomes called glycosomes and a single mitochondrion whose structure and function changes dramatically throughout the lifecycle. Very little is known about how these organelles interact. Peroxisome/glycosome biogenesis is regulated by Peroxins (Pexs). Homologs for fewer than 50% of eukaryotic peroxins have been identified in *T. brucei.* This scarcity of Pexs may reflect a streamlined biogenesis process or be a consequence of the long evolutionary distance between trypanosomes and other eukaryotes. We queried the Tritryp database for short sequences that function in glycosome-specific interactions with the reasoning that those domains may be more highly conserved than overall protein sequences. We identified a putative peroxin that we have named PIMM (Peroxin involved in mitochondria morphology). Epitope tagged PIMM localized to punctate structures consistent with glycosomes and PIMM-deficient cells exhibited growth defects, had swollen mitochondria and increased levels of reactive oxygen species, and were more sensitive to apoptosis triggers. These findings suggest an interaction between glycosomes and peroxisomes and current work is focused on resolving the function that PIMM plays in this communication.

Abstract #11

Developing a Multi-Institutional, Cross-Disciplinary Parasitology Course Undergraduate Research Experience

Dr. Amy Greene (Albright College - Chemistry and Biochemistry), Dr. Swati Agrawal (University of Mary Washington - Biology), and Dr. Paul Ulrich (Georgia State University – Program for Undergraduate Research in the Life Sciences, Biology)

Authentic research experiences and scientific collaboration are critical skills for undergraduates to develop. Over the last two years, we developed and taught collaborative molecular parasitology course-based undergraduate research experiences (CUREs) across three very different institutions and departments. Paul Ulrich, who has spearheaded CUREs using the trypanosomatid *Crithidia fasciculata* since 2014, was joined by Swati Agrawal (Biology, University of Mary Washington) and Amy Greene (Chemistry and Biochemistry, (Albright College). *Crithidia fasciculata* is a convenient non-pathogenic parasite model for undergraduates; its high density in serum-free media is amenable to cell biology and biochemical analysis. Undergraduates investigated various stressors and cell death, leveraging individual expertise and interests of each professor and course. This CURE is unique among multi-institutional CUREs in that students - not just PIs - from all three institutions interacted throughout the semester. Highlights of the courses included diverse speakers on molecular parasitology discussing the research process with our students, direct interaction of PI's with students at other institutions, shared project reports and conference presentations, professional-style graphical abstracts, and piloting a Slack platform to encourage communication. Our model for collaborative CURE research is being refined to overcome challenges of asynchronous engagement and aligning and accelerating the research process. Ultimately, our collaborative CUREs engage a larger number of undergraduates in an authentic parasitology community.

Abstract #12

The role of acetate utilization during primary infection by the fungal pathogen *Cryptococcus neoformans*

Perry Kezh and Kerry Smith
Department of Genetics and Biochemistry, Eukaryotic Pathogens Innovation Center, Clemson University, Clemson, SC

Cryptococcus neoformans, the pathogenic cause of fungal meningitis, accounts for an estimated 190,000 yearly deaths. Cryptococcosis resulting from this basidiomycetous fungi ranks as the fifth most fatal infectious disease behind AIDS, tuberculosis, malaria, and diarrhea. Environmental exposure during childhood is common, however most immunocompetent individuals never present with the infection. However, in the immunocompromised, primary infection follows inhalation of the spore form which subsequently colonizes other organs though hematogenous dissemination. This change of environment, from the soil to the human respiratory system, induces changes in metabolism. The alveolar macrophages, which present a first line of defense against infection, provide a glucose- and amino acid-poor environment differing greatly from that found in soil. Thus, the lack of available glucose and amino acids typically utilized in glycolytic function necessitates the use of alternate carbons sources to establish pulmonary infection. *C. neoformans* survival post inhalation hinges on alternative carbon sources such as acetate.

Following the cytosolic activation of acetate, utilization of the generated acetyl-CoA in the mitochondria requires transport in the form of acetyl-carnitine or succinate. The cryptococcal genes *acuL* (succinate/fumarate antiporter, CNAG_02288) and *acuH* (acetyl-carnitine translocase, CNAG_00499) are orthologous to transporters in *Aspergillus* and *Saccharomyces* necessary for the utilization of acetate. Characterization of AcuL and AcuH is thus far unreported in *C. neoformans*. Additionally, the carnitine shuttle requires the use of carnitine acetyl transferases (CATs) of which three, noted as Cat37 (CNAG_00537), Cat51 (CNAG_06551), and Cat42 (CNAG_05042), have been identified. Deletion mutants in *acuL*, *acuH*, *cat37*, *cat51*, and *cat42* display a lack of growth on acetate as a carbon source. Testing the proposed gene knockouts in various growth conditions displayed a defect in melanin production by the *acuH* mutant and a macrophage survival defect in the *acuL* mutant. Additionally, generated complements of said mutants restored growth on acetate, as well as melanin production and macrophage survival respectively. The insertion of each gene into the safe haven of the genome along with a mCherry tag confirmed the causation of phenotypes observed in the knockouts and allows for subcellular localization of proteins associated with these genes. Analysis of the acetate utilization pathway will foster a better understanding of how *Cryptococcus* adapts its metabolism to survive in the changing environments encountered during infection.

Abstract #13

Expanding the Genetic Toolkit of *Crithidia bombi*

Blyssalyn Bieber[1], Sarah Lockett[1], Faith St. Clair[1] & Megan L. Povelones[1]
[1]Department of Biology, Villanova University, Villanova, PA 19085, USA

Crithidia bombi is a monoxenous trypanosomatid parasite that infects bumblebees by adhering to the ileum of their intestinal tract. Parasites may persist within an infected colony or can be transmitted to neighboring colonies via the fecal-oral route as bees forage. *C. bombi* infection affects the reproductive success of a colony by reducing the fitness of the queen. To better understand the complex relationship between these parasites and their hosts and the implications this has on pollinator health and ecosystem services, we are establishing new genetic techniques for study of *C. bombi.* Using constructs for genetic modification of *Crithidia fasciculata*, a highly-related kinetoplastid, we episomally expressed cytoplasmic GFP in *C. bombi* wildtype cells. To our knowledge this is the first genetic modification of *C. bombi* to date and will be an important tool for in vivo visualization of *C. bombi* infection. We also expressed an episomal construct containing a c-terminally GFP tagged copy of *C. fasciculata* ribonuclease H1 (RNH1). Previous work tagging RNH1 in *C. fasciculata* found that the presence of alternate start codons led to its dual localization in both the nucleus and the kinetoplast. Similarly, we were able to localize RNH1 to both the kinetoplast and nucleus in *C. bombi.* Additionally, we have adapted methods for studying in vitro adhesion of *C. bombi. C. bombi* will adhere to both glass and untreated tissue culture plastic, in what appears to be a density dependent manner. Preliminary observations suggest that flagellar length is also correlated with cell density. We were able to observe rosettes after 48 hours of growth in both wild type and RNH1 tagged cells, indicating that genetic modification did not impact cellular adhesion. Expanding our genetic toolkit for *C. bombi* will help us better understand these important parasites and their impact on pollinator health.

Abstract #14

"Hey Tim, this is a TRAP" How BioID found TbTRAP1 neighboring TbTim17 in *Trypanosoma brucei*

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The protein translocase of the mitochondrial inner membrane in Trypanosoma brucei, TbTIM17, forms a modular complex in association with several other trypanosome-specific proteins. To identify transiently interacting proximal partner(s) of TbTim17, we used Biotinylation Identification (BioID) by expressing a modified biotin ligase-TbTim17 (BirA*-TbTim17) fusion protein in T. brucei. BirA*-TbTim17 was targeted to mitochondria and assembled in the TbTIM complex. In the presence of biotin, BirA*-TbTim17 biotinylated several mitochondrial proteins. Interestingly, TbHsp84/TbTRAP1, a mitochondrial Hsp90 homologue, was identified as the highest enriched biotinylated proteins. Interaction and colocalization of TbTim17 and TbHsp84 in T. brucei mitochondria was further validated by co-immunoprecipitation analysis and confocal microscopy, respectively. TbTim17 association with TbTRAP1 increased several folds during denaturation/renaturation of mitochondrial proteins in vitro, suggesting TbTRAP1 acts as a chaperone for TbTim17 refolding. Knockdown of TbTRAP1 reduced cell growth and decreased the levels of the TbTIM17, TbTim62, and mitochondrial (m)Hsp70 complexes. However, ATPase, VDAC, and Atom69 complexes, were minimally affected. In addition, the steady state levels of TbTim17, TbTim62, and mHsp70 were reduced significantly but Atom69, ATPase β, and RBP16 were mostly unaltered due to TbTRAP1 knockdown. Quantitative proteomics analysis also showed significant reduction of TbTim62 along with few other mitochondrial proteins due to TbTRAP1 knockdown. TbTRAP1 depletion did not hamper the import of the ectopically expressed TbTim17-2xMyc into mitochondria but reduced its assembly into the TbTIM17 complex, suggesting TbTRAP1 plays a critical role in the later process. This is the first report showing the role of TRAP1 in the TIM complex assembly in eukaryotes.

Abstract #15

Clec and *Sclra* as Possible Dectin-1 Homologues in Zebrafish

Erin Glass and Emily E Rosowski

The ubiquitous fungus, *Aspergillus fumigatus*, causes invasive fungal disease in immunocompromised patients. The receptors used by innate immune cells to recognize and respond to *Aspergillus* are largely unknown. Here, we use the larval zebrafish model and genetic tools like CRISPR to mutate predicted receptor genes and test effects on neutrophil migration, NFκB activation, and control of fungal germination in response to injection of zymosan, a beta-glucan particle that mimics infection of a fungus, or *Aspergillus*. *Clec4c* and *sclra* are predicted homologues of the Dectin-1 receptor in humans, a C-type lectin that recognizes beta-glucans found on the cell wall of fungi. In both experiments done, we injected gRNAs and Cas9 to knock down the *clec* and *sclra* genes, or targeted a luciferase sequence as a control. In immunocompetent zebrafish, the NFκB pathway is activated in response to beta-glucans. To determine if *clec* and *sclra* play a role in the activation of the NFκB pathway, larvae whose cells express GFP when the pathway is activated were injected with zymosan and imaged. Preliminary data indicates that these genes could play a role in beta-glucan recognition and activation of NFκB. Another function of the innate immune system is the migration of neutrophils to contain infection. To determine if neutrophil activity is altered by the knockdown of *clec* and *sclra*, larvae that have increased amounts of neutrophils in the absence of macrophages (*irf8-/-*) were injected with *Aspergillus* and imaging was done to score germination and invasive hyphae occurrence and area, and to enumerate neutrophils at the sight of infection. We are currently performing experiments and analyzing this data. In the future we will also be exploring the roles of other putative receptors, such as *tlr2*, *tlr9*, and *cd209*.

