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Poster Abstracts

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Presenting authors are shown in **green**. Poster session A (odd-numbered posters) begins Friday at 7:45 pm; poster session B (even-numbered) begins at 8:45 pm.

P1 Scf1 suppresses the innate immune response to systemic Candida auris infection

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Since its identification in 2009, Candida auris has spread across the globe, in part due to its potent ability to colonize skin and abiotic surfaces, which drives its high outbreak potential. Recently, the O'Meara group discovered an adhesin protein, Scf1, which is crucial for C. auris's colonization of abiotic substrates. Surprisingly, I found that the loss of Scf1 significantly reduced mortality and fungal burden in systemically infected mice, implicating the adhesin as a virulence factor beyond its role in attachment. However, its function during systemic infection is still unknown, as the mortality defect could be due to differences in fungal fitness in vivo, differences in host immune response, or a combination thereof. I will investigate each of these possibilities with an immunocompetent model of systemic infection and a combination of cell culture models, pathology, and RNAseq experiments. Thus far, I have observed a significant reduction in CFU burden, even at 3 d.p.i. I have also found that Δscf1 fungi induce slight differences in macrophage cytokine expression, defense response, and phagocytic uptake. Therefore, I am focusing my investigation on innate immune responses. Upcoming experiments will define the role of neutrophils and cytokine profiles to determine whether Δscf1 fungi exhibit virulence defects due to a differential innate immune response.

P2 Defining the Candidozyma auris pangenome

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Candidozyma auris is an emerging fungal pathogen that exhibits remarkable phenotypic variation and rapid adaptation in several clinically relevant traits, despite being seemingly incapable of sexual reproduction. The genetic mechanisms underlying the phenotypic variation that allows this rapid adaptation to occur are not well understood. This presents a significant obstacle to understanding the factors that make C. auris a successful and widespread pathogen in healthcare settings. However, the highly clonal nature of C. auris genomes presents a challenge when using bioinformatics tools and pipelines designed for eukaryotes in general. We have designed a workflow for efficient generation of a pangenome from short- and long-read C. auris sequencing data, including several methods originally designed for bacteria such as microbial GWAS (mGWAS). We applied this pipeline to a dataset of 694 isolates and identified several putative loci influencing keratinocyte adhesion in Clade IV, including the loss of a region on Chromosome VI overlapping the known adhesin gene ALS4112. We plan to use these analysis tools to better characterize the genetic diversity present in this species and how it contributes to the phenotypic trait variation that has made C. auris a successful healthcare-associated pathogen.

P3 Cryo-EM structures reveal unique protein components in the 43S translation initiation complexes of apicomplexan parasite Toxoplasma gondii

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Apicomplexan parasites, including Plasmodium spp. (the causative agents of malaria) and Toxoplasma gondii (the causative agent of toxoplasmosis), impose a substantial global health burden. However, the mechanisms underlying mRNA translation in these organisms remain poorly understood. Translation proceeds through initiation, elongation, termination, and ribosome recycling, with initiation representing the most highly regulated step. During initiation, eukaryotic initiation factors (eIFs) assemble on the 40S ribosomal subunit to form the 43S pre-initiation complex (43S), which is subsequently recruited to the 5' end of mRNA by the cap-binding complex eIF4F to form the 48S initiation complex.

A defining feature of apicomplexan mRNAs is their unusually long 5' untranslated regions (5' UTRs), with median lengths of ~800 nucleotides in T. gondii and ~600 nucleotides in Plasmodium spp., compared to ~200 nucleotides in humans. Here, we present the first high-resolution cryo-electron microscopy (cryo-EM) structure of the 43S translation initiation complex from the apicomplexan parasite T. gondii. This structure reveals a conserved core shared with yeast and human 43S complexes, along with two parasite-specific components, DDX60 and thioredoxin.

Functional analyses demonstrate that DDX60 is essential for parasite viability, as its depletion abolishes plaque formation and severely impairs replication. Ribosome profiling (Ribo-seq) and RNA sequencing (RNA-seq) further show that loss of DDX60 selectively suppresses translation of a subset of mRNAs.

Together, these findings establish the first structural model of a translation initiation complex from any apicomplexan parasite, providing new insights into the composition and regulation of protein synthesis in protozoan pathogens and advancing our understanding of parasite gene expression control.

P4 AurisCEN: a Candidozyma auris Co-Expression Network for gene function prediction

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Elucidating gene function remains a fundamental goal in molecular biology, especially among non-model organisms. However, molecular conservation and model organism biology does not always reflect functional conservation of a given gene, and complex relationships between genes are responsible for higher-order biological processes. One promising approach to address this is co-expression analysis, which relies on the principal that genes with similar expression patterns across multiple conditions are more likely involved in the same biological process through guilt-by-association. Here, we generated a co-expression network for Candidozyma auris, an emergent and critical priority fungal pathogen. We adapted a high-throughput RNAseq platform for use with C. auris and performed barcoded RNA sequencing across a panel of C. auris strains, including 32 unique transcription factor deletion mutants and 48 additional transposon insertion mutants, across 4 different growth conditions. This data was supplemented with bulk RNAseq data from 28 studies deposited in NCBI sequence read archive to yield a combined total of 1,251 individual RNAseq runs, which provided sufficient transcriptional diversity for a robust co-expression network. Retrospectively, AurisCEN shows high predictive performance of known gene function annotations, with an average AUROC of 0.79 across all GO terms. Additionally, AurisCEN recapitulates known biological networks, such as the ribosome complex and the ergosterol biosynthesis pathway. Future work will focus on using AurisCEN predictions to identify novel regulators of adhesion and echinocandin sensitivity with both computational and molecular characterization.

P5 Evaluating Thai and Holy Basil Extracts as Natural Therapeutics for Human Babesiosis

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Babesiosis is an emerging tick-borne zoonotic disease caused by intraerythrocytic protozoan parasites of the genus Babesia. In the United States, human babesiosis is primarily caused by Babesia microti in the northeastern and upper mid-western regions, while Babesia duncani has been reported mainly along the western United States. Manifestations include flu-like symptoms and hemolytic anemia; it poses a severe threat in immunocompromised individuals and blood transfusion recipients. Current treatment therapies rely on drug combinations such as azithromycin with atovaquone, or clindamycin with quinine; however, some reports suggest association with relapse and rising drug resistance. In this study, I evaluated the antiparasitic activity of methanolic extracts from Thai basil (Ocimum basilicum) and holy basil (Ocimum sanctum) against the intraerythrocytic stage of B. duncani. Dose-response assays revealed concentration-dependent inhibition of parasite growth, with Thai basil demonstrating greater effect than holy basil. The half-maximal inhibitory concentration (IC50) was determined to be 50µg/mL for Thai basil and 80µg/mL for holy basil. Standard anti-babesial drugs, including azithromycin and atovaquone, were tested in parallel using their established IC50 values of 5µM and 500nM, respectively, to evaluate potential combinational effects with basil extracts. Combination assays revealed a synergistic interaction between Thai basil extract and azithromycin, resulting in enhanced inhibition of B. duncani growth. MTT Cytotoxicity assay against HCT-08 cell line showed minimal cytotoxic effects, with sustained cell viability across high concentration ranges. Gas chromatography-mass spectrometry (GC-MS) fractioning and analysis of basil extracts identified eugenol as a major phytochemical compound and a likely contributor to the observed antiparasitic activity. Future studies will focus on evaluating the in vivo efficacy of these extracts in B. microti-infected mice to determine their potential for therapeutic drug development.

P6 Spatial CRISPR screen identifies Toxoplasma gondii secreted effectors modulating Human E3 Ubiquitin Ligase RNF213

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IFNγ-mediated responses are critical for immune defense against Toxoplasma gondii in humans. Across diverse human cell types, IFNγ stimulation induces ubiquitination of the parasitophorous vacuole (PV), the parasite's replicative niche, and leads to parasite restriction. It has been shown that the host E3 ubiquitin ligase RNF213 can recognize and be recruited to the PV membrane (PVM) shortly after parasite invasion, and that it plays a critical role in mediating PVM ubiquitination. However, the parasite-specific factors recognized by RNF213 on the PVM remain unknown. Using a T. gondii mutant defective in protein trafficking to the PVM (Δgra45), we have found that PV recognition by RNF213 is dependent on the localization of parasite secreted effector proteins to the PVM. Therefore, to understand the parasite factors required for T. gondii-RNF213 spatial interactions at the PVM, we leveraged a CRISPR library of 442 secreted effector protein knockout parasites and high-content imaging to measure RNF213 dynamics at the PVM. By applying the high-throughput, deep learning-based image analysis platform spaCR, we quantitatively compared the frequency of RNF213 recruitment to vacuoles containing mutant parasites with that observed for wild-type vacuoles. This pipeline allowed us to identify a few negative and positive regulators of RNF213's localization to the T. gondii PVM. Currently, we are in the process of validating candidate genes and characterizing the molecular mechanisms governing the critical recognition of T. gondii by RNF213. This will provide further insight into our understanding of the IFNγ-mediated anti-T. gondii response in humans and potentially inform the development of novel therapies to combat Toxoplasmosis.

P7 Unraveling the Mechanisms of Schistosome Death Triggered by Novel Thioredoxin Glutathione Reductase Inhibitors

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Schistosomiasis remains a major neglected tropical disease affecting more than 200 million people worldwide, with treatment still heavily dependent on praziquantel. Although praziquantel is effective against adult worms, its reduced activity against juvenile parasites highlights the need for new therapeutics with improved stage-specific efficacy. In our recent study published in Nature Communications, we identified a series of noncovalent inhibitors targeting thioredoxin glutathione reductase (TGR), a key enzyme required for redox homeostasis in schistosomes. These compounds showed strong in vivo efficacy in murine infection models, satisfied World Health Organization lead progression criteria, and demonstrated greater activity against juvenile worms than praziquantel. Here, we investigated the mechanism of parasite killing induced by these TGR inhibitors, focusing on whether death occurs through caspase-dependent apoptosis or alternative non-apoptotic pathways. Luminescence-based assays performed in adult worms and schistosomula revealed that TGR inhibitor treatment consistently produced marked increases in caspase-associated luminescence signals, indicating activation of apoptotic pathways. Lipid peroxidation assays were also conducted to assess oxidative membrane damage and the contribution of ferroptosis-associated processes. Similar responses were observed across multiple compounds in the series, suggesting a shared mechanism of action. To confirm pathway specificity, parasites were co-treated with the pan-caspase inhibitor Z-VAD(OMe)-FMK. Co-treatment significantly reduced luminescence signals and partially rescued parasite viability, supporting a central role for caspase activation in compound-induced death. In contrast, inhibition of non-apoptotic pathways, including ferroptosis-associated processes, produced only modest effects on luminescence and parasite survival despite detectable increases in lipid peroxidation. Overall, our findings demonstrate that noncovalent TGR inhibitors induce schistosome death predominantly through caspase-dependent apoptotic mechanisms, while oxidative stress pathways contribute to a lesser extent. This study provides important mechanistic insight into this emerging class of antischistosomal therapeutics.

P8 Defining Mechanisms of Cryptococcal Sterol Transport

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Ergosterol, a key fungal membrane lipid, must move from its site of synthesis (the ER) to other membranes to enable crucial cellular functions. This process and associated proteins represent an underexplored aspect of cryptococcal sterol homeostasis. To identify such proteins, we screened for spontaneous mutants that suppress the growth defects of cells lacking the retrograde sterol transporter Ysp2. Whole genome sequencing of suppressor isolates revealed numerous nucleotide polymorphisms in coding regions directly relevant to sterol biosynthesis, regulation and transport, which strongly validated the utility of our screen. Excitingly, a mutation in one unstudied gene, which would cause severe truncation of the encoded protein, suppressed multiple Ysp2 mutant phenotypes. Notably, this protein includes a predicted membrane phospholipid-binding domain, a feature associated with lipid transport-related proteins. As no sterol binding site is predicted in this protein, we hypothesized that it affects sterol transport indirectly, possibly through a mechanism involving interactions with membranes enriched with a phospholipid ligand. Subsequent characterization of this novel protein has revealed roles in lipid homeostasis, cell morphology, stress tolerance and virulence. In vitro studies of an exogenously expressed, truncated version of the protein suggest a phospholipid ligand, and confocal microscopy suggests the protein primarily occurs as puncta in the plasma membrane. Based on these results, our working model is that this protein associates with membranes enriched with its phospholipid ligand, possibly at plasma membrane-ER membrane contact sites, and influences sterol transport through a non-sterol binding mechanism. Current studies aim to further define its role in sterol transport, as well as its affinity for its phospholipid ligand in a membrane context. Overall, this work will illuminate the fundamental process of sterol transport in Cryptococcus neoformans, and may expand our understanding of cryptococcal ergosterol biology to reveal novel pharmacological targets.