Abstract #16

Investigating Variant Surface Glycoprotein Dynamics under Inhibition of Fatty Acid Synthesis

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The *Trypanosoma brucei* surface is coated with 10^7 identical copies of a single Variant Surface Glycoprotein (VSG), one of ~2500 VSGs encoded in the genome. VSG helps *T. brucei* evade host immune response by two mechanisms: antigenic variation (changing the surface coat) and endocytosis (internalizing immune complexes, recycling the VSG while degrading the immune components). For both strategies, the VSG glycosylphosphatidylinositol (GPI) membrane anchor and its two myristates are thought to play a key role in VSG mobility and trafficking. The role of fatty acid synthesis (FAS) in VSG function is unknown, but it is likely needed to maintain VSG GPI-anchor myristoylation. To reduce FAS, we use RNAi-mediated depletion of Acetyl-CoA Carboxylase (ACC), the first enzyme in the FAS pathway. To determine the effect of ACC RNAi on VSG dynamics, we assessed VSG half-life by surface biotinylation and streptavidin blotting. We found that ACC RNAi resulted in ~40% decrease in surface VSG half-life ($p<0.05$). The extent of biotinylated VSG internalization in ACC RNAi induced cells after 10 min. was double the extent seen in uninduced cells ($p<0.001$). We also examined the impact of ACC RNAi on endocytic trafficking. We found that ACC RNAi had no apparent effect upon receptor-mediated endocytosis using FITC-Concanavalin A or Alexa-488-transferrin; and the effect of ACC RNAi on fluid-phase endocytosis depended upon the marker, with endocytosis of FM 4-64 increased by 2-fold while FITC-dextran (500S) showed no change. Currently, we are examining two possible mechanisms leading to increased VSG turnover upon ACC RNAi: increased shedding in the media or increased intracellular degradation. Our preliminary data rules out degradation by proteasomes or lysosomes and reveals that ACC RNAi altered shedding of multiple surface proteins, including VSG. For the future, we will use proteomics to identify and quantify the proteins shed in the media, and we will investigate the mechanisms of their release.

Abstract #17

Trypanosoma brucei Bloodstream Form Regulates Glycosomal pH Different from Procyclic Form

Daniel H. Call, Erica Tovey, Lucas Eggers, Matthew Hyer, Emily Knight, James Morris, Kenneth A. Christensen

Little is known about how Kinetoplastida organisms regulate glycosome pH. Glycosomal pH likely affects glycolysis and other metabolic pathways, making its regulation important to survival. Previous work found *Trypanosoma brucei* procyclic form actively acidifies its glycosomes when starved using V-ATPase(s) and sodium/proton exchanger(s). It is unknown if the same is true for the bloodstream form since chemical pH sensors have not been successfully targeted to the glycosome in this life-stage. To overcome this challenge, we expressed a protein pH sensor, pHluorin2, with an appended peroxisomal targeting sequence in bloodstream form *Trypanosoma brucei*. The biosensor was localized to the glycosome as verified by colocalization with aldolase, a glycosome-resident enzyme. We measured relative pH change using flow cytometry. When starved, the bloodstream form acidifies its glycosome like procyclic form. Bloodstream form glycosomes appear to use only sodium/proton exchanger(s), not V-ATPases, as glycosome acidification was abolished when treated with EIPA but not bafilomycin. We identified two candidate sodium/proton exchangers and will test if they regulate glycosome pH by RNAi knockdown. These findings improve our understanding of the glycosome and its role in survival of *Trypanosoma brucei* and its relatives.

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Abstract #18

Host glycolytic metabolites serve as a vital fuel source for energy metabolism and intracellular survival of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that acquires vital nutrients from its host during infection. Previous literature revealed that host cells increase production of glycolytic intermediates upon *Toxoplasma* infection and lactate/pyruvate supplementation can partially rescue parasite growth when glycolysis is blocked, suggesting that *Toxoplasma* can take up lactate/pyruvate as a fuel source. However, the molecular mechanism of uptake from the host is unclear. Our bioenergetic analysis showed that *Toxoplasma* can ingest lactate and pyruvate and utilize these nutrients to fuel energy production. In fact, the glycolysis-deficient strain, Δ*hk,* lacking hexokinase, incorporated lactate/pyruvate to a greater extent than wildtype. To further elucidate how *Toxoplasma* transports lactate/pyruvate, we individually deleted three reported lactate/pyruvate transporters (FNT1-3) in wildtype and Δ*hk*. Our bioenergetic study showed that deletion of individual FNTs did not entirely prevent lactate/pyruvate acquisition. However, the removal of both FNT1 & FNT2 blocked lactate/pyruvate acquisition. A qPCR analysis showed that Tg*fnt2* is highly upregulated in the absence of Tg*fnt1,* suggesting functional compensation. Additionally, the Δ*hk*Δ*fnt1*Δ*fnt2* displayed a significant growth defect, indicating the importance of lactate/pyruvate ingestion for parasite survival. Further, the host's monocarboxylate transporter-1 (MCT1) is enriched on the parasitophorous vacuole membrane, suggesting that *Toxoplasma* hijacks the host's transporter for lactate/pyruvate incorporation. Collectively, our work presents a key nutrient acquisition pathway in *Toxoplasma gondii*.

Abstract #19

Cryptococcus neoformans GXM alters microglial cell migration and morphology in vivo

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Cryptococcal meningitis is an opportunistic disease particularly affecting immunocompromised patients. After inhaling yeasts/basidiospores, the encapsulated fungus *Cryptococcus neoformans* migrates from the respiratory system into the circulatory system. Ultimately this systemic infection has a predilection for the central nervous system, where the fungus invades and colonizes the parenchyma of the brain tissue. The mechanisms of crossing the blood-brain barrier have been characterized, however it is not well defined how the fungus is able to persist in the brain tissue to develop cryptococcal lesions or cryptococcomas. It is this mechanism of brain colonization that is the central question in this study. As the resident immune cells of the brain, microglia are critical in this process. To study how the fungus interacts with microglia during colonization, intracerebral infections were employed as the model to evaluate the immune response against fungal brain invasion. We hypothesized that the release of the fungal polysaccharide capsule, which is mainly made of glucuronoxylomannan (GXM), prevents microglial migration and activation. CXCR3-GFP-labeled mice were used to determine the microglial responses to the infection focusing on morphological and cell distribution changes. The cross-section analysis was conducted using confocal microscopy and Fiji analysis software along with H&E and Periodic-acid Schiff (PAS) stain. In comparison to the uninfected and acapsular *cap59* strain infection, brains infected with the wild-type H99 strain displayed an increased density of microglia around the site of infection. In addition, there were microglial morphological alterations. Future studies will describe the overall immune profile at the site of infection to determine the cerebral cryptococcosis dynamics over time.

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POSTER ABSTRACTS

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Abstract #20

Kinetic characterization of acetate kinase from the fungal pathogen *Cryptococcus neoformans* and phylogenetic analysis of eukaryotic acetate kinases

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Acetate kinase (ACK), which catalyzes the interconversion of acetate and acetyl phosphate (AcP) and plays crucial roles in acetate metabolism, was previously thought to be nearly ubiquitous in the Bacteria but present in only one genus of Archaea and absent in eukaryotes. We have identified putative genes encoding ACK in many fungi including the genome of the fungal pathogen *Cryptococcus neoformans*. Although ACK primarily partners with phosphotransacetylase (PTA) in bacteria and archaea, the absence of PTA in fungi suggests the possibility of a non-canonical pathway of acetate metabolism in *C. neoformans*. The presence in fungi of xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (Xfp), which converts phosphoketose sugars into acetyl phosphate (AcP), suggests that Xfp could partner with Ack to produce acetate. However, a kinetic characterization of a fungal acetate kinase has not been reported. Here we kinetically characterized the *C. neoformans* ACK (CnACK) and observed that CnACK preferentially utilizes Mg^{2+} as divalent cofactor and ATP as phosphate donor for the interconversion of AcP and acetate. Comparison of K_M and catalytic efficiency of CnACK for AcP and acetate suggests CnACK strongly prefers the acetate-forming direction of the reaction, which has metabolic implications *in vivo*. To gain a further understanding of the role of ACK in fungi and eukaryotes, we searched completed eukaryotic genome sequences and identified 526 species of Eukarya that possess putative ACKs. Phylogenetic analysis of these eukaryotic ACK (eACK) sequences suggests a composite picture of the evolutionary history of this enzyme in the domain Eukarya. This is the first report of the biochemical characterization of a fungal ACK. The potential for alternative pathways of acetate metabolism in *C. neoformans* involving ACK needs further study as acetate metabolism has been suggested to be implicated in cryptococcosis.

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Abstract #21

Identification of de novo biogenesis contribution to glycosome proliferation in Trypanosoma brucei

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Trypanosoma brucei is a parasite responsible for causing human African trypanosomiasis (HAT), an illness affecting people in the Sub-Saharan Africa, and nagana, a wasting disease in livestock. *T. brucei* forms part of a group of flagellated protozoans called Kinetoplastids, which compartmentalize most of the enzymes required for glycolysis in specialized peroxisomes called glycosomes. In other eukaryotes peroxisome proliferation occurs through the fission of existing organelles ***and*** de novo biogenesis from the endoplasmic reticulum (ER). While glycosome proliferation in *T. brucei* can occur through the fission of existing organelles and indirect evidence suggests glycosomes can be made *de novo*, this process has not been directly demonstrated. In yeast and mammalian cells, secretory pathway proteins Snf7, Sec20, Sec61p, and Sec16 are required for peroxisome biogenesis from the ER. To resolve the potential contribution of *de novo* biogenesis to glycosome proliferation in *T. brucei*, we are testing how silencing of secretory proteins listed above influences glycosome size, number, and remodeling. We generated RNA interference cell lines for homologs of each of the proteins using the pZJM silencing vector and followed glycosome remodeling using a fluorescent reporter system that enables the visualization of glycosome dynamics in live cells via flow cytometry. RNAi cell lines took longer to replenish glycosomes after induction of pexophagy, suggesting these secretory pathway proteins may be involved in glycosome biogenesis. Future work will concentrate on resolving the mechanisms that govern this process and the extent to *de novo* biogenesis contributes to glycosome proliferation in *T. brucei.*

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Abstract #22

The Mitochondrial Ubiquinone Synthesis of *Toxoplasma gondii*

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Toxoplasma gondii is an Apicomplexan parasite that infects approximately one third of the world human population. Toxoplasmosis can be fatal in immunocompromised patients. Current medicines available to treat toxoplasmosis have toxic side effects and require long term treatment for immunocompromised patients. Therefore, medicines with enhanced efficacy that target unique and essential metabolic pathways of *T. gondii* is of great interest to improve toxoplasmosis therapy. Ubiquinone (UQ) is an essential component of the mitochondrial electron transport chain (ETC) as it shuttles electrons from complex I or II to complex III. Moreover, very little is known about this pathway in *T. gondii* or other apicomplexan parasites. The *T. gondii* UQ synthesis pathway appears to diverge from the mammalian one and several enzymatic steps could represent novel therapeutic targets. In *Saccharomyces cerevisiae*, several Coq enzymes form a complex called ubiquinone synthome, which has not been characterized in *T. gondii*. The assembly of these peptides to form this complex is proposed to enhance catalytic efficiency and to minimize the escape of toxic intermediates due to their redox or electrophilic activities. We characterized two genes annotated as TGGT1_266850 (TgCoq3) and TGGT1_295690 (TgCoq5) using conditional gene disruption followed by various functional analysis assays. We found that TgCoq3 and TgCoq5 encode for two mitochondrial proteins that are essential for parasite growth and mitochondrial activities. To study the biochemical function of TgCoq3 we complemented the yeast Coq3 knockout strain with *TgCoq3* and found that it can be utilized in yeast to partially rescue the growth defect. To determine whether TgCoq3 forms part of a synthome we created C-terminal tagged cell lines with the TurboID biotin ligase and showed interaction between both TgCoq3 and TgCoq5. The future goal of this project is to determine the presence of the synthome protein complex using immunoprecipitation and mass spec analyses.

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Abstract #23

Identification and Characterization of Virulence Genes Required for Acetate Utilization in the Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is an invasive fungal pathogen that causes nearly one million cases of cryptococcal meningitis and over 190,000 deaths each year. *C. neoformans* can be known as an opportunistic pathogen because it takes advantage of individuals with compromised immune systems, specifically ones with HIV. *C. neoformans* is inhaled through the lungs, which is a low-glucose environment, so it is likely that it must use alternative carbon sources such as acetate to survive. The goal of this research is to identify genes in *C. neoformans* necessary for acetate utilization and then characterize their role in metabolism and pathogenesis.

We investigated deletions of the genes CNAG_02045, CNAG_02165, CNAG_04795, and CNAG_00613. CNAG_00613 was identified as one of fifteen genes required for acetate utilization in a screen of nearly 4000 gene deletion mutants. CNAG_02045, CNAG_02165, CNAG_04795 are part of a more recent 2000 Madhani gene deletion library that has not yet been screened but are expressed at a higher level when grown on acetate versus glucose. Spot assays were performed on YNB media with either 2% glucose, 0.2% glucose, 2% acetate, or 2% glycerol as carbon sources to determine the functions of these genes in the metabolism of these carbon sources. CNAG_00613 did not grow on YNB + NAT media, which was unexpected, and prevented further spot assay research from being conducted. The other three gene deletion mutants all grew on glucose, acetate, and glycerol.

Another one of the gene deletion mutants identified in our screen for acetate utilization genes was CNAG_00403, which we hypothesize encodes for gamma-butyrobetaine hydroxylase (BBox), which catalyzes the final step of carnitine biosynthesis. Thus, the biosynthesis of L-carnitine, which is the carrier of cytoplasmic acyl-CoA into the mitochondria, is necessary for acetate utilization. We are utilizing 3,5-pyridinedicarboxylic acid, an inhibitor of human BBox, to further examine the role of the carnitine biosynthesis pathway in acetate utilization.

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Abstract #24

Phosphoinositide phospholipase C is essential for the infective stages of *Trypanosoma cruzi* but is not involved in the synthesis of inositol pyrophosphates

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Phosphoinositide phospholipase C (PI-PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). In mammalian cells, IP_3 gates the IP_3 receptor, and stimulates Ca^{2+} release, while DAG remains in the membrane and stimulates protein kinase C. PI-PLC plays an essential role in cell signaling and it regulates many processes, such as cell division, secretion, and differentiation in these cells. In yeasts, IP_3 can be further phosphorylated at different hydroxyl positions producing IP_4 and IP_5 by inositol phosphate multikinase (Arg82), and IP_6 by inositolpentakisphosphate kinase (IPK1), producing the fully phosphorylated form known as inositol hexakisphosphate (IP_6) or phytic acid, which can be a precursor of the inositol pyrophosphates. Inositol pyrophosphates are characterized by the presence of single (PP-IP_4 and PP-IP_5) or double (PP_2-IP_3 and PP_2-IP_4) pyrophosphate moieties linked at different positions of the *myo*-inositol backbone. Here, we report the use of a CRISPR/Cas9-based strategy to generate *TcPI-PLC* knockout and complemented epimastigotes, as confirmed by PCR, Southern blot, and RT-PCR analyses. *TcPI-PLC*-KO epimastigotes were viable and able to undergo metacyclogenesis. However, *TcPI-PLC*-KO trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes. Capillary electrophoresis-electrospray ionization-mass spectrometry analyses of inositol pyrophosphates revealed that their synthesis was not affected in *TcPI-PLC*-KO epimastigotes, indicating that inositol pyrophosphate synthesis does not depend on the formation of IP_3 by TcPI-PLC and suggesting the presence of a lipid-independent pathway involved in their synthesis. In conclusion, our data shows that *TcPI-PLC* is dispensable in epimastigotes, but it is critical for the virulence of infective stages. In addition, deletion of *TcPI-PLC* does not affect the inositol pyrophosphate synthesis pathway.

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Abstract #25

Characterization of the Ato family of transporters in the fungal pathogen *Cryptococcus neoformans*

Will Betsill and Kerry Smith

Invasive fungal infections cause nearly one and a half million deaths annually, accounting for nearly 50% of all AIDS-related deaths. The basidiomycete *Cryptococcus neoformans* is the most frequent cause of fungal meningitis and is responsible for nearly half of the invasive fungal infections. The Centers for Disease Control estimates the yearly burden of cryptococcal meningitis to be nearly one million cases with greater than 190,000 deaths. AIDS is a major risk factor and mortality rates in AIDS patients range from 55-70% in Latin America and sub-Saharan Africa. Lung alveolar macrophages, which present a first line of host defense against *C. neoformans* infection, provide a glucose- and amino acid-poor environment, and nonpreferred carbon sources such as lactate and acetate are likely important early in establishment of a pulmonary infection.

The expectation is that the acetate transport will have a role in pathogenesis as other researchers have already shown that acetyl-CoA synthetase, the enzyme that activates acetate to acetyl-CoA for entrance into various metabolic pathways, is required for full virulence. We have shown that Ady2 (Ato1, CNAG_05678) is required for growth at concentrations of 0.2% or less of acetate as sole carbon source; however, at higher levels (0.5% and higher) of acetate, passive diffusion of acetate is sufficient for growth. Ato1 (Ady2) belongs to a three member Ato (ammonium transport outward) family of transporters in *C. neoformans*, but the functions of Ato2 (CNAG_05266) and Ato3 (CNAG_04787) are unknown. Previous research, in collaboration with the Levitz lab at Mass Chan Medical School, indicated a strain in which Ato1 and Ato2 were both deleted significantly attenuated virulence in *C. neoformans.* However, other phenotypes, of the double mutant strain, such as the reduced ability to grow at 37°C, were absent in either of the *a*to1 or *ato2* single mutants suggests the ‘double mutant’ strain may have other defects and that effect on virulence may not be the results of deleting both *ato1* and *ato2*. To resolve this, we are creating new double and triple knockouts of the Ato family using TRACE (Transient CRISPR-Cas9 coupled with Electroporation).

Abstract #26

Structure-activity relationship study of the Diazacyclobutene (DCB) motif against the protozoan parasites *Trypanosoma brucei* and *Trichomonas vaginalis*

William T. Bridges, Monireh Noori, Brock A. Miller, James C. Morris, Daniel C. Whitehead

The eukaryotic protozoan parasite *Trypanosoma brucei* is responsible for causing the neglected tropical disease Human African trypanosomiasis (HAT) endemic to sub-Saharan Africa. This infection, which is transmitted via bite of the native tsetse fly as a vector, can be fatal if the infected individual does not receive treatment. The typical therapeutic treatments for this disease are hindered by tedious treatment regimens, toxicity, adverse side effects, and/or antibiotic resistance. Our lab has developed succinct synthetic routes to access Diazacyclobutenes (DCBs), a class of heterocycles which are a novel molecular scaffold with reference to their biological activity. Multiple DCB derivatives have exhibited low EC_{50} values against blood-stream form *T. brucei brucei* and exhibit low-mammalian cytotoxicity. Utilizing the “parasite-hopping” paradigm, we have preliminary data that shows the DCB motif may also be biologically active towards the protozoan parasite *Trichomonas vaginalis*. This parasite is the cause of the most common non-viral sexually-transmitted disease in the world – Trichomoniasis. We plan to develop a sensitive, robust bioassay to test these DCB derivatives against *T. vaginalis*. This presentation will describe our preliminary biological results and our efforts to expand our library of DCB-based compounds for a thorough structure-activity relationship (SAR) against both parasite in order to identify the core pharmacophore responsible for anti-parasitic activity.

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Abstract #27

Comparing development of drug resistance by *Cryptococcus neoformans* to chemically distinct azole anti-fungal compounds

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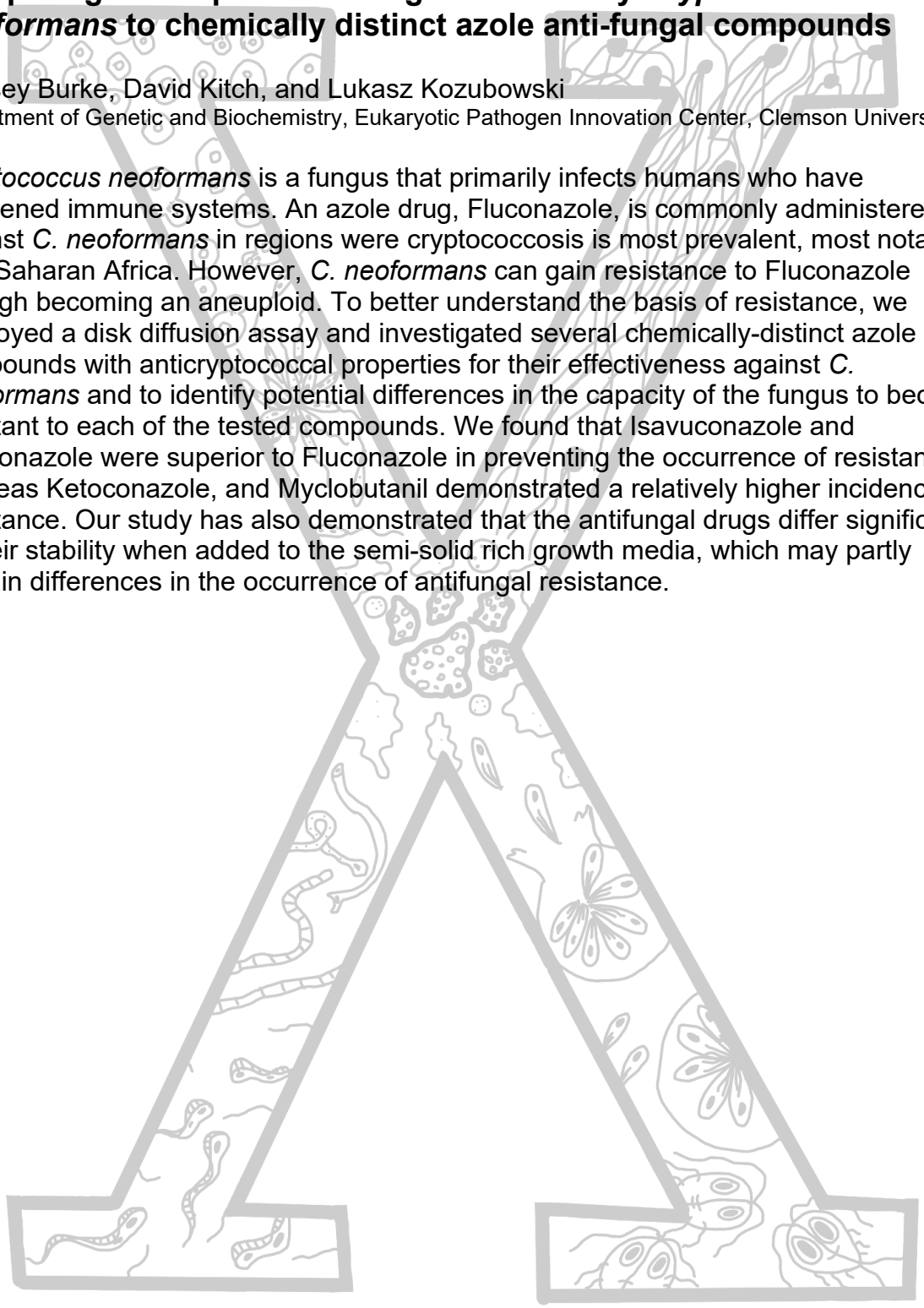
Cryptococcus neoformans is a fungus that primarily infects humans who have weakened immune systems. An azole drug, Fluconazole, is commonly administered against *C. neoformans* in regions were cryptococcosis is most prevalent, most notably Sub-Saharan Africa. However, *C. neoformans* can gain resistance to Fluconazole through becoming an aneuploid. To better understand the basis of resistance, we employed a disk diffusion assay and investigated several chemically-distinct azole compounds with anticryptococcal properties for their effectiveness against *C. neoformans* and to identify potential differences in the capacity of the fungus to become resistant to each of the tested compounds. We found that Isavuconazole and Voriconazole were superior to Fluconazole in preventing the occurrence of resistance, whereas Ketoconazole, and Myclobutanil demonstrated a relatively higher incidence of resistance. Our study has also demonstrated that the antifungal drugs differ significantly in their stability when added to the semi-solid rich growth media, which may partly explain differences in the occurrence of antifungal resistance.