P9 Histoplasma pathogenesis requires mannitol metabolism

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Mannitol is a main carbon-containing molecule in Histoplasma capsulatum, comprising about 15% of the total dry weight of the organism in vitro. Despite mannitol's prominence, its role in Histoplasma biology and potentially pathogenesis is unknown. CRISPR/cas9 methodology was used to create a deletion of the gene encoding mannitol-1-phosphate dehydrogenase (Mpd3), which synthesizes mannitol from glycolysis metabolites. This deletion resulted in an 87% reduction in mannitol. In a murine model of respiratory histoplasmosis, the mpd3 mutant showed attenuated virulence, confirming mannitol synthesis contributes to Histoplasma pathogenesis. Mannitol has been implemented in carbon storage, osmoprotection, and ROS protection in other fungi, but we have determined that is not true in Histoplasma. Mannitol synthesis has been proposed to be cyclical in other fungi, resulting in the exchange of NADH for NADPH to support anabolic reactions. To test this, we disrupted the degradative branch of the cycle by identifying the gene encoding mannitol dehydrogenase (Mdh4) in Histoplasma, deleting the gene via CRISPR/cas9, and confirming loss of mannitol dehydrogenase activity via enzyme functional assays. A murine infection done with the mdh4 mutant showed intact virulence. Therefore, we hypothesize that mannitol has a novel role in Histoplasma biology and pathogenesis independent of previously proposed methods.

P10 Investigating how malaria parasites build an effector protein translocon from an ancestral nutrient pore

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Obligate intracellular malaria parasites reside within a parasitophorous vacuole (PV) formed during invasion of their vertebrate host cell. Parasite development requires small molecule transport and effector protein export across this barrier and an oligomeric PV membrane pore formed by EXP2 is central to both processes. EXP2 is widely conserved among vacuole-dwelling apicomplexans, mediating solute exchange between the host cytoplasm and PV lumen. Remarkably, Plasmodium spp. have further functionalized this pore through a flange-like adaptor formed by PTEX150 which docks the HSP101 chaperone ATPase motor to form the Plasmodium Translocon of EXported proteins (PTEX). While PTEX is critical to parasite survival and disease pathogenesis, the molecular basis for transforming the EXP2 nutrient pore into a protein translocon remains poorly understood. Cryo-EM analysis revealed that the C-terminal assembly strand of EXP2 forms an augmented beta-sheet with HSP101, suggesting a possible interface for translocon assembly. To investigate the functional importance of this interaction, we used a DiCre-based inducible mutagenesis strategy to systematically interrogate EXP2 features in P. falciparum. Surprisingly, we find the augmented beta strand is completely dispensable, instead pinpointing residues in an adjacent linker region required for protein export and parasite survival. These residues are positioned near the HSP101 C-terminal domain in the cryo-EM structure, suggesting a role regulating HSP101 recruitment and/or activity and we are currently assessing their importance to PTEX integrity and nutrient pore function. Elucidating the molecular interactions that underpin expansion of EXP2 function will provide new insight into PTEX formation and identify potential vulnerabilities for antimalarial intervention.

P11 Elucidating the role of Parasite-derived extracellular vesicles in Human Babesiosis

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Human babesiosis is an emerging infectious disease caused by a bloodborne unicellular parasite called Babesia. One of the major species of Babesia that infects humans is Babesia microti, which is transmitted through tick bites during a blood meal. As a successful pathogen, Babesia has evolved mechanisms to evade and manipulate the human immune system, ensuring effective development and proliferation. One such mechanism is the secretion of extracellular vesicles. While extracellular vesicles have been shown to regulate host–pathogen interactions in related intracellular parasites, their role in modulating neutrophil responses during babesiosis remains unexplored. Neutrophils are a major white blood cell involved in innate immunity and act as first responders, accounting for approximately 70% of all WBCs in circulation. Neutrophils possess mechanisms to protect the host, such as cytokine secretion and the release of neutrophil extracellular traps consisting of the neutrophil's DNA, which have bound granules, such as myeloperoxidase, in a process known as NETosis. Here, we investigated the effect of Babesia microti – derived EVs using isolated mouse neutrophils and co-culture assays to assess cytokine secretion and NET formation. We found that Babesia microti EVs robustly trigger NETosis and activate the secretion of key inflammatory cytokines. Collectively, these findings reveal a previously uncharacterized role for Babesia microti EVs in modulating host neutrophil activity, providing mechanistic insight into host–parasite interactions during babesiosis, and highlighting the importance of EV-mediated signalling in the pathogenesis of B. microti infection.
Keywords: B. microti, extracellular vesicles, neutrophils

P12 Epigenetic and transcriptional control of Scf1

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Candidozyma auris outbreaks are driven by its ability to colonize diverse surfaces. Our previous work identified that the Scf1 adhesin is required for attachment to surfaces, and its expression is correlated to adhesion. However, the regulation of this adhesin was unknown. Using ATAC-seq we showed that the SCF1 promoter had a closed chromatin conformation in a low adherence strain and open in the high adherence strain. The SWI/SNF complex and the Tup1 repressor complex are global chromatin remodelers that can act in opposition to open or close chromatin to regulate expression. Through RNA-seq and qRT-PCR approaches, we found that Swi1 and Tup1 regulated the expression of SCF1. By investigating other components of each complex, we identified that although the SWI/SNF complex appears conserved between C. auris and C. albicans, the Tup1 complex appears re-wired in C. auris.

We then employed a systematic approach to identify transcription factors that regulate SCF1 downstream of these global chromatin regulators and found that most transcription factors involved in regulating adhesion in C. albicans have no effect on adhesion in C. auris. Instead, we identified that Efg1 is required for SCF1 expression. We show that Efg1 directly binds to the Scf1 promoter using ChIP. Additionally, complementation of the C. auris efg1 deletion with the C. albicans Efg1 rescues the adhesion defect. We propose a model in which C. auris employs canonical chromatin remodeling pathways to regulate accessibility of the SCF1 promoter to allow for binding of transcriptional regulators as a response to environmental cues.

P13 FLAM6 is a cAMP-binding protein that controls flagellar length, host cell infection, and vector colonization in Trypanosoma cruzi

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Trypanosoma cruzi is the etiological agent of Chagas disease, which remains a major neglected tropical disease with limited treatment options. The parasite transitions through four developmental stages in response to drastic environmental changes encountered in its digenetic life cycle. Cyclic AMP (cAMP) is a universal second messenger that plays a key role in eukaryotic cell differentiation and stress response. However, this signaling pathway remains poorly understood in T. cruzi, where protein kinase A (PKA) is cAMP unresponsive and other canonical effectors have not been identified. Flagellar Member 6 (FLAM6) is an axonemal protein localized to the flagellar proximal domain with two predicted cyclic nucleotide binding domains (CBDs). The total lysate of recombinant bacteria expressing a truncated version of FLAM6 containing both CBDs was used to conduct a cAMP binding assay. Our results indicate that FLAM6 is a cAMP-binding protein, suggesting a putative role as a cAMP effector in T. cruzi. Functional characterization of FLAM6 through gene knockout using CRISPR/Cas9-based genome editing revealed significant defects in parasite proliferation, metacyclogenesis, flagellar length, mammalian cell infection, and the ability to colonize the hindgut of the triatomine vector. To determine whether cAMP binding is required for FLAM6 function, a TcFLAM6R447A-AB cell line is being generated to test if loss of cAMP binding alone is sufficient to reproduce the phenotypes from the FLAM6-KO cell line. This cell line will provide direct evidence of the role of FLAM6 in the cAMP signaling pathway. The FLAM6 interactome analysis identified enrichment of proteins involved in translation, aminoacyl-tRNA ligation, phosphorylation, and intraflagellar transport. Together, these findings support the role of FLAM6 as a cAMP effector in T. cruzi. Our characterization of the first cAMP effector in this parasite will improve our understanding of the signaling pathways that govern parasite development and disease transmission in trypanosomes.

P14 Elucidating thermo-adaptive mechanisms and cell cycle progression in Cryptococcus deneoformans

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Human body temperature (37°C) serves as a key barrier to fungal infections, as most environmental fungi grow optimally between 25–30°C. However, the emergence of new fungal pathogens and the expanding range of existing ones underscore the ability of fungi to rapidly adapt to heat stress, posing a growing threat to human health. Despite this, the molecular and evolutionary mechanisms driving fungal adaptation to heat remain poorly understood. Cryptococcus deneoformans is an ideal model to study heat adaptation in fungi because it is less thermotolerant and less virulent than other disease-causing Cryptococcus species, primarily associated with skin infections and, more rarely, fungal meningitis. In a 30°C passaging experiment, I found that a spontaneous insertion of a transposable element into a single gene I have named (PRS1) conferred increased thermotolerance up to 39°C. To investigate the function of Prs1, pull-down and mass spectrometry analyses identified the phosphatase Pph22 as its primary interacting partner, along with two components of the NDC80 kinetochore complex. Because dephosphorylation of the NDC80 complex is required for stable chromosome attachment during mitosis, these interactions suggest a role for Prs1 in cell cycle regulation. Consistent with this hypothesis, the prs1Δ mutant exhibits faster cell division, increased viability at elevated temperatures, and altered G1 and G2 cell cycle dynamics compared to wild type. The mutant also displays increased resistance to cell wall and membrane stressors, while capsule and melanin production remain unchanged. Although the prs1Δ mutant showed enhanced survival in macrophage killing assays, this phenotype did not translate to increased virulence in an A/J mouse model of infection. Together, these findings reveal a mechanism promoting thermal adaptation while suggesting that thermotolerance alone is insufficient to drive pathogenesis. This distinction is increasingly relevant as climate change, antifungal resistance, and expanding susceptible populations elevate the threat of fungal disease.

P15 Determining the Role of GDH2 in the Apicoplast of Plasmodium falciparum

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The apicoplast of Plasmodium falciparum contains metabolic and antioxidant pathways that are attractive drug targets. These pathways require reducing power in the form of NAD(P)H, but it is unknown how the apicoplast produces it. One potential producer of reducing power is glutamate dehydrogenase (gdh2), which catalyzes the reversible conversion of glutamate and NADP+ to alpha-ketoglutarate and NADPH. To determine if gdh2 is essential, we used CRISPR/Cas9 to knock out the gdh2 gene in a parasite line engineered with a cytoplasmic mevalonate bypass pathway. This bypass system converts exogenous mevalonate to isopentenyl pyrophosphate, an essential product made by the apicoplast MEP pathway, allowing the parasite to survive even in the absence of a functional apicoplast. Parasites lacking gdh2 lost the apicoplast genome and were only able to survive when supplemented with mevalonate, indicating that gdh2 is essential for apicoplast maintenance and parasite survival. To ascertain if its essentiality is due to its role in providing NADPH, we are using a gdh2 conditional knockdown line for functional assays. First, we are complementing gdh2 deficiency with a mutated dihydrolipoamide dehydrogenase (E3) subunit of pyruvate dehydrogenase that will bind NADP+ instead of NAD+ to produce NADPH instead of NADH. Additionally, we are targeting a genetically encoded fluorescent sensor to the apicoplast to monitor NADPH/NADP+ levels indirectly. In parallel, we are testing the susceptibility of gdh2-depleted parasites to reactive oxygen species by exposing them to known redox stressors, including paraquat and the photosensitizer KillerRed. These assays will provide insight into the role of Gdh2 in the apicoplast.

P16 An epigenetic regulator, Set202, contributes to host adaptation and survival in the pathogenic fungus Cryptococcus neoformans

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Invasive fungal infections are a leading cause of mortality among immunocompromised populations, with an annual global incidence of 6.5 million and mortality exceeding 50%. Cryptococcus neoformans, a ubiquitous environmental fungus, causes cryptococcal meningoencephalitis (CME), a life-threatening infection with mortality rates ranging from 20% to 80%. Expanding at-risk populations, antimicrobial resistance, and limited knowledge of fungal biology and host-pathogen interactions exacerbate this threat. Understanding these interactions is essential for developing new therapeutic strategies.

Previous studies identified the C. neoformans gene SET202, encoding a putative histone methyltransferase (KMT), as important for competitive fitness in murine co-infection models. KMTs in other pathogenic fungi, such as Candida albicans, have been shown to regulate virulence traits, including antifungal resistance and dissemination. However, the function of SET202 remains uncharacterized.

Our findings show that deletion of SET202 alters macrophage interactions, increasing phagocytosis and reducing phagosomal membrane permeabilization, processes required for full virulence.

Furthermore, we have found that under host-like stress conditions (37°C and 5% CO_2), Set202 protein enables fungal growth, virulence factors production, survival in immune cells, and animal virulence, by functioning as a true histone 3 lysine 36 methyltransferase. These results suggest SET202 plays a critical role in fungal adaptation and survival during the transition from permissive environments to stringent host conditions, a key step in establishing infection and disease progression.

Collectively, prior evidence and our preliminary data strongly support further investigation of SET202's biological function. Elucidating its role in regulating fungal adaptation and disease progression will provide valuable insight for developing targeted antifungal therapies.