Abstract #28

Carnitine Biosynthesis in *Cryptococcus neoformans*

Dylan Carroll, Rodrigo Catalan-Hurtado, Kerry Smith

Fungal infections cause over one and a half million deaths annually, while the most common invasive species *Cryptococcus neoformans* kills more people each year than tuberculosis. In immunocompromised individuals, namely HIV/AIDS patients, *Cryptococcus* is extremely virulent and can spread to the brain causing meningitis. Due to increases in immunosuppressive drugs and a large population of humans living with some form of autoimmune disorders, cryptococcal meningitis cases have become prevalent. Furthermore, the cellular processes of fungal cells are similar to those in human cells that the identification of unique targets for the development of antifungal drugs is challenging.

As the site of *Cryptococcus* infection in the lungs provides a glucose- and amino acid-poor environment, the Smith lab hypothesizes utilization of acetate is likely important early in establishment of pulmonary infection. One of fifteen mutants identified in a genetic screen of the Madhani gene deletion libraries to find genes crucial for acetate utilization was CNAG_00403. We hypothesize this gene encodes for gamma-butyrobetaine hydroxylase (BBox), a mitochondrial protein that catalyzes the synthesis of L-carnitine from gamma-butyrobetaine in the final step of carnitine biosynthesis. L-carnitine assists in the transport of cytoplasmic generated acyl-CoA into the mitochondria to be broken down as part of the Krebs Cycle to produce energy for the fungi.

The CNAG_00403 deletion mutant can grow on YNB + 2% glucose but not YNB + 2% acetate. Addition of L-carnitine to the mutant on YNB + 2% acetate restores growth. Furthermore, if this hypothesis is correct, the addition of the substrate gamma-butyrobetaine should not rescue growth on 2% acetate + YNB. This mutant also has decreased melanin production, a known virulence factor for *Cryptococcus*, when tested on melanin-inducing conditions. We are further examining the growth condition in which the CNAG_00403 mutant is required and investigating the role of the carnitine biosynthetic pathway to determine if it could serve as a drug target for *Cryptococcus*.

Abstract #29

Further characterizing acetate production in the fungal pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans, an invasive opportunistic fungal pathogen, is the most frequent cause of fungal meningitis resulting in greater than 190,000 deaths per year worldwide. Current methods of treatment are inadequate for most of the infected population; therefore, it is imperative to find novel therapeutic targets. *C. neoformans* has been shown to upregulate genes encoding products involved in the production of acetate during murine infection. The xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (Xfp) – acetate kinase (Ack) pathway, previously thought to be present only in bacteria, converts phosphoketose sugars to acetate thought acetyl-phosphate. The pyruvate decarboxylase (Pdc) and acetaldehyde dehydrogenase (Ald) pathway, found in other fungi, converts pyruvate to acetate through acetaldehyde. The role that acetate production enzymes play during primary infection is yet unknown. In this study, we observed the growth of acetate production gene knockouts, Δ*pdc*, Δ*ald5,* Δ*ack*, Δ*xfp2* and the double mutant Δ*pdc*Δ*ack* on various carbon sources and stress conditions. Three mutants, Δ*xfp2,* Δ*ack* and Δ*pdc*Δ*ack*, presented decreased growth on xylose, while only Δ*xfp2* and Δ*ack* exhibited reduced growth on acetate. Furthermore, preliminary data shows that all five mutants showed impaired growth on the cell membrane stress sodium dodecyl sulfate, but only Δ*xfp2,* Δ*ack* and Δ*pdc*Δ*ack* displayed a defect on growth with the cell wall stress congo red. Additionally, Δ*xfp2* and Δ*ack* presented decreased growth on the oxidative stress, hydrogen peroxide. Interestingly, Δ*pdc,* Δ*ald5* and Δ*pdc*Δ*ack* produced more melanin, an important virulence factor, than wildtype.

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Abstract #30

Characterization of interkingdom microbial interactions in pigeon excreta

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The study of polymicrobial interactions has been useful to understand the evolution of virulence in specific microbial species. The pathogenicity of microorganisms in mammals and plants can be altered depending on their interactions with other microbes in their natural habitat. Pigeons travel all over the world and can transport microorganisms from one place to another. Pigeon excreta provides a rich content of nitrogen, phosphate, and potassium for the successful growth of microorganisms and has been identified as a habitat for potentially pathogenic microbes including bacteria, fungi, and parasites. In this study, we analyzed the microbial communities and the potential interactions between interkingdom prokaryotic and eukaryotic microbes in pigeon excreta samples using next generation sequencing. We found that bacteria had the highest index of diversity. We identified medically important bacteria in our samples including *Staphylococcus aureus, Clostridium perfringens, Bacillus anthracis and Salmonella enteritidis.* Among the eukaryotic microorganisms, the genus *Gregarinasina* was the only protozoa found in a significant percentage. Most of the fungi species found were related with plant, animal, or human diseases. Among the medically important fungi identified were *Cryptococcus neoformans, Malassezia obtusa, Aspergillus species, and Candida albicans. Cryptococcus* species, which are typically associated to pigeon excreta, were abundant in samples where *Salmonella* species were present and its relative abundance correlated with the presence of pathogenic bacteria including *Enterobacter cloacae, Azosporilum brasiliense, Escherichia coli, Pseudomonas, Klebsiella, S. enterica, Streptococcus and S. aureus*. Our studies are significant because they help us to understand the interactions of microorganisms in their natural environment and the impact of these relationships in infection and disease in the human host.

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Abstract #31

Understanding Fatty Acid Uptake in *T. brucei* Through Gene Knockouts

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Trypanosoma brucei is a parasite that causes African Sleeping Sickness, a disease that impacts humans and livestock in sub-Saharan Africa. This disease can be deadly, and there are limited safe and effective treatments available for the people of this region. Our goal is to find new ways to treat this infection by gaining a better understanding of the metabolism of the parasite. The purpose of my project is to understand the function of genes related to the uptake of fatty acids in *T. brucei*. At the moment, little is known about the proteins involved in fatty acid uptake. We are focusing on two genes, FATP and LACS5, that are believed to play a role in the uptake process. This assumption is based on these genes having related functions in other organisms. To do this, we are creating knock-out *T. brucei* cell lines for FATP and LACS5 to test what happens to fatty acid uptake in these parasites when FATP or LACS5 is genetically deleted. We are using Gibson Assembly to generate constructs that directly replace each allele with antibiotic resistance genes. Once the constructs are completed and validated, they will be transfected into *T. brucei* to generate the knock-out cell lines, followed by validation and phenotypic characterization. By identifying genes essential for fatty acid uptake, we can understand how the parasite acquires essential nutrients from its host. This work may eventually enable us to create better treatments for African Sleeping Sickness and potentially cure a disease that kills thousands yearly.

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Abstract #32

A role for the Rsp5 ubiquitin ligase and its interactome in the pathogenesis of *Cryptococcus neoformans*

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The short ubiquitin polypeptide serves as a “death warrant” that condemns misfolded and spent proteins to proteasomal degradation. However, recent studies have also elucidated a regulatory role for ubiquitination in addition to its role in protein degradation, including the regulation of the location, activity, and expression of proteins with the attachment of mono- or poly-ubiquitin tags. During this form of posttranslational modification, the 76-amino acid ubiquitin polypeptide is covalently linked to substrate proteins by the three-component ubiquitination system consisting of the ubiquitin activating, conjugating, and ligase enzymes. One such ubiquitin ligase, Rsp5, is known to be required for the survival of the human fungal pathogen, *Cryptococcus neoformans*, in a murine infection model and for growth at elevated temperatures. In our hands, an *rsp5*Δ mutant strain is also unable to grow in the presence of various cellular stressors, such as Congo red, sodium chloride, and caffeine. This ubiquitin ligase is known to interact with adaptor proteins, most notably arrestin-like (or ART, arrestin-related trafficking adaptor) proteins to facilitate recognition of substrate proteins. We have previously identified four arrestin-like (Ali1-4) proteins in *C. neoformans*, and showed that Ali1 contributes to cytokinesis, likely through interactions with Rsp5. Recently, we have also shown that Rsp5 interacts with Ali2 to regulate the localization of a Fur4-like transporter. In addition, a high-throughput differential ubiquitination screen revealed several targets of Rsp5 that are involved in stress response and, therefore, also potentially in pathogenesis. Rsp5 and its adaptor network are likely involved in the regulation of multiple pathogenesis-related pathways, which will form the basis of further studies.

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Abstract #33

Role of Cell Density in Encystation of *Entamoeba histolytica* in Axenic Culture

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Entamoeba histolytica is a parasitic protozoan that causes amoebic dysentery through the consumption of contaminated food and water contaminated with the cyst form which is encapsulated in a hard chitin cell wall. The cysts then undergo excystation to the trophozoite form in the human host. Trophozoites can undergo encystation to convert back to the cyst form and are shed in feces to continue disease spread. Encystation has been primarily in the reptile pathogen *Entamoeba invadens* but recent developments now allow these studies to be conducted directly in the human pathogen.

In this project, I investigated glucose limitation and cell density to enhance the rate of in vitro encystation. Cells were grown in standard glucose medium and transferred to medium lacking glucose at different starting cell densities for 24 to 48 hours. Initiation of encystation was assessed using reverse transcriptase PCR (RT-PCR) of two genes shown to be regulated during early encystation. The *Jacob* gene encodes a protein that forms part of the chitin cell wall and its gene is expressed early in encystation. The gene encoding heat shock protein 90 (Hsp90) is downregulated during initiation of encystation. I examined expression of these two genes as a means to assess whether the conditions tested cause earlier initiation of encystation than the current standard encystation conditions.

The optimal conditions determined here will be combined with other factors to improve synchronization of encystation in *E. histolytica* for future studies.

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Abstract #34

Determining the role of p22^{phox} in macrophages with hosts infected by *Aspergillus fumigatus*

Lynette Goins and Emily E. Rosowski

Aspergillus fumigatus is a fungus capable of causing diseases such as invasive aspergillosis in immunocompromised individuals. With resistance to azole antifungals increasing, understanding how the innate immune system responds to *A. fumigatus,* specifically in the first responder cells macrophages, is needed. Macrophages help in the prevention of hyphal growth of *Aspergillus.* LC3-associated phagocytosis (LAP) is one of the pathways in macrophages that can target *A. fumigatus* to the lysosome for degradation. As part of this pathway, the NADPH oxidase 2 complex (NOX2) is recruited to produce reactive oxygen species (ROS) within the phagosome. Within this complex is p22^{phox}, a cytosolic protein needed for the stabilization of NOX2. However, the exact role p22^{phox} plays in macrophages to promote the clearance of *A. fumigatus* is not fully understood. Therefore, we will look at the function of p22^{phox} in macrophages using zebrafish as an *in vivo* model. Using confocal microscopy, we seek to quantify the rate of *A. fumigatus* germination and ROS production in macrophages in p22^{phox}-deficient larvae as compared to wild-type larvae. Further, we aim to determine how the absence of p22^{phox} may impact the efficacy of anti-fungal drugs. By studying this protein, we will gain novel insights into how macrophages respond to *A. fumigatus* spores and hyphae during the early stages of infection.

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Abstract #35

Interaction between osmotic regulation and temperature stress in *Crithidia fasciculata* and other contractile vacuole-containing organisms

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Most organisms undergo regulatory volume decrease in response to hypotonic stress. The contractile vacuole (CV) organelle complex in trypanosomatids, amoebas, ciliates, and other single-celled eukaryotes controls expels excess water from the cell at a rate highly dependent on the environmental temperature. Faster CV cycling occurs at higher temperatures, following an Arrhenius linear relationship between inverse temperature and natural log of CV rate. The kinetics of regulatory volume decrease in *Crithidia fasciculata* at different temperatures was studied using cell death assays, a light scatter assay for cell shape changes, and a ninhydrin assay measuring release of small amines (amino acids) during initial cell swelling. At higher temperatures, the cells were much more sensitive to hypotonic stress, perhaps due to overwhelming the CV cycling mechanisms. The unique and understudied CV organelle in human parasites presents an interesting target for anti-parasitic drug development, especially when connections between other stress response pathways are better understood.

Abstract #36

Investigating the structure-activity of Oxadiazon derivatives for developing an effective therapy for *Toxoplasma Gondii* infections

Samuel Kwain, Rajib Islam, Zhicheng Dou,* Daniel C. Whitehead*

Toxoplasma gondii, a major species within the Apicomplexa phylum and is believed to infect approximately one-third of the human population worldwide. While *T. gondii* infections are generally asymptomatic and infrequently diagnosed in immunocompetent individuals, the parasite can cause severe and sometimes fatal diseases in people with impaired immune systems. Current treatments involve combinatorial therapy which can cause severe side effects in some patients. We previously reported that inhibition of the plant-like protoporphyrinogen oxidase (PPO) enzyme in the de novo heme biosynthesis pathway in *T. gondii* offers a potential druggable target to develop an effective therapy against this parasite. We showed that oxadiargyl, an analog of the herbicide, oxadiazon that inhibits the PPO in the heme and chlorophyll biosynthesis in plants is capable of inhibiting the *T. gondii* PPO. Interestingly, structural modification of the oxadiargyl with benzyl azides by means of an alkyne/azide click reaction resulted in new oxadiazon derivatives some of which showed potent inhibitory activity against the *T. gondii* PPO with IC_{50} values below 2.5 μM. Based on this interesting result, we have synthesized a library of 23 new oxadiazon derivatives based on the modification of oxadiargyl with a series of aliphatic azides containing other functional groups for a structure-activity relationship study. We believe that this study will further broaden our understanding of the structural effectiveness of oxadiazon derivatives and will help us develop an effective therapy against toxoplasma infections in humans and other animals.

Keywords: *Toxoplasma gondii*, protoporphyrinogen oxidase, Oxadiazon, oxadiargyl, structure-activity relationship.

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Abstract #37

Putative GPI-anchored protein Fgr41 impacts cell wall organization in *C. albicans*.

Ainsley King

Candida albicans is a pathogenic fungus that can cause life-threatening infections. Several different antifungals can be used to treat *C. albicans* infections; however, resistance to many of these drugs is becoming an increasingly serious problem. One potential alternative method of treating these infections is to increase immune recognition (and therefore clearance) of the pathogen. The cell wall of *C. albicans* contains β(1,3)-glucan, which triggers a robust pro-inflammatory response, making it a good target to leverage for immunotherapy. However, an outer layer of mannosylated proteins covers, or masks, this layer of β-glucans, severely hindering immune recognition. If this masking could be reduced, then immune recognition would increase. Our lab has discovered that a largely uncharacterized protein, Fgr41, plays a role in the organization of the *C. albicans* cell wall; it was found previously that this protein is downregulated during treatments that cause unmasking, such as treatment with the echinocandin caspofungin and overexpression of the MAP3K *STE11*. We therefore knocked out *FGR41* and found that this mutant has increased unmasking of β(1,3)-glucan. Furthermore, we determined that Fgr41 activity can actively cause β(1,3)-glucan masking, as overexpression of *FGR41* causes a reduction in unmasking caused by *STE11* overexpression. Finally, we confirmed that the *fgr41Δ/Δ* mutant exhibits some filamentation defects, including a reduction in the length and number of hyphae in early growth stages. It remains to be determined if this is related to its impacts on the cell wall. Further characterization of this protein will allow us to better understand the factors that control cell wall organization and structure.

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Abstract #38

Development of novel chemical inhibitors against the infection of *Toxoplasma gondii*, a human protozoan pathogen

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Toxoplasma gondii is an obligate intracellular parasite, which infects almost 40 million people and causes severe complications or may be fatal in immunocompromised individuals. There are a few commercial antibiotics against toxoplasmosis, but they show considerable side effects in certain populations. The different features in nutrient metabolism between *Toxoplasma* parasites and their host cells draw attention to these pathways for therapeutics. Recent genetic studies in our lab reported that the heme biosynthetic pathway in *Toxoplasma* is indispensable. The intracellular survival and acute virulence of *Toxoplasma* parasites are severely compromised when they encounter heme deprivation. Some steps within the parasite's heme metabolism displayed plant-like features. Therefore, targeting the heme pathway of *Toxoplasma* has therapeutic potential. The primary structure analysis of protoporphyrinogen oxidase (PPO), the enzyme catalyzing the penultimate reaction within the heme biosynthetic pathway, is more closely related to the plant counterpart compared to mammalian orthologs. Our latest work has synthesized a group of oxadiazon derivatives showing high potency against parasite growth with low toxicity in human cells. In this study, we modified the side chain of oxadiazon and generated a small library of oxadiazon derivatives with improved aqueous solubility. Currently, we are measuring their potencies against intracellular replication of *Toxoplasma* parasites and will further explore the extent to which the top candidates suppress the parasite's mitochondrial functions.

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Abstract #39

Synthesis and computational studies of Enolase inhibitors as therapeutic leads for *Naegleria fowleri* infection

Samuel Kwain, Jillian E. Milanes, Kerrick C. Rees, Brian N. Dominy, James C. Morris, and Daniel C. Whitehead

The pathogenic free-living amoebae *Naegleria folweri* can cause severe and nearly always fatal illness in infected individuals. Currently, there is a paucity of treatment options for the infections caused by this parasite in humans. A recent investigation of human enolase 2 (ENO2) phosphonate inhibitors revealed that (1-hydroxy-2-oxopiperidin-3-yl)phosphonic acid (HEX) and its structural analogs – in particular (1,5-dihydroxy-2-oxopyrrolidin-3-yl) phosphonic acid (SF2312) – elicit potent inhibitory activity against *N. fowleri* ENO (*Nf*ENO) with the former being the more potent inhibitor (IC_{50} value of 0.14 ± 0.04 μM). To gain insight into how these compounds bind and inhibit the (*Nf*ENO) enzyme, we performed a molecular docking simulation of the compounds with the X-ray crystal structure of *Nf*ENO (PDB 7UGH) using Autodock Vina. The docking model revealed that HEX and SF2312 bind strongly to the active site of *Nf*ENO with binding affinities of –8.6 and –8.5 kcal/mol respectively. Detailed analysis of the binding poses of the *Nf*ENO-HEX and *Nf*ENO-SF2312 complexes show that the hydroxamate moiety present in these compounds chelates the sodium metal ion and also interacts through hydrogen bonding with other important catalytic residues at the active site of the *Nf*ENO enzyme. This result suggests that HEX and SF2312 have the desired structural and electrostatic complementarity to fit and interact effectively at the active site of *Nf*ENO. The main functional groups present in HEX and its analogs are the hydroxamate and the phosphonate moieties. In the current effort, we hope to replace the phosphonate group with sulfonate and other functional groups and probe their binding properties and inhibition efficacy with the *Nf*ENO parasite using the docking model and experimental bioassay. This would help us develop a potent small molecule inhibitor against the *N. folweri* parasite. This presentation will describe the synthesis and docking evaluation of HEX and SF2312 as potential inhibitors of *Nf*ENO.