P17 Growth phase influences virulence in Candidozyma auris systemic infection models

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Candidozyma auris is a growing public health concern, capable of causing long-term contamination of healthcare settings, skin colonization, and life-threatening bloodstream infections. However, C. auris pathogenesis is not well understood, which is exacerbated by limitations and discrepancies in existing animal infection models. Further, the effects of C. auris growth phase on virulence have not been examined, despite growth phase being linked to virulence in many bacterial species. To address this question, and to develop an immunocompetent murine model of infection, we directly compared log-phase and stationary-phase C. auris systemic infection in immunocompetent C57BL/6J mice at high and low doses of infection. Systemic infection with high-dose log-phase C. auris results in rapid mortality between 2 h and 1 day post-infection, whereas stationary phase C. auris results in significantly extended survival. We observed that C. auris initially colonizes multiple organs but is rapidly cleared from the lungs and spleen, while kidney fungal burdens remain stable. Mice infected with high-dose log-phase C. auris exhibited symptoms consistent with septic shock and disseminated intravascular coagulation, including fibrin-associated blood clotting in multiple organs and decreased serum fibrinogen levels, suggesting that coagulation may drive rapid mortality. Further, log-phase C. auris-induced coagulation can be recapitulated in an in vitro hemagglutination assay, while Candida albicans does not cause hemagglutination, suggesting specific features of C. auris drive coagulation. Future work will characterize the molecular determinants of C. auris-induced coagulation, possibly revealing new treatment strategies for septic shock and disseminated intravascular coagulation in C. auris candidemia patients.

P18 Functional Characterization of TcCIF1 in the Life Cycle of Trypanosoma cruzi

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Trypanosoma cruzi, the etiological agent of Chagas disease, undergoes a highly coordinated cell division process that requires the ordered duplication and segregation of key organelles. In addition to the conserved eukaryotic cytokinesis machinery, trypanosomatids rely on lineage-specific regulators. One such protein, CIF1 (cytokinesis initiation factor 1), is essential in T. brucei for posterior cell-end formation and cleavage furrow progression. We are investigating the role of CIF1 in T. cruzi using CRISPR/Cas9-based tools to generate knockout and endogenously tagged cell lines. Deletion of TcCIF1 markedly impaired epimastigote proliferation, cytokinesis, and differentiation into metacyclic trypomastigotes. Endogenous cMyc-tagged TcCIF1 localized to the proximal end of the flagellum, near the basal bodies and FAZ, and allowed us to monitor TcCIF1 expression across all major life-cycle stages. Using double-tagged cMycCIF1/cMycCIF2 cell lines, we detected an interaction between TcCIF1 and TcCIF2 by IFA and co-immunoprecipitation followed by western blot, which was further supported by mass spectrometry analysis of cMycCIF1-interacting proteins. In conclusion, we demonstrate that TcCIF1 is required for epimastigote cytokinesis in T. cruzi and interacts with TcCIF2, another predicted cytokinesis regulator. We also identify an important role for TcCIF1 in metacyclogenesis. Ongoing work is assessing the impact of TcCIF1 on parasite infectivity and intracellular proliferation in host cells.

P19 Fast and Scalable Flow Cytometry Assay Reveals a Damaged Population and Complements the CFU for Quantifying Post-Stress Survival in Diverse Yeasts

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Quantifying post-stress survival is an essential and critical step in studying pathogenic microbes, including opportunistic yeast pathogens. Colony Forming Unit (CFU) remains the most widely accepted method owing to its low set-up cost and robust result. Viability dyes that assess plasma membrane integrity are a fast and scalable alternative to the CFU especially if coupled with flow cytometry. However, its application in yeast studies is inconsistent due to the lack of standardized protocol and reporting standards, making it hard to compare results across studies or with CFU. In this work, we systematically characterize a two-dye (SYTO 9 / PI) LIVE/DEAD assay coupled with flow cytometry to quantify post-stress survival across diverse yeast species. We found: 1) the staining buffer composition has a profound effect on the results; we identified 0.85% saline solution as the optimal buffer producing the least artefacts; 2) a subpopulation in the post-oxidative stress samples show enhanced SYTO 9 fluorescence with minimal PI staining; further experiments suggest this population is consistent with damaged cells; 3) our results caution that viability dye-based results, such as the commonly used PI, measure different quantities than CFU, whose results can be drastically different in absolute values even though the trends are consistent across a range of stress doses. We conclude that our optimized SYTO 9/PI viability assay offers a great complement to the CFU assay by offering a faster turnaround, higher throughput, and distinguishing a damaged population from the live and dead cells. We successfully applied the optimized assay in diverse yeast species following hydrogen peroxide treatment and against Amphotericin B treatment in a pathogenic yeast, demonstrating its wide applicability.

P20 Paralogs of the Candida albicans TLO gene family form interconnected functional networks with incomplete redundancy

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Gene duplication typically fails to confer a selective advantage to an organism, prompting their removal from a population. In the rare instance that duplication either does not incur a fitness cost or it enhances fitness, gene families can form through repeating the duplication process. While the function of gene duplicates has been studied in detail, little work has explored how repeated duplication impacts paralog redundancy and may restrict the emergence of new paralogs or novel function. Here, we constructed a panel of single deletion mutants for each of the 14 members of the Candida albicans telomere-associated (TLO) gene family to test the redundancy in molecular and biological function among paralogs from a lineage-specific expansion. Tlo proteins function as interchangeable subunits of the Mediator transcriptional regulatory complex and have the potential to alter gene expression and an array of cellular responses. Redundancy was the most common outcome, being observed for approximately 80% of the phenotypic assays in strains lacking single TLO genes. However, mutants for all 14 paralogs displayed non-redundant functions in phenotypes ranging from carbon utilization to in vivo virulence. Analysis of gene expression in single TLO mutants found similar trends in redundancy, and loss of single TLOs disproportionately affected genes involved in filamentation, adhesion, redox reactions, and transporter activity at the cell surface. Importantly, sequence divergence between paralogs positively correlated with the frequency of altered phenotypes in single TLO mutants, indicating the acquisition of non-redundant function with increased evolutionary distance. Double mutants lacking two TLO genes produced both positive and negative synergistic phenotypes, suggesting that crosstalk or coordinated regulation is common among paralogs. Together, this study demonstrates that recently emergent paralogs acquire non-redundant functions despite often retaining redundancy with other gene family members to form a highly interconnected functional network.

P21 Functional Characterization of the Histoplasma Siderophore Biosynthetic Gene Cluster and Non-Cluster Siderophore Modification Enzymes

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The fungal pathogen, Histoplasma capsulatum (Hc), causes disease in both immunocompromised and immunocompetent hosts by proliferating within host macrophages. Acquisition of iron is a particular challenge for intracellular pathogens as host cells limit the availability of essential micronutrients. To counteract this nutritional immunity of the host, Hc secretes iron-scavenging siderophores into the phagosome. The production of siderophores is necessary for Hc pathogenesis as yeasts which lack Sid1, the monooxygenase responsible for the first step in siderophore production, have attenuated virulence in a murine infection model, specifically following activation of macrophages by T-cells. We have identified 4 siderophore species produced by Hc pathogenic yeasts, which includes two coprogen-type molecules. To define the necessary factors for siderophore biosynthesis, we performed functional tests of the gene products encoded in the Histoplasma biosynthetic gene cluster (BGC) through deletion of each gene. Surprisingly, not all of the BGC genes are required for siderophore synthesis and growth of Histoplasma in restricted iron and in macrophages. By combining genetics and untargeted mass spectrometry of yeast culture filtrates, we determined that three BGC genes contribute to the core siderophore structural components including fusarinine, and a non-ribosomal peptide synthetase assembles these into higher-order hydroxamates including dimerum acid and coprogen-type siderophores. Using a candidate gene approach, we identified a methyltransferase enzyme (Cmt1) encoded by a gene outside of the siderophore BGC that is responsible for methylation of coprogen B. With these key factors identified for synthesis of the repertoire of Histoplasma siderophores, we are now in a position to test which siderophore molecules facilitate Histoplasma growth in the iron-restricted environment of the macrophage providing fundamental insight into how fungal pathogens overcome host-imposed iron restriction.

P22 Investigating Cryptosporidium parvum thrombospondin protein cpTSP2 in macrogamont development

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The apicomplexan protozoan parasite Cryptosporidium is a major cause of diarrheal illness globally and chronically infects children, people, and young ruminants with weakened immune systems. Despite widespread infection, there are no adequate drugs or vaccines for the treatment of cryptosporidiosis in vulnerable populations. Oocysts enter the host's gastrointestinal tract and excyst four sporozoites that invade intestinal epithelial cells. After three asexual replication cycles, the parasites enter a sexual cycle. The microgamonts (male) and macrogamonts (female) develop synchronously until the microgametes egress and fertilize the macrogamonts, forming a zygote that later forms an oocyst. The molecular mechanisms that facilitate macrogamont maturation and gamete signaling leading to fertilization are not known. C. parvum thrombospondin repeat-containing protein (CpTSP2) contains Lin-12/Notch domains, known to have a canonical role in cell-cell signaling. Peak cpTSP2 expression is observed during the sexual stages. Given its potential signaling capacity, we hypothesize that CpTSP2 is involved in macrogamont development or in communication with microgametes, facilitating fertilization. To visualize CpTSP2 localization, a stable transgenic C. parvum line was generated with a hemagglutinin tag at the C-terminus of CpTSP2 using CRISPR/Cas9 editing and IFN-γ knockout mice. During the sexual cycle, CpTSP2 is confirmed to localize only within macrogamonts by immunofluorescence assays (IFA) using female-specific antibodies (anti-COWP1, anti-DMC1). Some macrogamonts show an intracellular CpTSP2 localization with the protein in the cytoplasm; larger macrogamonts exhibit punctate expression of CpTSP2 near the membrane. CpTSP2 localization and predicted domains suggest a role in macrogamont development and/or fertilization. Our plans include using the DiCRE conditional ablation system to delete the signaling/adhesive portion of the CpTSP2 and determine the effect on macrogamont maturation and fertilization. Insight gained on the mechanisms of macrogamont development and fertilization will guide future strategies of reducing disease burden and transmission.

P23 A kelch protein redox survival mechanism licenses killing by artemisinins.

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Frontline antimalarial artemisinin (ART) derivatives that induce oxidative damage also show potential activity against other eukaryotic cells, but redox mechanisms of ART-susceptibility in broadly across eukaryotes and their relationships to malarial counterparts, if any, remain unclear. We previously reported that the Plasmodium falciparum Kelch13 (K13), a malarial marker for ART-resistance (ART-R), binds the oxidant heme in vitro. Here, we show that heme at nanomolar concentrations specifically binds and stabilizes ectopically expressed K13 in Du145 tumor cells. K13 also binds and regulates the major mammalian redox transcription factor Nuclear factor erythroid 2-related factor 2 (Nrf2). Heme-binding dysregulates Nrf2, which is reversed in ART-induced death (ART-death). K13's kelch domain confers heme-induced stabilization and ART-death activities to its closest mammalian orthologue Kelch-like ECH-associated protein 1 (KEAP1; the canonical regulator of Nrf2). Chemical or genetic elevation of K13 enhances ART-death even in excess heme in two independent tumor cell lines. Together, these data reveal a redox survival mechanism that licenses ART-death in mammalian cells with implications for malaria parasites and other eukaryotic pathogens.

P24 PTEX components are important for Plasmodium transmission to the mosquito

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In the vertebrate host, Plasmodium parasites invade target host cells and develop within a parasitophorous vacuole (PV). Intracellular survival depends on protein export and solute transport across the PV membrane mediated by the Plasmodium Translocon of Exported proteins (PTEX) composed of EXP2, PTEX150 and HSP101. Transmission to the insect vector occurs following a blood meal when ingested gametocytes mature and fuse in the mosquito midgut to generate a zygote which then transforms into a motile ookinete, traverses the midgut epithelium and forms an oocyst for sporozoite development. While PTEX is essential for vertebrate infection, it is thought to be dispensable in the insect vector where oocyst development occurs in the extracellular space; however, PTEX expression and function have not been evaluated during early insect stages. Interestingly, we found EXP2, PTEX150 and HSP101 are expressed in Plasmodium berghei ookinetes with signal distributed in puncta throughout the cell and concentrated around crystalloids, clustered vesicular structures thought to function in protein storage and transport during the ookinete to oocyst transition. To query the importance of PTEX in the mosquito, we employed a DiCre conditional knockout strategy. Strikingly, deletion of exp2, ptex150 or hsp101 in mature gametocytes severely impacted oocyst formation. Importantly, complementation under the control of an ookinete-specific promoter rescued oocyst formation but not asexual development in the vertebrate host, confirming a critical role in mosquito infection after fertilization. Collectively, these results reveal an unexpected role for PTEX components in parasite transmission to the mosquito vector, suggesting a function in ookinete midgut penetration and/or early oocyst development.

P25 The burning questions: Understanding host-fungal interaction dynamics during thermal wound infection

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Military conflicts in the 21st century have been marked by a shift towards asymmetric warfare that is characterized by increased use of improvised explosive devices (IEDs) and incendiary drone strikes in active conflict zones and neighboring residential areas. Burn wound injuries (BWIs) in both military personnel and local civilians are amongst the most devastating forms of trauma caused by these blasts and secondary fungal infections are a consistent concern within these wounds. Reports have found that between 8-44% of severe BWIs develop fungal infections. Once infected, mortality is 8 times more likely and the necessity for limb amputation is tripled. Yet, despite the high prevalence and risks associated with fungal burn infections, few studies have been directed at assessing how fungi cause disease within the burn niche and how the host subsequently responds to pathogen presence. To better elucidate host-fungal interaction dynamics at the burn wound interface, we expanded upon an existing zebrafish model of thermal injury that recapitulates many features of human burn wound pathology to establish a fungal burn infection model. Here, we show that Candida albicans, the most common cause of fungal burn infections, successfully colonizes burn tissue and is effectively cleared in immunocompetent fish. Mechanistically, we identify distinct roles for macrophages and neutrophils in facilitating fungal clearance from the burn site, where macrophages control fungal burden and neutrophils primarily control invasive hyphal growth. Accordingly, we find that C. albicans mutants with increased exposure of the immunogenic cell wall epitope ß(1,3)-glucan are more readily cleared during burn wound infection, highlighting a role for immune system evasion for successful fungal colonization in this niche. Together, our findings support the use of zebrafish larvae as a model to study host-fungal interaction dynamics during burn wound infection and provide important insights into host-fungal interactions within the burn wound niche.

P26 A ubiquitin-like protein influences apicoplast protein import in malaria parasites.

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The genus Plasmodium contains an essential four membraned plastid called the apicoplast. Most apicoplast proteins are nuclear encoded and are trafficked through the plastid membranes via the TIC, TOC, and SELMA translocons. In related organisms, SELMA involves the participation of a ubiquitinylation cascade for protein movement across the plastid membranes. However, the ubiquitin analog for SELMA in Plasmodium has not been elucidated. This work aims to characterize the ubiquitin-like protein PfAUBL (Pf3D7_0815700) and its role in apicoplast protein import. Unlike other ubiquitin proteins, PfAUBL lacks a C-terminal diglycine motif, containing three transmembrane domains at the C-terminal end instead, and has no identified homologs in related apicomplexans. Ultrastructure expansion microscopy showed PfAUBL localized in the apicoplast membranes, while immune-EM imaging revealed signal in the apicoplast membranes and small cytoplasmic vesicles. Proximal labeling experiments using PfAUBL as bait identified some components of the SELMA translocon among the top hits, as well as many apicoplast localized proteins and components of vesicular trafficking machinery, suggesting that PfAUBL participates in these functions. Utilizing the TetR aptamer knockdown system we found that PfAUBL is required for parasite growth in the asexual blood stage. Western blot analysis on PfAUBL-deficient parasites revealed a block in apicoplast lumen protein processing, suggesting a block in SELMA-associated apicoplast import. We hypothesize that PfAUBL is involved in vesicular trafficking to the apicoplast, and protein import across the apicoplast membranes. Gaining an understanding of PfAUBL function and how the SELMA translocon transports apicoplast-bound cargo will provide new insights into apicoplast homeostasis and growth of this essential organelle.

P27 Characterization of Toxoplasma gondii dense granual protein GRA5 as a modulator of toxoplasmosis disease severity

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Toxoplasma gondii is a highly prevalent protozoan parasitic infection that can lie dormant in an encysted stage within its human host's central nervous system, notably the brain, for the hosts lifespan. While this chronic T. gondii infection persists asymptomatically, it holds the potential for reactivation which can result in devasting neurologic consequences. While T. gondii is the only species in its genus, the genetic diversity across the species is vast and it is clear that different strains of T. gondii heterogenous phenotypic presentation in its host. Three cases of reactivant toxoplasmosis, one fatal, at the University of Wisconsin – University Hospital prompted an investigation into using human cerebrospinal fluid (CSF) to identify T. gondii-specific peptides within human CSF for strain identification. One peptide in specific, dense granual protein GRA5, was identified and observed to have peptide sequence heterogeneity, with differing amino acid residues even within strains of the same Restriction Fragment-Length Polymorphism genotype (#7). In vivo studies demonstrate differences in pathogenic potential, providing evidence for increased virulence in these strains compared to a clinically relevant strain, ME49. In vitro characterization by means of plaque assay, invasion assays, and replication assays provide greater characterization of these different RFLP genotype #7 strains within the acute period of infection. Lastly, we begin to investigate the heterogeneity of these other highly abundant peptides identified in the CSF analysis to identify if any hold potential as additional strain-specific biomarkers.

P28 Investigating the role of parasite G protein coupled receptors in sensory processes and their potential as novel anthelmintic targets

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Parasitic nematodes infect billions of people and are primarily controlled by a limited arsenal of anthelmintics. Concerns about drug resistance and contraindications with co-infections underscore the urgent need for new compounds with diverse mechanisms of action. G protein-coupled receptors (GPCRs) are important drug targets in human medicine and are known to mediate essential behaviors in the model organism, Caenorhabditis elegans. However, their roles in similar behaviors in parasitic nematodes as well as their potential as anthelmintic targets remain largely unexplored. First, we aimed to explore the anthelmintic potential of a specific class of GPCRs, the biogenic amine GPCRs (BAGPCRs). We first profiled whole-organism effects of aminergic compounds known to target human BAGPCRs against C. elegans and the human parasitic nematode Brugia malayi. We found that they produced impaired development in C. elegans and motility in B. malayi microfilariae as well as altered secretory activity in adult B. malayi. To identify receptors underlying these effects, we performed phylogenetic analyses which revealed three GPCRs and one ligand-gated ion channel as candidate dopamine receptors in B. malayi. We used a heterologous expression platform to characterize two GPCRs (Bma-dop-1 and Bma-dop-2) as dopamine receptors and found that both receptors displayed species-specific differences in biogenic amine sensitivity between human, C. elegans, and B. malayi BAGPCRs. These findings suggest that parasite dopamine receptors possess distinct pharmacological properties that could be exploited to develop selective anthelmintic agents. Second, we aim to define the role of parasite GPCRs in chemosensory processes. I will express putative receptors from Brugia malayi, a human parasitic nematode, in C. elegans neurons along with a GCaMP fluorescence biosensor. I will then develop a novel assay to detect fluorescence as a proxy to GPCR activation in response to chemical stimuli to identify which GPCR(s) are participating in known chemosensory behaviors in B. malayi.

P29 Bioinformatic analysis of Toxoplasma alleles that may suggest symptomatology in the chronic phase.

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Background: One third of the world is seropositive for Toxoplasma gondii. Current medical dogma suggests all of these individuals have latent, but metabolically active cysts in their hearts and brain. Whether these “asymptomatic” cysts contribute to epilepsy and other diseases is disputed.
Toxoplasma has 6 major clades. Two polymorphic genes are potentially linked to greater human disease. Rop16 has divergent kinase alleles that either do or do not activate host Stat6. Phosphorylation of Stat6 in the neuron possibly contributes to erratic electrical activity and subsequent lowering of the seizure threshold.
The second polymorphism is in Gra5 and also has two alleles. We identified this protein (peptides from both alleles) in the spinal fluid of three human patients with reactivated toxoplasma. The patient with the “virulent” allele (based on mouse data) was rare lethal case without HIV.
Results: We identified over 35 sequenced Rop16 alleles and over 23 Gra5 sequences that could be classified. The most common combination was a less virulent Gra5 and Rop16 stat activating combination (present in all type II Clade D strains sequenced). All four possible combinations were seen. Clade A (type I) and Clade C (type III) appear to have 2 uniform, but different combinations). There was no single strain in Clade E with both alleles sequenced. Clade B appears to have a uniform Rop16 kinase allele but varying Gra5 alleles. The small number of strains that have had both alleles sequenced limits the applicability of these results.
Conclusions: More sequencing is needed particularly of diverse isolates with sequence data on both Rop16 and Gra5, especially Clades B, E and F. It is possible though that sequence variation in these two genes and others explain some of the inconsistency of clinical studies associated toxoplasma seropositivity with an increase in epilepsy.

P30 Targeting Candidozyma auris adhesion with AI-designed miniproteins

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Candidozyma (Candida) auris is an emerging pathogen that is easily transmissible in healthcare settings worldwide. C. auris robustly colonizes human skin and frequently contaminates abiotic surfaces such as medical devices. Nearly 90% of C. auris isolates are resistant to at least one class of antifungals and pan-resistant untreatable infections have already been seen in clinics. Therefore, there is an urgent need to identify novel treatment strategies that prevent C. auris colonization and infection. The physical attachment of fungal cells to surfaces is the first step in colonization and biofilm formation. In fungal cells, this attachment is mediated by proteins exposed at the cell surface known as adhesins. Previously, the adhesin Als4112 was identified as essential to the adhesion of C. auris to skin, and the adhesin Scf1 was identified as essential to the attachment of C. auris to abiotic surfaces and biofilm formation. We hypothesized that directly targeting these adhesins would reduce C. auris skin colonization, attachment to abiotic surfaces, and biofilm formation. To test this, we used AI methods including BindCraft and Boltz2 to design miniprotein binders to target the cell surface adhesins Als4112 and Scf1. We then cloned and expressed these miniproteins in E. coli, and demonstrated direct interaction with C. auris cells using immunofluorescence. We used C. auris adhesin mutants to examine specificity of binding. We then tested whether the Als4112-targeting miniproteins can disrupt fungal attachment to human skin keratinocyte cells and whether the Scf1-targeting miniproteins can inhibit attachment to abiotic surfaces and biofilm formation. This work aims to establish a novel anti-adhesion strategy to limit skin and abiotic surface colonization to reduce the transmission reservoir of multidrug-resistant C. auris.

P31 Blood-feeding frequency impacts fitness of malaria parasites and their mosquito host

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Female mosquitoes blood-feed to acquire nutrients for egg production, often doing so multiple times to produce multiple clutches of eggs. Anopheles mosquitoes transmit malaria parasites, which belong to the genus Plasmodium and kill nearly half a million people annually. Despite their virulence to vertebrates, several studies have demonstrated that these parasites impose no fitness cost on their vector. Furthermore, an additional blood meal after infection can accelerate P. falciparum development in A. gambiae, suggesting a second influx of nutrients during Plasmodium development can benefit transmission potential. However, whether the number and timing of blood meals relative to infection shapes the parasite's development and interaction with the mosquito vector has remained unexplored. This is important for understanding how iterative blood feeding affects malaria transmission potential in nature, since access to vertebrate hosts can vary outside the laboratory. To address this, we offered P. berghei-infected A. stephensi additional naïve blood meals at different frequencies and assessed parasite prevalence and intensity compared to females that received only the infectious meal. We also assessed mosquito fecundity, longevity, expression of genes controlling egg production, and nutrient storage in the fat body relative to naïve females. Sporozoite loads increased with blood-feeding frequency, suggesting that additional blood meals can provide further resources for parasite reproduction. However, while P. berghei modulated mosquito gene expression and metabolism, infection only subtly impacted mosquito fitness. Taken together, these results indicate that malaria-infected mosquitoes that blood-feed more frequently may transmit the parasite more efficiently to their vertebrate hosts.

P32 Development of novel antifungal compounds targeting the Cdc14 phosphatase

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Invasive fungal diseases are a rapidly evolving threat to human health. Driven by antifungal resistance and limited number of effective antifungal drugs, there is a dire need for the identification of novel targets and therapeutics with unique modes of action. Our group recently demonstrated that the Cdc14 phosphatase, which is invariant in Dikarya fungi, is required for virulence of Candida albicans in mouse models of invasive candidiasis. Cdc14 is also important for virulence in several phytopathogens, highlighting this phosphatase as a potential target for broad-spectrum disease control. The unique active site structure and substrate specificity of Cdc14 indicate that the enzyme is amenable to selective inhibitor development. Here, we report a novel set of competitive inhibitors targeting the Cdc14 phosphatase family based on existing phosphotyrosine mimetic warheads and demonstrate their potential as lead antifungal compound. Our inhibitors exhibit broad activity against Cdc14 enzymes from diverse fungal pathogens and minimal activity against other related phosphatases. The compounds effectively suppress hyphal growth of Candida albicans in solid media and in mice, a phenotype previously associated with Cdc14 loss of function mutants. One inhibitor significantly extended mouse survival and exhibited no toxicity in a common model of invasive candidiasis. Further optimization and characterization of this novel scaffold is ongoing. These compounds lay the foundation for development of a novel antifungal therapeutic with a unique target and mode of action.