Keywords: inhibitors, *Naegleria fowleri*, molecular docking, hydroxamate moiety, pathogen

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Abstract #40

Pathogenicity of *Naegleria fowleri* isolates vary significantly in the mouse model of primary amoebic meningoencephalitis

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(*equal contributions)

Naegleria fowleri, colloquially known as “brain-eating amoeba”, causes an acute, fatal disease called primary amoebic meningoencephalitis (PAM). *N. fowleri* is commonly present in warm freshwater and soil and although PAM is a rare disease, it results in >97% mortality rate. One persisting question is why few people succumb to disease when so many are potentially exposed? We hypothesized that clinical isolates vary in inherent virulence and these variabilities affect the minimum infectious dose required to induce PAM. Utilizing a mouse model of PAM, we intranasally inoculated clinical isolates of *N. fowleri* (Nf69, V067, V631, Villa Jose, and V596) at three concentrations per mouse: 100, 1000, and 5000 amoebae. Results showed significant variability in onset of severe disease and fatality rates among isolates and within genotypes. The highest infectious dose (5000 amoebae) induced 100% morbidity from all isolates except V067(87.5%), with a large variance in onset of endpoint symptoms from 4 days post inoculation (dpi) to > 20 dpi. Remarkably, V596 produced 100% mortality with as few as 100 amoebae. Concurrently we assessed *in vitro* pathogenicity by comparing feeding rates among isolates seeded onto Vero cells. We observed similar variability in feeding rates for 12 *N. fowleri* isolates and although not all isolates were tested *in vivo*, our data suggest a correlation between an increased feeding rate *in vitro* and virulence *in vivo*. Overall, these results support our hypothesis of inherent differences in pathogenicity between isolates that result in variance in minimum infectious doses. We acknowledge support from NIH (R03AI141709 to DEK and 5T35OD010433-14 to ES) and the Georgia Research Alliance to DEK.

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Abstract #41

Mutation of predicted Pdr1 phosphosites restores fluconazole sensitivity in *Candida glabrata*

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Resistance to azole-class antifungals in *Candida glabrata* and *Saccharomyces cerevisiae* is mediated by Pdr1, a $Zn(II)_2Cys_6$ transcription factor that initiates the transcription of drug efflux pump genes. Many $Zn(II)_2Cys_6$ transcription factors are regulated by phosphorylation, and we hypothesized that Pdr1 activity is regulated by phosphorylation as well. To test this hypothesis, we predicted phosphosites in *C. glabrata* Pdr1 using the NetPhos Yeast server and selected high-probability sites based on comparison with known phosphosites in *S. cerevisiae* Pdr1. A *pdr1*Δ *C. glabrata* strain was complemented with Pdr1 variants containing alanine or glutamic acid at predicted phosphosites. Spot dilution assays and agar gradient diffusion assays identified 5 variants that restored sensitivity to fluconazole and 1 variant that increased fluconazole resistance. Currently, mass spectrometry phosphoproteomics is being used to confirm potential phosphosites and identify additional sites for investigation. Our future work will explore how phosphorylation affects Pdr1 localization, protein-protein interactions, protein-DNA interactions.

Abstract#42

Therapeutic Effect of Palmitoylethanolamide During Brain Infection with *Cryptococcus neoformans*

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Cryptococcus neoformans (Cn) is an encapsulated neurotropic fungal pathogen and the causative agent of cryptococcosis in humans. Infection typically occurs upon inhalation of fungal spores, followed by colonization of the lungs and dissemination into the bloodstream. Cryptococcal meningoencephalitis is the most severe form of infection and results from the ability of the fungus to cross the blood-brain barrier and reside in the meninges. Current recommended treatment with Amphotericin B (AmpB) disrupts the fungal membrane through an ergosterol-binding mechanism. Though effective, the potency of AmpB and its associated nephrotoxicity at fungicidal levels have prompted investigation into alternative methods of treatment for cryptococcosis. Palmitoylethanolamide (PEA) is an endogenous lipid compound characterized by previous studies as an immunomodulatory molecule capable of controlling and reducing neuroinflammation. This study investigates the therapeutic effects of PEA during cryptococcal infection to identify a potential alternative treatment for cryptococcosis. To determine whether PEA demonstrates fungicidal activity, we exposed *Cn* to varying concentrations of PEA alone and in combination with AmpB. The results showed that the combination group reduced fungal growth more effectively than AmpB alone when compared with the control, although PEA alone had no significant reduction. To evaluate the immunomodulatory effects of PEA treatment *in vivo,* we infected C57BL/6 mice intracerebrally with *Cn* and treated with AmpB, PEA, or a combination. We observed that the combination of PEA and AmpB extended the median survival by 8 days from that of the AmpB-treated group. Fungal burden was quantified from brain tissue for 3- and 7-days post-infection, and PEA reduced the fungal burden more effectively when combined with AmpB than AmpB treatment alone. The results suggest PEA confers a therapeutic synergistic effect during murine cryptococcosis when combined with AmpB and support further investigation of this compound as a potential supplement to AmpB treatment in humans.

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Abstract #43

The Role of Glucose Deprivation and Conditioned Media in *Entamoeba histolytica* Encystation

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Entamoeba histolytica is a pathogenic protist associated with intestinal infections, causing amoebiasis in ~100 million people and killing tens of thousands worldwide each year. Its life cycle consists of a cyst stage and a trophozoite stage. Infection begins via the ingestion of food and water contaminated with mature cysts, which excyst into the trophozoite form in the small intestine and go on to colonize the large intestine. *Entamoeba invadens*, a reptile pathogen, has previously served as the model for studying encystation as reproducible encystation could not be accomplished in the human pathogen. *E. invadens* is induced to encyst under conditions of osmotic stress, glucose deprivation, and high cell density, with mature cysts produced within 72 hours. We have now established *in vitro* encystation in *E. histolytica* and can study this phenomenon directly in the human pathogen, although the time for production of mature cysts is longer than for *E. invadens*. The overall goal of this project is to optimize encystation conditions to decrease the time frame for encystation of *E. histolytica* to that for *E. invadens*. To follow the onset of encystation, we monitored expression of the *Jacob* gene encoding a primary component of the chitin cell wall, one of the earliest morphological events in encystation. Our results are shown.

Abstract #44

Characterizing azole drugs in the fungal pathogen *Cryptococcus neoformans*

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Understanding mechanisms of anti-fungal drugs in the pathogenic fungus, *Cryptococcus neoformans*, is crucial for improving anticryptococcal therapy. We have previously determined that presence of
anti-oxidants in growth medium can reverse the fungal inhibition caused by the azole drug, fluconazole. However, treatment with the azole drug, fluconazole, presents the problem of resistance of *C. neoformans* to fluconazole treatment. Hence, we screened several azole drugs, including voriconazole, propiconazole, cyproconazole, miconazole, isavuconazole, tebuconazole, myclobutanil, and ketoconazole to determine the following: (1) ability to inhibit growth of H99 strain of *C. neoformans*, (2) MIC of the azole drugs in H99 strain of *C. neoformans*, (3) ability to induce Reactive Oxygen Species (ROS) in H99 strain of *C. neoformans*, and (4) ability of the anti-oxidant vitamin C to reverse growth of H99 in the presence of each of the drugs. We found that all azole drugs inhibit growth of H99 and we identified the MIC of the drugs against H99. Isavuconazole, voriconazole, and propiconazole showed the largest zone of inhibitions and the smallest MIC among all the drugs tested. However, growth of H99 in presence of these drugs (voriconazole, propiconazole, and isavuconazole) and vitamin C was reversed. Other drugs that showed decreased inhibition of H99 in the presence of vitamin C were ketoconazole and cyproconazole. Inhibitory effects of miconazole, tebuconazole, and myclobutanil were not affected by vitamin C. We found that all the drugs whose inhibitory functions were lowered by vitamin C also induced higher Reactive Oxygen Species (ROS) production in H99. We concluded that while all the eight azole drugs tested inhibited H99 growth, only five drugs (voriconazole, propiconazole, isavuconazole, ketoconazole, and cyproconazole) induced production of ROS in *C. neoformans.*

Abstract #45

Benzamidobenzoic Acids (BABA) as Novel Drug Scaffold for Treatment of Human African Trypanosomiasis (HAT)

Soham Panda, Sabrina Pizzaro, Meredith T. Morris, James C. Morris, Daniel C. Whitehead

Human African trypanosomiasis (HAT), also known as African sleeping sickness, is caused by the eukaryotic parasite *Trypanosoma brucei,* and is transmitted by blood-feeding tsetse flies. This disease, if untreated, is typically fatal. Most of the drugs currently used in the treatment of HAT have variable efficacy, adverse side effects or may require long-term treatments. This necessitates the identification of new chemotherapeutic approaches in trypanosomes for the treatment of the disease. Bloodstream forms (BSF) of *T. brucei* generate ATP exclusively through glycolysis. The first step in this glycolytic pathway is mediated by the hexokinase enzymes, TbHK1 and TbHK2, rendering them potential therapeutic targets for the treatment of HAT. Inhibition of these enzymes has been proven to be lethal to the parasite, and genetic manipulation has validated their status as a target. A high-throughput screening of 220,000 compounds to discover inhibitors of TbHK1 yielded three distinct scaffolds: isobenzothiazolinone, isobenzoselenazolinone, and benzamidobenzoic acid (BABA). Our research solely focuses on one of the scaffolds – BABA, which was further optimized using structure-activity relationship studies. Previous studies have demonstrated the successful localization of molecular cargo in the glycosomes (where TbHKs are compartmentalized) when modified by the addition of a peroxisomal targeting sequence (PTS1) – specific sequence(s) of 3 amino acids. This information was then used as the inspiration to develop optimized BABAs modified with the PTS1 sequence via an intervening linker to further improve the targeting of the inhibitor into the glycosome. Current work involves modification of the peptide-drug conjugate by varying the PTS1 sequence. This research is being pursued not only to improve the potency of the existing peptide-drug conjugate but also to gain a better understanding of the cellular mechanisms governing the delivery of the uptake in trypanosomes.

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Abstract #46

The Effects of the Fatty Acid Transport Protein (FATP) Inhibitor Grassofermata on Fatty Acid Uptake in *Trypanosoma brucei*

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The protozoan eukaryotic pathogen, *Trypanosoma brucei*, is the causative agent of Sleeping Sickness in humans and Nagana in livestock and is found predominantly in Sub-Saharan Africa. The pathogen is transmitted into mammalian blood and other tissues when an infected tsetse fly, the pathogen's vector, consumes a blood meal. As a result, *T. brucei* is adapted to survive in different host conditions through the pathogen's diverse life cycle and ability to transition from the tsetse midgut stage, the procyclic form (PF), to the mammalian bloodstream form (BF). Vaccine developments and eradication of the parasite have been stunted due to the pathogen's innate ability to change between the life stages, as they are vastly different in their metabolic requirements. The highly antigenic Variant Surface Glycoprotein (VSG) plays a key role in the immune evasion strategies of *T. brucei*, including antigenic variation whilst in the mammalian bloodstream. The VSG membrane anchor requires two myristates, fatty acids (FAs) relatively scarce in the host. *T. brucei* appears to use de novo synthesis to generate myristate for VSG anchors, while relying on uptake to acquire longer, more common FAs from the host. Unlike FA synthesis, little is known about the molecular mechanisms governing FA uptake in *T. brucei*. Grassofermata (GF) is a known inhibitor of Fatty Acid Transport Proteins (FATPs) in yeast and mammals. FATPs couple FA translocation across the membrane with its esterification to CoA, catalyzed by an intrinsic acyl-CoA synthetase (ACS) activity. *T. brucei* lacks predicted FATP homologs, but do possess six ACSs, therefore, we hypothesized *T. brucei* might be sensitive to GF. Experimentation has shown that GF treatment inhibits uptake of both medium and long chain fluorescently labeled FA (BODIPY-C12 and BODIPY-C16, respectfully) in both BF and PF. The EC50s for uptake inhibition of BODIPY-C12 were found to be ~ 157μM and 95μM, for the BF and respectively, while the EC50's for BODIPY-C16 inhibition were slightly lower at ~85μM and ~72μM. GF treatment did completely block FA uptake, as we observed maximal inhibition of 70-75%. The EC50s for cell killing after 48 hr were found to be much lower, ~18μM and ~4.5μM for BF and PF respectfully. GF treated cells after incubation with BODIPY-C12/C16 revealed two distinct populations, a second un-expected population of dimmer cells appeared that increased with increasing GF concentration. Inhibition of endocytosis by incubation at 4°C did not affect the emergence of the dim population upon GF treatment. Currently, we are utilizing fluorescent microscopy to analyze the patterns of incorporation in the BF and PF in an effort to identify and understand this second dimmer population. Based on these findings, it is concluded that GF treatment partially inhibits FA uptake, and the residual BODIPY-C12/C16 uptake may be due to other non-endocytic uptake mechanisms.