P33 Elucidating factors that alter uptake of Cryptococcus neoformans by human microglia

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Cryptococcus neoformans (Cn) is a globally distributed, environmental fungal pathogen of high health concern, particularly to immunocompromised individuals. Although acquired by inhalation, it spreads to the central nervous system (CNS),where it causes disease that is invariably lethal without treatment and carries high mortality even with intervention. Why microglia, the tissue-resident macrophages of the CNS, are unable to control and clear the infection is not known. While due in part to cryptococcal antiphagocytic proteins, microglial deficiency in controlling Cn infection has not been fully explained. Previous work has demonstrated that microglia exhibit limited phagocytosis of Cn compared to alveolar macrophages. To determine if particle size impacts microglial phagocytosis, uptake assays were conducted exposing C20 immortalized human microglia to 2, 5, and 8 micron latex beads, both unopsonized and opsonized with either human serum or human complement, and phagocytic indices were measured. Two micron beads were found to be phagocytosed at higher levels than either 5 or 8 micron beads, both of which were phagocytosed at low levels, indicating that particle size is a contributing factor to microglia phagocytic ability. Similarly, to determine if Fc receptors play a role in microglial phagocytosis, uptake assays were conducted to compare uptakes of IgG-coated latex beads and uncoated latex beads by C20 microglia, and the resulting phagocytic indices were measured. IgG-coating increases bead uptake by microglia, indicating that Fc receptors play a role in microglial recognition of particles and subsequent phagocytosis. An uptake assay screen was conducted to identify Cn mutants with increased uptake by C20 microglia. Phenotypic characterization of these hits is currently underway to elucidate the specific fungal qualities that alter microglial uptake.

P34 Using Proteomic Methods to Reveal Novel Candidate Substrates of Candida albicans Hyphal Regulatory Proteins

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Fungal pathogens cause invasive infections that result in millions of deaths annually. The opportunistic pathogen Candida albicans is dimorphic, growing as both yeast and hyphal cells, with both morphologies essential for proliferating invasive infections. However, the molecular mechanisms that establish and maintain the hyphal state remain incompletely understood. We are studying a hyphal growth regulatory network consisting of the Cdc14 phosphatase and the Mob2/Cbk1 and Hgc1/Cdc28 kinase complexes. Although these enzymes are each required for hyphal development, their specific substrates and the molecular mechanisms by which they coordinately promote hyphal growth remain unclear. To better understand how this network regulates hyphal morphogenesis, I am employing complementary targeted proteomic approaches. To identify candidate substrates and regulators of Cdc14, Mob2/Cbk1 and Hgc1/Cdc28, I used TurboID proximity labeling in yeast and hyphal cells. The TurboID biotin ligase domain is genetically fused to a protein of interest, enabling rapid biotinylation of proximal proteins in vivo. Biotinylated proteins are enriched by streptavidin affinity capture and identified using mass spectrometry. My TurboID data have revealed known and novel interactors and substrates of each key regulatory enzyme in both morphologies. By filtering for known consensus phosphorylation motifs, I have identified the strongest candidate substrates. In parallel, I am using an auxin inducible degron (AID)-coupled phosphoproteomics method to identify phosphorylation sites with altered abundance immediately following acute degradation of each respective regulatory enzyme. Integration of these proteomic datasets is expected to reveal high-confidence identification of direct substrates and their regulatory phosphorylation sites. Future work will involve phosphosite mutations to determine how substrate phosphorylation controls hyphal development. Defining these substrates will advance our understanding of how the Cdc14, Mob2/Cbk1 and Hgc1/Cdc28 regulatory network contributes to C. albicans pathogenicity.

P35 Fungal Avoidance of Host Defenses: Trehalose Biosynthesis in Cryptococcus

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Trehalose-6-phosphate synthase (TPS1), part of the trehalose biosynthetic pathway, was identified as a promising potential drug target for treating systemic fungal infections such as those caused by Cryptococcus neoformans. Deletion of TPS1 gene led to avirulence in C. neoformans. Previous work in the lab showed that TPS1 conveys protection to C. neoformans against pulmonary innate and residential host defenses to establish infection in the lungs, in a T- cell independent manner. This project investigated whether exposure of mice to a strain of Cryptococcus neoformans that lacked TPS1 would alter the adaptive immune response, even if it is not required for clearance of the mutant. To test this, mice were infected intranasally with 2 live-doses of a TPS1-deficient (tps1Δ) mutant strain of C. neoformans at 2 week intervals. Following their recovery from the tps1Δ strain, they were infected intratracheally with the highly virulent H99 strain of C. neoformans, in a lethal challenge. Mice that were initially exposed with tps1Δ showed improved rates of survival over a 70 day infection period and lower fungal burdens in multiple organs compared to the control group that was mock-infected with PBS. Immune analysis of the vaccinated mice showed a protective polarization of the T cell responses. Subsequent depletion of CD4+ T cells via treatment with α-CD4 antibody hindered the effectiveness of the initial immunization and led to no significant difference in survival rates between the tps1Δ-exposed and the PBS-exposed mice. Thus, the tps1Δ strain has the potential to interact with the adaptive immune system as a vaccine and provide long-term protection.

P36 Cell Cycle Arrest Strategies of Related Yeast Species Under Phosphate Conditions

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Sensing and responding to nutrient availability is crucial to an organism's survival. Phosphate is an essential macronutrient - a building block of nucleic acids, phospholipids, proteins, etc. - that yeast must acquire from the environment. Under phosphate limitation, whether cells continue to reproduce or enter cell cycle arrest carry grave consequences for survival and fitness. In this study, we investigate how four related yeast species, Saccharomyces cerevisiae, Nakaseomyces glabratus (Candida glabrata), Kluyveromyces lactis, and Candida albicans diverge in their temporal dynamics of entering cell cycle arrest upon phosphate limitation.

Using both flow cytometry and microscopic analysis, we show that all four species eventually reach 60 - 75% of cells in G1/G0 phase after 6 hours of phosphate starvation. However, the underlying dynamics show intriguing differences. K. lactis and C. albicans both show a relatively steady increase in the fraction of G1/G0 arrested cells. In contrast, S. cerevisiae shows a rapid, transient increase in G1/G0-arrested population during the first hour, followed by a quick recovery before increasing again to the plateau. C. glabrata shows an opposite pattern, an initial mild decrease in the fraction of G0/G1-arrested fraction before a rapid increase, reaching the plateau before the other three species. A high-resolution time course confirmed these trends, suggesting that these species adopt different strategies of cell cycle arrest response to the same nutrient starvation signal. Furthermore, these cellular response dynamics are supported by gene expression dynamics, revealing the potential genetic basis for the divergent behaviors.

Our findings demonstrate the dynamics of entry into cell cycle arrest entry during phosphate limitation diverged between these four yeast species and are paralleled by divergent transcriptional responses. We speculate that this divergence reflects species-specific adaptations. Our study serves as a model for understanding the evolutionary principles for starvation responses as species adapt to unique environments.

P37 Investigating the function of MCM10 in Candidozyma auris

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Faithful replication of the genome requires the coordinated unwinding of double-stranded DNA at bidirectional replication forks by multi-subunit helicase complexes. The MCM10 gene has been characterized as an essential gene in Saccharomyces cerevisiae, where it acts as a critical initiation and processivity factor required to activate these helicase motors. However, in our recent work investigating gene essentiality in C. auris, we identified multiple inserts in MCM10, suggesting that this gene is not essential in C. auris.

We are creating mutants to assess the role of MCM10 in phenotypes related to replisome stability, replication fork protection, and genotoxic stress tolerance. To test the effects of this gene on Candida auris, we are using a CRISPR-Cas9 gene-editing system to generate a stable Δmcm10 mutant strain. We will assay sensitivity to DNA damaging agents and measuring chromosomal break frequencies via pulsed-field gel electrophoresis. Through these approaches, we expect to understand more about the biological function of Mcm10 and gain insight into why it is not essential in C. auris. Investigating this gene within Candidozyma auris is vital to determine if the essential replication function is conserved, which could reveal novel therapeutic vulnerabilities targeting the core DNA replication machinery of this pan-resistant organism.

P38 Evaluating phenotypic and genotypic variation across Candidozyma auris clinical isolates

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Since its first report in 2009, Candidozyma auris cases have steadily risen globally, leading the WHO to classify it as a critical priority fungal pathogen in 2022. Following the introduction into a healthcare system, C. auris is able to quickly become an established pathogen within the hospital where it has high mortality rates among immunocompromised individuals. Its efficient survival and transmission in hospitals is bolstered by its ability to grow at high temperatures, live on plastic surfaces for extended periods of time, and resist disinfectants commonly used in hospitals. However, many of the genetic factors contributing to these phenotypes remain unknown and understudied.

Here, we demonstrate that there is high variation between and within clades in phenotypes related to environmental persistence and transmission. We have screened a collection of 160+ C. auris clinical isolates for their desiccation tolerance, growth at 42°C, and growth in the presence of the quaternary ammonium salt cetyl trimethyl ammonium bromide. These phenotypic data, along with genotypic data obtained through whole genome sequencing, will be used to conduct a microbial Genome Wide Association Study (mGWAS). This will identify genetic factors that contribute to C. auris persistence in hospitals, providing insight into the mechanism of C. auris spread.

P39 mRNA pseudouridylation as a potential regulator of translational control driving differentiation in Toxoplasma gondii

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Toxoplasma gondii is an obligate intracellular protozoan parasite that has two distinct asexual life stages in the intermediate host: slowly replicating bradyzoites and rapidly replicating tachyzoites. Translational control is induced by tachyzoite stress, triggering their conversion into bradyzoites. BFD1 is a master regulator transcription factor of bradyzoite differentiation, whose mRNA is subject to translational control through interaction with the RNA-binding protein BFD2/ROCY1. To further understand how BFD2 drives stress-dependent translation of BFD1, we elucidated the BFD2 interactome. Our findings indicate that BFD2 forms a complex with MKT1 and PBP1, proteins, known to mediate translational regulation and development in other systems. Additionally, we identified PUS2 as a novel interactor of MKT1. PUS2 is a pseudouridine synthase that mediates the isomerization of uracil to pseudouridine, a conserved RNA modification that may impact stability and structure. Notably, a previously conducted forward genetic screen independently identified PUS1 as being implicated in bradyzoite formation, highlighting the importance of RNA pseudouridylation in parasite latency. We are now testing the hypothesis that BFD1 mRNA translation is regulated via pseudouridylation by generating PUS1 and PUS2 conditional mutants. Future work will use these mutants to identify pseudouridine sites using direct RNA-seq that modulate mRNA translation, which will be confirmed through mutational analysis of target transcripts.

P40 Transcriptomic, behavioral, and morphometric differences between Schistosoma mansoni miracidia recovered from different host tissues

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Schistosomiasis is a neglected tropical disease caused by human-infective parasitic flatworms of the genus Schistosoma. Adult male and female parasites pair in the mesenteric vasculature; females lay eggs that traverse the intestinal wall to be excreted, but a significant proportion become trapped in host tissues, especially the liver, eliciting granulomatous immune responses that underlie most disease pathology. Schistosoma mansoni is the primary lab model for research due to the abundance and ease of harvesting. Liver-derived eggs are almost exclusively used to maintain the life cycle and to study miracidia and subsequent larval stages, despite intestine-derived eggs being more representative of the continuation of the parasite's natural life cycle. Recent evidence shows that eggs from the liver or intestine have key transcriptomic and antigenic differences, which profoundly affect experimental outcomes. To determine whether these differences extend to the miracidia stage, we compared miracidia hatched from mouse liver and intestine-derived eggs, sequencing their transcriptomes and assessing their behaviors over time in an arena allowing for high-resolution tracking. We found that while transcriptomic profiles of miracidia are distinguishable based on egg tissue origin, only a small subset of genes is differentially expressed. Further, the behavioral profiles of miracidia that developed in different niches were significantly different. Future experiments will investigate whether these differences persist in cercariae, the subsequent stage of schistosome development, to determine the duration of this divergence across life cycle. Additionally, reproducing these results in other S. mansoni strains (e.g., the Liberian strain) would help evaluate whether the findings are consistent across strains with different isolation and husbandry histories. These findings underscore the importance of parasite source in experimental design and interpretation, with significant implications for maintenance of laboratory life cycles.

P41 Extraocular color discrimination by Schistosoma mansoni

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Schistosomiasis is a neglected tropical disease that affects over 250 million people worldwide and is caused by schistosomes, parasitic flatworms with a complex life cycle. Miracidia, the first larval stage of schistosomes, infect snails as intermediate hosts, where they mature into a larval stage capable of infecting humans. Miracidia detect and interpret light to navigate their environment and locate themselves within microhabitats likely to contain potential snail hosts, but the photosensory behavioral program, neuronal structures and pathways, and molecular events involved in this response are poorly understood. Indeed, schistosome miracidia are rare among trematodes in that they have no identifiable photosensory anatomical feature. The purpose of this study was to analyze the movement of Schistosoma mansoni miracidia in response to light to determine minimal photon threshold, potential chromatic preference, and the quantitative ethology of the light response. Miracidia were loaded into arenas and exposed to light gradients, including white, red, blue, and/or green light. Different combinations of light gradients were used to investigate chromatic preference. As expected, and consistent with previous work, miracidia are phototactic at even low levels of light. Notably, chromatic preference experiments demonstrated that miracidia are attracted to all visible wavelengths tested but have a strong preference for green over blue or red light. Future experiments will explore the integration of light and chemical perception, and the ecological implications of these behaviors, using mesocosm experiments with snails and miracidia under different light conditions to determine if color has effects on host-seeking. Understanding this sensory coordination could be key to developing new strategies to reduce the population of infected snails and the spread of schistosomiasis.

P42 Deep learning–based pose tracking of Aedes aegypti larvae reveals chemosensory and photosensory behaviors

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Aedes aegypti is an important arbovirus vector responsible for transmitting diseases such as dengue, Zika, and yellow fever, which collectively cause millions of symptomatic cases globally each year. Consequently, extensive research has been conducted to understand the basic biology of these vectors and inform public health initiatives worldwide. The sensory behaviors which allow adult mosquitoes to forage and locate hosts have been thoroughly investigated, while less is known about photosensory and chemosensory behaviors of the larvae. We conducted behavioral assays using custom-built arenas and an array of high-resolution cameras to evaluate the response of Aedes aegypti larvae exposed to light and chemical gradients. By analyzing poses using deep learning models that allow for precise tracking of individual anatomical features, we confirmed that starved mosquito larvae accumulate near concentrated areas of chemical cues associated with food. The platform is now being used to investigate methods of motion used by larvae to navigate light gradients, and future efforts will explore the effects of combining photosensory and chemosensory stimuli on larval movement behavior.

P43 Living with a killer: how coevolved Saccharomyces cerevisiae become killer toxin resistant

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Killers abound among Saccharomyces cerevisiae. Virally encoded toxic peptides provide an advantage against susceptible neighbors, but what evolutionary strategies are open to susceptible yeast for toxin resistance? How susceptible strains evolve can provide insights into fungal defense and toxin evasion strategies. Understanding these mechanisms may aid in the discovery of new anti-fungals.

In an experimentally co-evolved population, susceptible yeast evolved resistance to virally encoded K1 type toxin. A fixed missense variant in the gene SSK1 was identified as a genetically dominant determinant of toxin resistance. SSK1 is an intermediate regulatory signal transducer of the HOG osmoregularity pathway and part of a two-component system. Implicated in fungal adaptation under other conditions, metazoans lack such systems, making them appealing anti-fungal drug targets.

An ionophore, K1 toxin causes cell death through loss of membrane integrity, but also interacts with components of the cell wall. To determine how mutant SSK1 confers toxin resistance we challenged strains with stressors targeting various cell membrane and cell wall components. Preliminary results suggest mutant SSK1 increases resistance to compounds targeting the membrane, but decreases resistance to beta-glucan and chitin targeting dyes. This suggests SSK1 engages in crosstalk with the cell wall integrity (CWI) pathway and resistance comes at a cost to cell wall defenses. Early results from a pilot RNA-seq experiment suggest cells carrying mutant SSK1 have increased responsiveness of components of the HOG and CWI pathways to a cell wall disrupting compound compared to cells carrying the reference SSK1 allele. In addition to being part of a signaling system not found in animals, SSK1s vulnerability to tradeoffs in evolving resistance to toxins suggest its potential as a target for anti-fungal treatments.

P44 Investigating the Role of Phosphatidylinositol 3-phosphate in the Cryptococcal-Containing Phagosome

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Cryptococcus neoformans' ability to survive within the phagosomes of immune cells is one of the main drivers of infection. These cryptococcal-containing phagosomes (CCPs) within macrophages allow for a permissible environment for the fungi. Although studies have begun to characterize the fungal factors contributing to the CCP, there is a major knowledge gap in the specific host factors that influence its formation. We have found that a significant population of CCPs are labeled with both phosphatidylinositol 3-phosphate (PI3P) and lysosome-associated membrane protein 1 (LAMP1), which are early and late endosomal markers, respectively. Of these, the host lipid PI3P is required for the proper maturation of phagosomes and reports from other microbial pathogens suggest that prolonging its association to the pathogen-containing phagosome inhibits its maturation. Given that we previously found that up to 30% of CCPs exhibited this hybrid phenotype of PI3P and LAMP1 association in THP-1 cells, we aim to determine the effect of PI3P on the CCP's properties. THP-1 cells were infected with KN99 C. neoformans and BY4741 S. cerevisiae for up to 8 hours with select wells treated with LY294002, a PI3 Kinase inhibitor. Cells were then stained with antibodies against EEA1, an early endosomal marker, or LAMP1, a lysosomal marker. While there was no change in LAMP1 association, cells treated with LY294002 had a decrease in EEA1 association. When testing for changes in uptake, we observed a significant decrease in KN99 uptake when treated with LY294002 with no change in BY4741. While the delay of EEA1 association in PI3P-depleted conditions is unsurprising, the continual association of LAMP1 suggests little change in the manipulation of phagosomal maturation. The change in uptake specific to KN99 suggests an alternative method of phagocytosis. We plan to investigate this phenomenon further with internal properties of the CCP environment, LC3 studies, and siRNA knockdown.

P45 Characterizing the 1-CysPrx Gene Promoter in the Malaria Parasite Plasmodium berghei

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The malaria parasite Plasmodium spends a significant portion of its life within a mosquito, which transfers the infection from person to person. While inside of the mosquito, Plasmodium faces many challenges to its survival, such as the damaging effects of reactive oxygen species (ROS) produced in the mosquito midgut during bloodmeal digestion. In order to defend itself against ROS, Plasmodium upregulates the expression of antioxidant proteins while inside the mosquito. This project focuses on the antioxidant 1-cysteine peroxiredoxin (1-CysPrx), and investigating the identity of the transcription factor binding site in the DNA promoter region of the 1-CysPrx gene. The results of this research will provide a key insight into the regulation and expression of the Plasmodium antioxidant defense system.

P46 The immunoregulatory role of iNOS during cryptococcal infection: Limiting neuroinflammation and CNS damage

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Cryptococcus neoformans (C. neo) is an environmental fungal pathogen that results in severe infections in immunocompromised individuals. C. neo infection has a high mortality due to the limited number of therapeutic options, as many antifungals are toxic or ineffective. Inducible nitric oxide synthase (iNOS/NOS2) is a hallmark of classically activated myeloid cells with known antimicrobial functions, but its role in regulating CNS immunity during cryptococcal meningoencephalitis (CM) remains unclear. In this study, we investigated the contribution of iNOS to fungal control, neuroinflammation, and CNS pathology using a murine model of cryptococcal infection. Wild-type (WT) and Nos2-knockout (Nos2-/-) mice were infected intravenously with 1 × 10^3 C. neo strain H99 and monitored for survival, neuroinflammation, and neurological disease. Despite comparable fungal burdens in the brain and peripheral organs, Nos2-/- mice exhibited significantly accelerated mortality relative to WT controls. iNOS deficiency was associated with increased blood–brain barrier permeability and exacerbated CNS pathology. Characterization and analysis of brain immune response revealed increased cytokine production, including IL-1a, IL-1b, and IFNg in Nos2-/- mice, suggesting that iNOS plays a key role in regulating the CNS immune response during CM, independent of fungal burden. Interestingly, CD4 T cell depletion failed to rescue the early mortality observed in iNOS-deficient mice, suggesting that this phenotype occurs independently of CD4 T cell-mediated adaptive immune responses. Together, these findings demonstrate that iNOS is dispensable for fungal clearance during CM but plays a critical immunoregulatory role in limiting excessive neuroinflammation and protecting against immune-mediated CNS damage.

P47 Evaluating The Impact Of Protease Inhibitors On The Survival Of Babesia microti and Babesia duncani

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Human babesiosis is an emerging malaria-like tick-borne disease caused by apicomplexan parasites of the Babesia genus, posing a significant risk to immunocompromised patients. In the United States, human babesiosis is primarily caused by Babesia microti and B.duncani[JT2.1]. The current treatments for babesiosis are limited to high-dose, toxic drug regimens derived from anti-malarial therapies, which have [JT3.1]shown increasing cases of drug resistance. Proteases play an important role in the parasite life cycle and metabolism, making them a promising drug target. In this study, we assessed the potential of protease inhibitors as compounds for alternative treatments of babesiosis. A protease inhibitor library consisting of 184 compounds was screened using a SYBR Green fluorescence assay to quantify drug efficacy. We identified 30 compounds that showed more than 75% inhibition of B. duncani [JT4.1]growth in under in vitro conditions. Many of these compounds are known for their anti-inflammatory, anticancer, and antibacterial properties, and represent promising candidates for further research. Drug dose-response assays with drug concentrations from 19.5 nM to 10 µM were then used to determine the IC50 of the major hits[JT5.1] that were identified. The calculated IC50 values ranged from 4200 [JT6.1]nM to 72 nM, with the best two compounds being carfilzomib, with an IC50 of 86 nM, and epoxomycin with an IC50 of 72 nM for B. duncani. Ossirene was a standout as a novel compound with an IC50 of 454nM for B. duncani, and 173 nM for B. microti. Ossirene is a synthetic immunomodulatory compound with known antiviral, anti-inflammatory, and anti-tumor properties. It is a potent inhibitor of IL-10 and caspase -1, and further studies to are in progress to understand its mechanism of action and in vivo efficacy. [JT7.1]This study provides a foundational basis for advancing therapeutic options for babesiosis beyond current drug limitations.

P48 Developing Expansion Microscopy for whole arthropods to investigate the biology of arthropod-borne Parasites.

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Light microscopy is the most widely used tool in the study of cell biology but many of the subcellular structures of parasites are too small to see even with the best light microscopes. Recently, a technique called expansion microscopy that physically enlarges parasites has revolutionised parasite cell biology. To date, expansion microscopy has only been applied on either parasites grown in vitro or from isolated host tissues. We wanted to perform expansion microscopy on whole mosquitoes, to simultaneously visualise the ultrastructure of both malaria parasites and their mosquito hosts, but the presence of the Chitin-rich mosquito cuticle prevents expansion. Here, we develop expansion microscopy for whole mosquitoes by digesting the cuticle with enzymes. We validate that the mosquitoes expand as expected, showing exquisite preservation of mosquito anatomy. Utilising a novel ‘deep-imaging’ microscope configuration, we were able to image through the entire expanded mosquito; capturing both host and parasite ultrastructure. Additionally, we have validated this technique for ticks and fruit flies, highlighting its versatility across arthropods. While developed to investigate cell biology, application of this technique could also greatly improve the resolution of spatial omics techniques in the study of vector-pathogen interactions.

P49 RNAcross: An Interactive Platform for Cross-Species Transcriptomic Visualization

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RNA-seq analysis is now a routine bioinformatic task supported by standardized tools. However, most of these tools are designed to interrogate single species. Comparative studies of gene expression is a powerful approach that is becoming increasingly common, and yet there is a lack of methods that integrate homology with gene expression to support this analysis. To fill this gap, we developed an interactive web application called RNAcross for exploratory analysis of time-course RNA-seq data across multiple species. This app takes a gene-expression matrix and sample metadata, along with a precomputed orthology table (e.g., from OrthoFinder), as input data. The app provides an interface where the user can interactively query gene orthology relationships, visualize and compare their expression dynamics using a variety of useful plot formats. The graphic elements of the plots are highly customizable, and high-quality figures can be saved to a local device. In addition to single gene analysis across species, the app also offers gene-set visualization tools that can be used to compare orthologous pathways and networks. Lastly, to make the tool useful for any lab with suitable datasets, we designed the app to allow users to upload their own input data without any curated reference. To illustrate its utility, we used RNAcross to analyze phosphate-starvation time-courses from four budding yeast species and demonstrate that the core PHO-regulon dynamics are conserved across all four species despite ~200 MY of divergence; we also identified intriguing differences in ribosomal gene expression and other pathways, suggesting divergence in the transcriptional response to this common stressor. This motivates new questions and hypotheses about how stress responses evolve as species occupy and adapt to distinct environments.

P50 Characterization of Inositol Metabolism in Aspergillus fumigatus

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Aspergillus fumigatus is a ubiquitous pathogenic mold known for its versatile metabolism including its ability to use multiple carbon sources. This flexibility in its metabolism allows A. fumigatus to infect diverse niches and aids in its ability to be a successful opportunistic pathogen. Cerebral Aspergillosis (CA) is a rare disease in which A. fumigatus invades and establishes infection in tissue of the central nervous system (CNS). This is a critically fatal fungal disease, but the mechanism in which Aspergillus invades and thrives in this niche is largely unknown. Within the brain inositol, a key metabolite in both fungi and host, is about 200X times more concentrated than the bloodstream. In Cryptococcus neoformans, inositol metabolism displays an important role in cerebral pathogenesis. However, inositol metabolism is completely uncharacterized in A. fumigatus. We hypothesize inositol metabolism may play a similarly important role in A. fumigatus pathogenesis and may play a role in its ability to thrive within the brain. To address this hypothesis, we have developed single genetic mutants of important enzymes required for de novo synthesis and utilization of inositol using CRISPR-Cas9 technology to study phenotypic and pathogenic changes. Based on basic phenotyping and staining we have observed changes in the fungal cell wall of these genetic mutants and a potential link to the production of chitin/chitosan. Preliminary animal studies highlight a role for these enzymes in pulmonary pathogenesis. Work is still ongoing to determine its role in cerebral pathogenesis.