Abstract #47

Use of Peroxisomal Targeting Sequences in Drug Delivery

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In the parasite *Trypanosoma brucei*, several essential metabolic pathways, including glycolysis, localize to specialized peroxisomes called glycosomes. Using *in vitro* assays, we identified small molecule inhibitors of enzymes within these pathways. However, the *in vivo* activity of some of these inhibitors is lower than *in vitro* studies predict. One possibility is that these inhibitors are not effectively targeted to the glycosome. Exogenous cargo, including small molecule inhibitors of enzymes, can been targeted to glycosomes by appendage of a type 1 peroxisomal targeting sequence (PTS1), a C-terminal tripeptide motif that targets proteins to these organelles. Building on this concept, we appended a PTS1 sequence to a TbHK1 inhibitor. This modified inhibitor exhibited increased trypanocidal activity. With the goal being optimized delivery of drugs by modification with PTS1 tripeptides, we are quantitating how different PTS1 sequences influence glycosomal targeting. Using fluorescein isothiocyanate (FITC) coupled to different PTS1 sequences, we monitored uptake into trypanosome cells via flow cytometry. We have identified a variety of factors that can impact assay reproducibility including incubation time, presence of FBS in the culture media used during incubations, and compound concentration. Optimization of cytometry protocols, along with microscopy to compare distribution of the label, is ongoing to strengthen our understanding of exogenous cargo uptake in the African trypanosome. We will discuss the assay conditions and specific amino acid sequences that influence PTS-FITC uptake.

Abstract #48

LPMO (-like) proteins and their role for *Cryptococcus neoformans* growth and virulence

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Fungi need to adapt quickly to environmental stresses to thrive. The fungal cell wall, which supplies support and integrity to the cell, is an essential compartment to react and interact with the surrounding environment. Rapid changes within the carbohydrate composition and architecture occur in response to environmental stresses. Lytic polysaccharide monooxygenases (LPMOs) are mononuclear copper-enzymes, secreted by microbes to assist in the first steps of remodeling and degradation of cell wall carbohydrates. While recent studies have established an important role for LPMOs in plant pathogenic microbes, studies about the role of LPMOs in human pathogenic microbes are scarce and limited to bacterial LPMOs.

The human fungal pathogen *Cryptococcus neoformans* (Cn) encodes for 4 potential LPMO or LPMO-like proteins. Initial phenotypic analysis of loss-of-function mutant strains, for 3 out of the 4 predicted LPMO-encoding genes, identified one gene, which encodes for the copper binding and release protein Cbi1, to function in copper import as well as maintaining cell wall integrity during low copper stress. Interestingly no *in vitro* LPMO activity was identified for the Cbi1 protein, suggesting it to be a member of an inactive LPMO-like X325 protein family. Unlike *CBI1*, none of the other LPMO genes are regulated by copper. Interestingly, further analysis of their transcriptional profiles identified the *CEL1* gene to be up-regulated during a murine lung infection, as well as in conditions associated with stresses occurring within the mammalian host (high temperature and alkaline pH). In line with this finding, the *cel1*Δ mutant strain is avirulent in a murine model of infection. Downstream analysis of virulence associated phenotypes identified the *CEL1* gene to be required for thermotolerance as well as cell wall integrity, and efficient cell cycle progression in presences of host mimicking stresses. Cel1 is secreted but retained in its own cell wall. Based upon those findings, we propose that, in contrast to other known LPMOs which are mostly targeting exogenous carbohydrates, Cel1 likely promotes intrinsic fungal cell wall remodeling events essential for adaptation to the host environment.

Taken together, our recent work has demonstrated that 2 out of 4 encoded LPMO or LPMO-like proteins have important roles in adaptation during environmental stresses, showing the importance of LPMO and LPMO-like proteins for *Cn* growth and virulence.

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Abstract #49

Optimizing encystation of *Entamoeba histolytica*: the effect of short chain fatty acids

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Entamoeba histolytica, the causative agent of amoebiasis, undergoes conversion between the amoeba form that colonizes the large intestine and the cyst form that spreads disease through contaminated food and water. We have developed a method for encystation of *E. histolytica* in laboratory culture and are now investigating signals and genes involved in this process. We are using reverse transcriptase PCR (RT-PCR) of the Jacob gene, a lectin involved in forming the chitin cell wall early in encystation, as a marker to detect entry into encystation instead of tracking encystation progress over the current week-long encystation protocol. Previous research has shown acetate and propionate, short chain fatty acids present in the large intestine, trigger encystation initiation. We found that lower acetate concentrations resulted in earlier *Jacob* expression but that propionate had no effect even in combination with acetate. We also examined the role of acetyl-CoA synthetase (ACD) in acetate enhancement of encystation initiation. ACD catalyzes the conversion of acetate/propionate to acetyl/propionyl-CoA. We examined *Jacob* expression in wild-type and an *ACD* RNAi mutant and found that acetate had a reduced effect on encystation initiation in the *ACD* mutant. Our results suggest that ACD plays a key role in acetate enhancement of encystation. We will examine the role of both ACD and acetate in the future and how other environmental signals influence encystation.

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Abstract #50

Role of IL-6 on *Cryptococcus neoformans* brain infection

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Cryptococcus neoformans is an opportunistic encapsulated fungal pathogen that causes life-threatening meningoencephalitis in immunosuppressed individuals. The mechanisms by which the fungus transmigrate through the blood-brain barrier (BBB) and invades the central nervous system are poorly understood. Our data shows that the increased production of IL-6 in the murine brains is directly associated with increases in fungal load in the central nervous system. IL-6 is pyrogenic and acts on the hypothalamus, the thermoregulator in mammals. Hyperthermia has been shown to dysregulate the BBB, thus, the impact of IL-6 on hyperthermia and *C. neoformans* brain infection was investigated in *in vivo*. We infected intravenously wild-type (WT) C57BL/6, IL-6 knock-out (KO) and IL-6 KO treated with recombinant IL-6 (rIL-6; 40 pg/g) mice to assess the role of this cytokine on the survival, hyperthermia, and systemic fungal colonization 3- and 7-days post infection (dpi). IL-6 KO mice showed significantly faster mortality than WT and IL-6 KO + rIL-6 treated mice. IL-6 KO mice evinced higher fungal burden than WT and IL-6 KO + rIL-6 in lungs, blood, and brain tissues. WT and IL-6 KO + rIL-6 treated mice showed higher temperature compared to IL-6 KO mice. These results suggest that IL-6 plays a critical role in the host's temperature regulation, fungal survival, systemic cryptococcosis progression, and brain colonization.

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Abstract #51

Characterization of the extracellular vesicles secreted by *Naegleria fowleri*

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The free-living amoeba, *Naegleria fowleri* causes primary amoebic meningoencephalitis, an acute brain disease with a case mortality rate of >97%. Several factors contribute to this considerable degree of mortality including delayed diagnosis, ineffective therapeutics and lack of understanding of the amoebic pathogenesis. The understanding of the molecular basis for parasite-host interactions is also lacking, thus, we endeavored to unravel the mechanistic basis used by *N. fowleri* in relaying cell-cell communications. Apparatuses termed extracellular vesicles(EVs) have been implicated as mediators of intercellular communication. EVs are secreted by originating cells and house signaling molecules that elicit a response in recipient cells. We hypothesized that *N. fowleri* produces EVs and packages smallRNAs and other cargo into them to be secreted and elicit a response in recipient cells. To test this hypothesis, secreted components from amoeba-conditioned media were extracted and purified followed by enumeration and size determination with Nanoparticle Tracking Analysis. Scanning electron microscopy confirmed the EV morphology and we show images of amoebae secreting these vesicles. The nucleic acid contents were characterized by deep-sequencing of smallRNAs within the EVs which revealed highly prevalent small RNAs that we are validating with qPCR. Mass spectrometry analyses has also identified thousands of proteins associated with the secreted vesicles. Fusion assays using the self-quenching R18 dye also show that these EVs are taken up by host-cells and other amoebae. This as well as future work will expand the knowledge of the intracellular interactions among these amoebae and subsequently deepen the understanding of the mechanistic basis of PAM.

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Abstract #52

Probing Fatty Acid Uptake Mechanisms in Trypanosoma brucei Using BODIPY-Labeled Fatty Acids

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Trypanosoma brucei readily acquires a range of lipids (from fatty acids to sterols) from its mammalian and tsetse fly hosts. However, little is known about the molecular mechanisms mediating fatty acid uptake in *T. brucei* and its contribution to parasite survival. Given the redundancy of the apparent *T. brucei* uptake repertoire, we undertook a biochemical characterization of fatty acid uptake in the bloodstream (BF) and procyclic form (PF) of *T. brucei* using fluorescent BODIPY-conjugated fatty acids. We found that BODIPY-fatty acid uptake was partially saturable (BODIPY-C16 but not -C12), was impaired below 4°C, and exhibited a preference for longer-chain fatty acids. Fluorescence microscopy of live cells showed that BODIPY-C12 and -C16 labeled punctate structures and other intracellular membranes. We assessed the impact of protein-mediated uptake by digesting surface proteins with Proteinase K and found that surface proteolysis did not affect BODIPY-C16 or -C12 uptake. Reduction of cellular ATP levels by 80-90% with metabolic inhibitors (2-deoxyglucose ± sodium azide) reduced BODIPY-C12 uptake by 70%. Finally, we assessed the effect of Triascin C, an inhibitor of Acyl-CoA Synthetases (ACSs), on fatty acid uptake and cell viability. ACS catalyzes the ATP-dependent esterification of fatty acids to Coenzyme A. Triascin C inhibited BODIPY-C12 uptake in BFs and PFs with EC_{50}s of 1.2 µM and 0.8 µM, respectively, with maximal inhibition of 10-30%. Triascin C killed both BFs and PFs with 48 h IC_{50}s of 3.1 µM. and 1.6 µM, respectively. Altogether, these data are consistent with a model for *T. brucei* free fatty acid uptake mediated mainly by passive diffusion, followed by intracellular trapping by ACSs.