P51 The path of peace: investigating a molecular mechanism of Candida albicans commensalism

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Candida albicans is a normal member of the human microbiota, colonizing skin and mucosal sites like the oral cavity, gut, and vaginal tract. In some cases, C. albicans can opportunistically invade tissue and spread to the bloodstream. Our ability to prevent the commensal-to-pathogen transition is hindered by our lack of understanding of the commensal state. While C. albicans commensalism is often framed as merely a lack of virulence, it is in fact an active state that requires ongoing molecular interactions between fungal and host cells. Our preliminary data hints at one possible mechanism: activation of host TGFbeta1 by an isolate with commensal behavior (529L). TGFbeta1 is a multifunctional cytokine with broadly anti-inflammatory effects. 529L, but not a more virulent C. albicans strain, induces expression of genes regulated by Tgfbeta1 signaling in oral epithelial cells. 529L supernatant is sufficient to produce this effect, implicating a secreted fungal factor. Furthermore, inhibiting the Tgfbeta1 pathway increases internalization of 529L cells by host cells. Our working model is that 529L induces a tolerant host response via TGFbeta1 activation in order to sustain its superficial colonization of epithelial cells. Ongoing work is focused on identifying the secreted fungal molecule and investigating whether Tgfbeta1 is directly activated.

P52 Determining the impacts of the mammalian lung environment on Cryptococcus spore germination

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Cryptococcus is an environmental yeast capable of causing fatal meningitis in humans. Cryptococcus causes disease from inhalation and then disseminates from the lungs to the brain via poorly understood mechanisms. To cause disease Cryptococcus must germinate from a spore into a replicating yeast. In cryptococcal infections germination occurs early, but the kinetics of this process and their effect on dissemination and disease are unknown. We aim to understand how both spore properties and germination environment impact germination kinetics and dissemination in mammalian disease. To evaluate Cryptococcus germination in vitro we developed a high throughput, quantitative germination assay (QGA). Using QGAs, we determined that spores germinate synchronously in response to glucose over ~12 hours in vitro. In vivo, little is known about how germination is triggered or what kinetics occur. To understand germination in a lung environment, we are developing a germination medium for QGAs from mouse lung homogenate (LH). Preliminary data show that spores germinate in LH media, but do so slower and less synchronously than in synthetic glucose medium. RNA-seq data from germination in LH medium show that germinating spores induce transcripts associated with host environment responses, such as the host environment associated transcription factor Pdr802. Simultaneously, we are using a mouse model of intranasal infection to determine germination timing in vivo. We discovered that in vivo germination kinetics are slower than in vitro, and approximately 20% of spores fail to germinate. Overall, these findings indicate that host lung and lung-like environments lead to germination profiles that differ substantially from standard in vitro germination. Studying the characteristics of spore germination in physiologically relevant environments promises to inform our understanding of early events in Cryptococcus infection and provide insights into how spores mediate fatal cryptococcal disease.

P53 An Alternative Genetic Mechanism to K1 Toxin Resistance in Saccharomyces cerevisiae

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Two dsRNA viral genomes infect some killer yeasts: the viral satellite ScV-M1 that encodes a toxin and antidote, and its helper virus L-A. We previously identified a gain-of-function mutation in SSK1, a member of the HOG osmoregulatory pathway, that drives resistance to K1 toxin in coculture experiments. K1 toxin resistance is also observed in coculture experiments where the sensitive yeast populations were allowed to evolve, and the killer populations were replaced with the ancestor every ~25 generations. Resistance arose in replicate populations around generation 400, and did not involve the previously identified SSK1 allele under this alternative selective regime. This work aims to identify the genetic basis/molecular mechanism of this resistance, as well as any associated trade-offs. While the previously identified SSK1 allele was present at low frequencies in the starting population, it appears to have been outcompeted by a different lineage that we believe might have achieved a more fit or 'elegant' molecular mechanism. Our preliminary data suggest not only a different evolutionary route to toxin resistance but also a potential fitness advantage over yeast harboring the toxin-resistant SSK1 allele. We are currently working to elucidate the molecular mechanisms and trade-offs of this unknown mutation in driving K1 toxin resistance.

P54 Mapping the genetics of parasex in Candida albicans

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Meiosis generates genetic diversity and facilitates adaptation in many eukaryotes but is absent in Candida albicans, a genetically-diverse fungal pathogen. Instead, C. albicans can undergo parasex, an irregular form of ploidy cycling that achieves the conventional meiotic outcomes of independent assortment and segregation. In the parasexual cycle, two euploid (2n) cells fuse to form a tetraploid (4n) mating product. While the 4n cell can stably propagate, growth on high-sugar media induces extensive genetic recombination and ploidy reduction through mitotic divisions (i.e., concerted chromosome loss). Surviving progeny are typically aneuploid, highly recombinant, and show greater phenotypic variability than their euploid parents. While the functional parallels between parasex and meiosis have recently been resolved, the molecular factors required for parasex remain largely unknown. To address this gap, I will define loci that mediate parasex in C. albicans via genetic screens. As an orthogonal approach to understand the requirements for parasexual function, I will comparative genomics approaches to infer genes associated with different sexual programs among species in the Candida genus.

P55 Quantifying the Phylogenetic Pattern of Oxidative Stress Resistance in Pathogenic and Benign Yeasts

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Oxidative damage is a major weapon used by our immune system to fend off fungal pathogens. Limited phylogenetic survey found pathogenic fungi exhibiting a higher oxidative stress resistance (OSR) than non-pathogenic species. However, this trend has not been systematically tested. The Saccharomycotina subphylum is well-suited to test the relationships between OSR and pathogenic potential, as it contains multiple, independently derived opportunistic pathogens in phylogenetically distant clades.

In this study, we quantify the resistance against the common reactive oxygen species, hydrogen peroxide, in 20 budding yeast species, which include at least three clades of independently derived pathogenic lineages along with their low pathogenic potential relatives.. We combine several well established methods, including Minimum Inhibitory Concentration (MIC), growth curve, and time-kill assays to quantify resistance to hydrogen peroxide. Multiple isolates of diverse origins are profiled for several key species, enabling estimation of intra-specific phenotypic variability. This data enables us to directly test the relationship between OSR and pathogenic potential under a phylogenetic comparative framework in the Saccharomycotina subphylum, laying the groundwork for identifying genetic and evolutionary mechanisms underlying these key virulence-associated traits.

P56 Characterizing Cytokine-Expressing C. neoformans Strains for Effects on the Cryptococcal Phagosome

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C. neoformans is a leading cause of death in patients with HIV and other immunocompromised patients. Contributing to its pathogenicity is its capacity to survive intracellularly inside the phagosome of macrophages, allowing it to avoid immune clearance but also to spread systemically. Previous studies have implicated the polarization of macrophages as a critical factor in controlling the infection, with a Th1 response associated with clearance and a Th2 response with susceptibility to infection. The effect the Th1- or Th2-associated cytokines have on macrophage phagocytosis of C. neoformans and on the cryptococcal phagosome has not been fully explored. Here we hope to elucidate the effect of the cytokines IFNγ, IL4, IL5, and IL17 on the macrophage response to C. neoformans and the mechanism by which these cytokines affect the properties of the cryptococcal phagosome. We have created KN99 strains expressing the human version of these cytokines and the strains demonstrate no obvious cell wall defects under 30C and 37C growth conditions, maintain melanization to L-DOPA, and the ability to form capsules. Additionally the cytokine strains overall retain growth rates similar to the background strain. Thus, they serve as a promising tool to study macrophages' ability to clear C. neoformans under various cytokine conditions.

P57 Characterization of an unusual and essential translation initiation factor 4G subunit (TgIF4G3) in Toxoplasma gondii

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Translational control is employed by organisms to rapidly reshape their proteome and respond to diverse environmental conditions. Toxoplasma gondii is a protozoan pathogen that transforms from replicating tachyzoites to dormant bradyzoite cysts under stress. Translation control mediates the initial steps of T. gondii stage conversion, but the mechanisms remain poorly characterized. Like other eukaryotes, T. gondii initiates translation using the eukaryotic initiation factor 4F complex (eIF4F) which includes the eIF4G scaffold protein. T. gondii has three paralogs of the eIF4G scaffold protein: TgIF4G1, TgIF4G2 and TgIF4G3. TgIF4G3 is unusual in that it lacks the domain to bind the mRNA cap-binding protein and form the canonical eIF4F complex. As TgIF4G3 is essential in tachyzoites, we fused it to a mini-auxin inducible degron (mAID) tag to examine the effects of TgIF4G3 depletion. Parasites lacking TgIF4G3 show profound replication and viability defects within 24 hours. A puromycin incorporation assay suggests that TgIF4G3 depletion does not significantly impair global protein synthesis. We also performed Ribo-Seq to look at changes in ribosomal association with mRNA transcripts and found that depletion of TgIF4G3 did not cause a significant shift in ribosome associated transcripts. We are currently testing whether TgIF4G3 regulates a small subset of crucial mRNAs during specific steps of the lytic cycle. Insights from the study of TgIF4G3 will likely illuminate vital roles for translational control during the lytic cycle and potentially reveal novel targets for drug development.

P58 Homologous recombination is more mutagenic than CRISPR-Cas9 in Candida albicans

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Candida albicans is an opportunistic fungal pathogen that is of concern in clinical settings. Genetic modification of C. albicans in the laboratory is critical to determining genotype-phenotypes relationships that contribute to its virulence, pathogenicity, and antifungal resistance. Two popular methods of genome modification are homologous recombination (HR) and CRISPR-Cas9 gene editing. These two methods have been used to great success; however, unintended genomic changes can occur during the genome modification process. A robust, controlled comparison of unintended genetic changes between the two methods is lacking. Here, we constructed a total of 180 deletion mutants in three genes (GAL1, SIR2, or ADE2) using HR and CRISPR-Cas9. Targeted gene deletions were built in one of two genetic backgrounds: a diploid lineage (2N) and an aneuploid lineage (2N+1) to determine how karyotypic imbalance impacts the mutational effects of transformation. Twenty independently constructed mutants for each gene using each method underwent whole genome sequencing and were analyzed for karyotypic and genomic changes to determine the mutagenicity of each method. Genome modification by HR resulted in greater chromosomal copy number changes and loss of heterozygosity events when compared to CRISPR-Cas9. Taken together, our analysis demonstrates that HR produces a greater frequency of genomic changes compared to CRISPR-Cas9 and advocates for the use of CRISPR-Cas9 in strain construction.

P59 Functional characterization of Histoplasma capsulatum chitinases

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Histoplasma capsulatum is a dimorphic fungal pathogen which can cause severe lung infection in both immunocompetent and immunocompromised people. Chitin, a core constituent of the fungal cell wall, is crucial for cell structure and protection of fungi from extracellular stressors. However, structural changes during morphological transitions as well as yeast cell division require remodeling of cell wall components, including chitin. To better understand how chitin impacts Histoplasma cell structure and cell wall dynamics, we characterized the eight chitinases (chitin hydrolyzing enzymes; Cts1-Cts8) encoded within the Histoplasma genome. For functional tests, we generated strains depleted of each chitinase via RNA-interference (RNAi), as well as CRISPR/Cas9-mediated genome editing. Individual chitinase depletion did not alter the overall level of cell wall chitin. However, depletion of Cts2, and to a lesser degree depletion of Cts4, increased yeast cell aggregation. The aggregation of Cts2-deficient cells requires cell growth and division suggesting Cts2-deficient yeasts have defects in cell separation following division. Either supernatant from Cts2-producing yeasts or addition of purified Cts2 protein can prevent aggregation of Cts2-deficient cells, indicating Cts2 acts in trans to facilitate cell separation. Yeasts lacking Cts2 and Cts4 are fully virulent in vivo. Interestingly, the yeasts in lung tissue homogenates, as well as from infected macrophages, are completely disaggregated. This suggests that a host factor disaggregates yeasts during infection, which may facilitate more dissemination of Histoplasma following respiratory infection. To identify this host factor, we are testing both the necessity and sufficiency of mammalian Chit1, an enzymatically active chitinase that is highly expressed in macrophages, for disaggregation of Histoplasma yeasts. Combined results from this work will define the role of both pathogen and host chitinase activity in fungal biology and infection.