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Abstract #53

Identification of Genes Required for Acetate Utilization in *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen that causes cryptococcal meningitis, a potentially fatal infection that affects immunocompromised individuals, especially those with AIDS. According to the CDC, cryptococcal meningitis is responsible for 181,000 deaths worldwide each year. Metabolic flexibility by *C. neoformans* provides a means for nutrient assimilation and growth in diverse host microenvironments. At an early stage of infection, *C. neoformans* must survive in alveolar macrophages, the first defense mechanism in the lungs, which contain limited amounts of glucose, forcing *C. neoformans* to survive on nonpreferred carbon sources, such as acetate. Therefore, we hypothesized that genes that are needed for acetate utilization are important in establishing cryptococcal infection and contribute to virulence. Gene CNAG_00236, which was previously identified in a genetic screen to identify genes necessary for growth of acetate as a carbon source, was further examined to assess whether the gene was necessary for growth on acetate and its possible role in virulence. Genes CNAG_05310, CNAG_07403, and CNAG_03697, which were all expressed more than log 2 higher on acetate than glucose, were also investigated in this manner. *C. neoformans* also requires a functional glycolytic pathway for disease. The mitochondrial pyruvate carrier (MPC) is a two-subunit carrier, designated MPC1 and MPC2, located in the inner mitochondrial membrane for transport of pyruvate, generated by glycolysis. We are characterizing the ability of a MPC2 mutant (CNAG_00092) from the Madhani gene deletion library to grow on different carbon sources. A MPC1 mutant (CNAG_00270) has never been successfully created, so we are currently working on creating and testing Δ*mpc1* and Δ*mpc1mpc2* on an array of carbon sources. Understanding the roles of these genes will help us understand whether they are necessary for the creation of branched-chain amino acids or aid in acetyl CoA creation to be used in the TCA cycle. As the current treatments for cryptococcal meningitis are not always effective, identifying genes in *C. neoformans* that are required for virulence may be an important step in the development of more effective treatments for cryptococcal meningitis.

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Abstract #54

Role of Hsp 90 in regulating sensitivity of *C. neoformans* to fluconazole

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Cryptococcus neoformans is an encapsulated yeast, found in the lungs of immunocompromised hosts. In the human host, *Cryptococcus* is subject to multiple forms of environmental stress, including heat stress. Heat shock protein 90 (Hsp90) is an essential protein that has been shown to protect fungal cells against extreme temperature in the human host and to assist in proper cell division. Hsp90 has also been implicated in resistance to the anti-fungal drug fluconazole. To better characterize the role of Hsp90 in fluconazole resistance in *Cryptococcus* we addressed the following questions: 1. Does concomitant partial inhibition of Hsp90 and treatment with fluconazole lead to increased sensitivity to fluconazole and 2. Does prolonged partial inhibition of Hsp90 prior to treatment with fluconazole lead to subsequent increase in development of fluconazole resistance. Using growth assays, disk assays, and a standardized e-test to determine the Minimum Inhibitory Concentration (MIC) of fluconazole, we found that indeed, pharmacological partial inhibition of Hsp90 with radicicol, concomitant with fluconazole treatment leads to increased sensitivity to fluconazole. However, initial pre-treatment of *Cryptococcus* with sub-inhibitory concentration of radicicol followed by exposure to fluconazole leads to development of fluconazole resistant *Cryptococcus* colonies. We characterized the resistant colonies, which show a lower MIC to fluconazole compared to cells that were never exposed to radicicol and fluconazole. Cells derived from the resistant colonies exhibit slower growth on drug-free media, and lose resistance to the drug after passaging in drug-free media. Based on the fact that resistance to fluconazole is lost when fluconazole is removed from the environment, we hypothesize that compromising Hsp90 function during the pre-treatment with radicicol triggers aneuploidy in *Cryptococcus neoformans*.

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Abstract #55

Further Investigations into the Roles of Arginine Biosynthesis in the Fungal Pathogen *Cryptococcus neoformans*

Arohi Singhal and Kerry Smith

Cryptococcus neoformans is an invasive human fungal pathogen that can lead to fungal meningitis in immunocompromised patients, particularly those with HIV. It is the fifth most lethal disease in the world, causing the deaths of about 181,000 people yearly. Multiple virulence factors, like capsule formation, melanin synthesis, carbon utilization, and amino acid biosynthesis, are important for its pathogenicity. We have previous demonstrated that the arginine biosynthesis pathway is a critical amino acid biosynthesis process in virulence. We have examined the roles of glutamate N-acetyltransferase (CNAG 01238), acetylornithine transaminase (CNAG 05134), and arginosuccinate synthase (CNAG 00930) in arginine biosynthesis. Although CNAG_01238 is required for arginine biosynthesis, the deletion mutants were surprisingly still able to grow on minimal media in the absence of arginine. Additionally, a melanin assay was performed on all knockouts to explore virulence in depth. Deletion of mutants of CNAG_05134 and CNAG_00930 did not show a defect in melanin production, in contrast CNAG_01238 shows a melanin production defect which is restored by the addition of arginine. Despite the global significance of cryptococcal meningitis, current treatments are inadequate as the gold standard therapy is based on half century old drugs that have a wide range of liabilities and shortcomings. Our investigations into arginine biosynthesis may identify targets that can lead to the development of new anti-fungal drugs.

Keywords: Arginine auxotrophy, an anti-fungal drug, virulence,

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Abstract #56

The interactive impact of various abiotic conditions on the growth of *Naegleria fowleri*

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Naegleria fowleri is the cause of primary amoebic meningoencephalitis (PAM), a deadly infection that occurs when the free-living amoebae enters the nose via freshwater and travels to the brain. PAM infections are generally associated with exposure to elevated water temperatures during recreational water activity, as *N. fowleri* naturally thrives in freshwater and soil. While environmental and laboratory studies have sought to identify what environmental factors influence the presence of this amoeba, many questions remain. Our study examined the interactive effects of temperature (15, 25, or 35 °C), pH (6.5, 7.5, or 8.5), and salinity (0.5%, 1.5%, or 2.5% NaCl) on *N. fowleri* growth in deionized water and environmental water. One strain of *N. fowleri* was used in ATP luminescent assays for these experiments. Our results indicated that *N. fowleri* growth was highest at 25°C, and multiple interactive effects occurred between abiotic factors. Interactions varied somewhat by water type but were largely driven by temperature and salinity. Lowered temperatures increased *N. fowleri* persistence at higher salinity levels, and low salinity (0.5% NaCl) concentrations supported *N. fowleri* growth at all temperatures. Our research provided an experimental approach to assess interactive effects influencing *N. fowleri* persistence. As climate change impacts water temperatures, additional understanding of the microbial ecology of *N. fowleri* will be needed to decrease pathogen exposure.

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Abstract #57

Glucocorticoids suppress neutrophil control of *Aspergillus* hyphal growth in zebrafish larvae

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Glucocorticoids are potent anti-inflammatory and immunosuppressive drugs which are prescribed for patients with inflammatory disorders and for patients who have undergone transplant surgery. Long-term use of these drugs increases patients' susceptibility to opportunistic infections, such as *Aspergillus fumigatus*. Invasive aspergillosis is one of the main fungal infections in patients undergoing glucocorticoid therapy which can lead to death in >50% of infected patients.

Glucocorticoids are potent repressors of NF-κB, a major pathway that activates inflammatory responses. However, whether glucocorticoid-mediated suppression of NF-κB is the main factor that allows for development of invasive aspergillosis *in vivo* is not known. Our goal is to identify the molecular and cellular immune mechanisms that glucocorticoids suppress to make the host susceptible to invasive aspergillosis. Using a zebrafish larva-*Aspergillus* infection model, we find that exposure to dexamethasone—a commonly used glucocorticoid—significantly decreases host survival. When exposed to dexamethasone, larvae lacking neutrophils succumb to the infection at a higher rate than larvae lacking macrophages, suggesting that dexamethasone predominantly suppresses neutrophil-mediated responses. Through daily, confocal imaging of infected larvae, we find that neutrophils fail to control hyphal growth in the presence of dexamethasone. Larvae with mutations in *irf8*, which lack macrophages, but have an increased number of neutrophils, also fail to contain hyphal growth with dexamethasone exposure. In line with previous findings, dexamethasone suppresses NF-κB activation in *Aspergillus*-infected zebrafish larvae. To test the significance of NF-κB, we used CRISPR/Cas9 to target the NF-κB pathway activator Ikkγ. Similar to dexamethasone exposure, *irf8* mutant larvae injected with guide RNAs targeting Ikkγ also fail to control *Aspergillus* hyphal growth and succumb to the infection. Downstream of NF-κB, the mechanisms that neutrophils activate to prevent invasive hyphae—neutrophil degranulation, reactive oxygen species, and neutrophil extracellular traps—are currently under investigation. Collectively, we find that NF-κB activation promotes neutrophil control of *Aspergillus* hyphae in zebrafish larvae.

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Abstract #58

Therapeutic Effect of Palmitoylethanolamide During Brain Infection with *Cryptococcus neoformans*

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Cryptococcus neoformans (Cn) is an encapsulated neurotropic fungal pathogen and the causative agent of cryptococcosis in humans. Infection typically occurs upon inhalation of fungal spores, followed by colonization of the lungs and dissemination into the bloodstream. Cryptococcal meningoencephalitis is the most severe form of infection and results from the ability of the fungus to cross the blood-brain barrier and reside in the meninges. Current recommended treatment with Amphotericin B (AmpB) disrupts the fungal membrane through an ergosterol-binding mechanism. Though effective, the potency of AmpB and its associated nephrotoxicity at fungicidal levels have prompted investigation into alternative methods of treatment for cryptococcosis. Palmitoylethanolamide (PEA) is an endogenous lipid compound characterized by previous studies as an immunomodulatory molecule capable of controlling and reducing neuroinflammation. This study investigates the therapeutic effects of PEA during cryptococcal infection to identify a potential alternative treatment for cryptococcosis. To determine whether PEA demonstrates fungicidal activity, we exposed *Cn* to varying concentrations of PEA alone and in combination with AmpB. The results showed that the combination group reduced fungal growth more effectively than AmpB alone when compared with the control, although PEA alone had no significant reduction. To evaluate the immunomodulatory effects of PEA treatment *in vivo,* we infected C57BL/6 mice intracerebrally with *Cn* and treated with AmpB, PEA, or a combination. We observed that the combination of PEA and AmpB extended the median survival by 8 days from that of the AmpB-treated group. Fungal burden was quantified from brain tissue for 3- and 7-days post-infection, and PEA reduced the fungal burden more effectively when combined with AmpB than AmpB treatment alone. The results suggest PEA confers a therapeutic synergistic effect during murine cryptococcosis when combined with AmpB and support further investigation of this compound as a potential supplement to AmpB treatment in humans.

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