P60 Determining mechanisms of translational control for factors driving bradyzoite differentiation in Toxoplasma gondii

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Apicomplexan parasite Toxoplasma gondii converts from replicating tachyzoites to its latent bradyzoite stage in response to cellular stress. Stress initiates the integrated stress response (ISR) by phosphorylating the alpha subunit of eukaryotic translation initiation factor 2 (TgIF2alpha), which dampens global protein synthesis and favors preferential translation of select transcripts. Preferentially translated mRNAs in stressed tachyzoites include those encoding factors that drive bradyzoite differentiation, such as the bradyzoite formation deficient 1 (BFD1) transcription factor and the RNA binding protein BFD2/ROCY1. T. gondii appears to employ different mechanisms to drive preferential translation of these mRNAs. BFD1 is preferentially translated under stress in a cap-independent manner that involves BFD2 binding to BFD1 mRNA. BFD2 is preferentially translated in a cap-dependent manner, but the mechanism remains unknown. We hypothesize that inhibitory upstream open reading frames (uORFs) in the BFD2 5′-untranslated region (UTR) suppress translation unless TgIF2alpha is phosphorylated. We are generating mutant parasites that model a defect in TgIF2alpha phosphorylation, having determined that a direct mutation of the serine 71 phosphorylation site is likely lethal. We will use luciferase reporter assays to elucidate how uORFs in the 5′-UTR of BFD2 may modulate translation. Our results will provide crucial insight into how T. gondii differentiates into bradyzoites, allowing it to persist for the remainder of its host's life.

P61 Identifying Cryptococcus spore-enriched proteins and their roles in spore coat restructuring during germination

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To colonize new environments, including a mammalian lung, fungal spores must undergo germination into a vegetatively growing cell type. For Cryptococcus, this necessitates significant morphological changes in which a small, ovoid spore becomes larger and rounder as a yeast emerges laterally from the original spore structure. Functions such as spore coat degradation and new cell wall synthesis are likely crucial for spores to undergo the changes required for germination into yeast, yet little is known about the molecular pathways involved in these processes. To determine how protein identities and levels change during germination, we carried out a comprehensive analysis of the spore proteome during germination (at 6 time points over 10 hours). From this dataset, we identified 19 highly spore-enriched carbohydrate active enzymes (CAZymes) that we hypothesize are key players in spore coat restructuring during germination. Among these proteins are a chitin synthase, an endochitinase, an endo-1,3-beta glucanase, and a polysaccharide deacetylase, suggesting that cell wall modification plays a crucial role in the germination process. We are creating strains with targeted knockouts of the genes encoding these proteins to determine how their deletion impacts spore germination. We will evaluate the rates and efficiency of spore germination at both single-cell and population levels using quantitative germination assays (QGAs), and we will identify differences in spore carbohydrate levels and localization using fluorescence microscopy. These studies promise to reveal fundamental aspects of spore biology and identify mechanisms by which germination occurs, opening the door to more targets for prophylactic antigerminant therapies.

P62 Determining the individual function and effect of structural variation among the TLO gene family members of Candida albicans

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The clinical relevance of Candida species coincides with the expansion of several gene families involved in pathogenesis, such as adhesins and aspartyl-proteases. C. albicans is the most clinically important Candida species and has experienced an even greater expansion of another gene family, the telomere-associated (TLO) genes. TLOs are present as 14 paralogs broken into three architectural groups (α, β, and γ) in the genome reference strain and are functional homologs of the Med2 subunit of the yeast Mediator transcriptional regulatory complex. However, their individual molecular and biological roles in pathogenesis and adaptation remain unknown. To elucidate their individual roles, a complete TLO knockout was constructed in the genome reference strain using CRISPR-Cas9 mutagenesis. This tlo null mutant served as the platform to build a panel of single TLO addback strains. The tlo null strain grew primarily as pseudohyphae in rich medium conditions and could be complemented by addition of any TLOα or TLOβ gene but not most TLOγ genes. Similarly, other simple phenotypes, such as filamentation and cell wall sensitivity, split similarly along architectural group of the TLO re-integrated into the null background. Individual TLOα genes and the TLOβ gene seem to have a differential impact on the ability to switchto the opaque mating state, however the role of TLOγ is unclear and requires further study. Interestingly, reintegration of a truncated TLOγ gene, TLOγ4, into the tlo null background phenocopied complementation with TLOα and not TLOγ genes in some assays. Current efforts seek to further understand the structure-function relationship of the TLOγ group. We are also performing transcriptional analysis and continuing phenotypic analysis, specifically around pathogenesis, of this panel of strains to further determine the function of individual paralogs.

P63 Cryptococcus spore germination can occur in the absence of oxygen

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Because invasive cryptococcosis is caused by fulminant yeast growth, Cryptococcus spores must germinate into yeast to cause disease in mammalian hosts. Our lab used a high-throughput screen of small molecules to identify novel germination inhibitors. We discovered one class of compounds that presumably targets complex II of the electron transport chain (ETC) in a fungal-specific manner. Although yeast (obligate aerobes) cannot grow in the presence of ETC inhibitors or in anaerobic environments, we discovered that spores can germinate in the absence of detectable oxygen. This calls into question the need for ETC function and the mechanism of complex II inhibitors during germination. These paradoxical findings reveal a substantial knowledge gap in how respiration and metabolism operate during the energy-intensive process of germination. To evaluate the role of the ETC in germination, we tested inhibitors of ETC complexes and conducted transcriptomic analyses on spores germinating hypoxically. Our data indicate that ETC function is important for hypoxic germination, including a role for the cyanide-independent alternative oxidase (AOX1). Future experiments will determine the unique and potentially targetable mechanisms of cellular respiration in germinating Cryptococcus spores, including evaluating potential roles of non-oxygen terminal electron acceptor pathways.

P64 High-Throughput Fluorometric Screening Reveals Novel Antileishmanial Scaffolds and Lead Compounds

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Leishmaniasis is a vector-borne neglected tropical disease endemic in over 90 countries, affecting approximately 12 million people worldwide, with nearly 1 million new cases reported annually. The disease is caused by obligate intracellular protozoan parasites of the genus Leishmania, which proliferate within the acidic phagolysosomes of host macrophages. It presents in multiple clinical forms, ranging from disfiguring cutaneous lesions to fatal visceral infections if left untreated. Despite its global impact, there is currently no approved vaccine, and existing treatments are limited by high cost, toxicity, prolonged treatment regimens, and the emergence of drug resistance, highlighting the urgent need for new therapeutic strategies. To address this need, our laboratory developed a high-throughput fluorometric screening platform utilizing mCherry-expressing Leishmania parasites to identify novel antileishmanial compounds from the DIVERset-EXP chemical library. Previous screening efforts identified a 2,4-diaminoquinazoline (2,4-DAQ) scaffold with potent antileishmanial activity. Building upon these findings, the present study characterizes two additional enriched chemical scaffolds, 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) and pyrazolo[5,1-c][1,2,4]triazine (PTZ), as well as several singleton compounds identified within the DIVERset-EXP chemical library. Selected lead compounds were evaluated for in vitro efficacy against multiple Leishmania species and life stages to determine half-maximal inhibitory concentration (IC_{50}) values. Several compounds demonstrated potent antiparasitic activity comparable to reference drugs, including miltefosine (MLT) and amphotericin B (AmpB). Cytotoxicity profiling in differentiated human THP-1 macrophages revealed low toxicity for select compounds, consistent with MLT, supporting a favorable preliminary therapeutic index. Collectively, these findings demonstrate the effectiveness of fluorescence-based high-throughput screening as a robust platform for antileishmanial drug discovery and highlight promising lead compounds for further optimization, target validation, and mechanistic studies.

P65 Salted and Burned: Survival of Candida albicans in synthetic burn wound exudate

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Secondary fungal infection by Candida albicans represents a major complication following thermal injury, as it increases both the morbidity and mortality rates for inflicted individuals. If not quickly resolved, C. albicans can form biofilms within the burn wound, further complicating disease control while increasing the risk for chronic burn wound infection. Despite this, we know very little about how fungi colonize and persist with the burn niche. To survive, C. albicans must metabolize available nutrients from burn wound exudate, a fluid discharge from burned skin, and necrotic tissue in its surrounding environment. To better understand the metabolic requirements for C. albicans survival and proliferation within burn wounds, we created a synthetic wound exudate (SWE) that mimics the chemical and nutritional composition of primary human burn wound exudate. Using a single gene knock out library of C. albicans, we analyzed the growth of 674 mutants to identify genes that are necessary for fungal survival in SWE. Growth analyses have identified 35 genes necessary for growth in synthetic wound exudate. Gene ontology analysis of hits has implicated trace element uptake, metabolic plasticity, pH response, and osmotic and general stress response pathways as important factors for growth in SWE. We have also linked growth defects in SWE with impaired biofilm formation, as loss of SNF4 (metabolism switching) and PRA1 (zinc sequestration) attenuated biofilm formation in the synthetic wound exudate when compared to the wildtype control. Collectively, the development of SWE has provided a novel platform for the identification of fungal processes needed for growth within the burn wound environment and has generated a list of potential genes for future studies aimed at assessing the role they play in burn wound infection.

P66 Extracellular Vesicle-Derived Spliced Leader RNA As a Minimally Invasive Molecular Biomarker For Leishmaniasis Detection

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Leishmaniasis is a vector-borne disease complex caused by protozoan parasites of the genus Leishmania, with clinical manifestations that vary depending on the infecting species. The most severe form, visceral leishmaniasis (VL), is primarily caused by L. infantum and L. donovani and affects internal organs such as the liver and spleen. If left untreated, VL can be fatal. Although cutaneous leishmaniasis can often be diagnosed through skin biopsy, VL diagnosis remains challenging because its symptoms are nonspecific and overlap with those of other diseases. Current diagnostic approaches may require invasive procedures including bone marrow or splenic aspiration which carry risks and may produce inconclusive results. Therefore, sensitive and minimally invasive diagnostic alternatives are needed. Extracellular vesicles (EVs) are membrane-bound particles released by cells that transport proteins, lipids, and nucleic acids between cells. During parasitic infections, EVs may contain parasite-derived molecules and therefore have potential as a source of diagnostic biomarkers. Spliced Leader (SL) RNA is a highly abundant and conserved non-coding RNA sequence found in all Leishmania species and has also been identified in parasite-derived EVs. In this study, we investigated SL RNA as a potential molecular diagnostic target for leishmaniasis by evaluating its detection in EVs derived from parasite culture media and infected samples using quantitative PCR (qPCR) and droplet digital PCR (ddPCR). SL RNA signals were successfully identified across both in vitro and in vivo sample types. Overall, these findings demonstrate the feasibility of detecting SL RNA in diverse biological samples and support its further evaluation as a sensitive and less invasive diagnostic marker for Leishmania infection.

P67 Glucose Limitation and Amino Acid Metabolism Define a pH-Modulating Transcriptional Program Underlying Candida glabrata Phagosomal Persistence

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Candida glabrata can persist within macrophages despite harsh conditions in the phagosome. Previous studies suggest that during host infection, C. glabrata may adapt to nutrient limitation by utilizing alternative carbon sources, including amino acids. Amino acid catabolism requires removal of amino groups, generating ammonia that can raise environmental pH. This alkalinization counteracts the acidification that is part of the maturation process for phagolysosomes; since the acidic pH is crucial for the activity of the proteases to breakdown the fungal cell walls, the alkalinization could help create a permissive intracellular niche for fungal persistence and survival. These observations suggest that nutrient availability directly influences pH modulation and may contribute to macrophage survival. However, the transcriptional program linking amino acid utilization to pH modulation remains unresolved. We hypothesized that glucose limitation activates an amino acid-responsive transcriptional state that supports alkalinization and may contribute to phagosomal persistence.
To define this program, we performed RNA-seq on C. glabrata grown in %1 casamino acid medium from pH 4.0, sampled at baseline, t450 min, and t690 min, alongside glucose-supplemented controls. Principal component analysis separated baseline, casamino acid, and glucose-supplemented samples, indicating that growth on amino acids as alternative carbon sources induces a distinct transcriptional state. Differential expression analysis identified casamino-specific genes by requiring significant changes relative to baseline and matched glucose controls. This classified genes into t450min-specific, sustained, and t690min-specific groups, including 124 early-specific, 58 sustained, and 37 late-specific genes. The response was predominantly inductive, with more upregulated than downregulated genes at both time points.
GO enrichment of sustained upregulated genes highlighted amino acid and nitrogen metabolism, transport, mitochondrial/TCA-associated metabolism, lipid and sterol biosynthesis, and cell wall-associated processes. Candidate genes included regulators and enzymes linked to gluconeogenesis, amino acid transport, nitrogen metabolism, mitochondrial function, and cell wall remodeling.
Together, these data define a nutrient-responsive transcriptional program linking glucose limitation, amino acid metabolism, and pH modulation in C. glabrata. Ongoing functional studies will test whether prioritized candidates contribute to alkalinization and macrophage survival

P68 Determining Sex Differences in Spore-Mediated Cryptococcosis Using a Mouse Model

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Human cryptococcosis exhibits a well-established sex bias, with males experiencing higher disease incidence and poorer clinical outcomes than females. The underlying bases for these differences (i.e., biological, cultural, social, etc.) are poorly understood. Although extensive research has been conducted on the virulence mechanisms of Cryptococcus, the effects of sex and sex hormone differences on infection outcomes remain largely unexplored. To evaluate a potential role for biological sex in cryptococcal disease, we infected both male and female mice (as determined by anogenital distance at weaning) with Cryptococcus and evaluated disease progression and outcomes. Male and female C57/BL6 and BALB/c mice were infected intranasally with either spores or yeast (B3501 and B3502 strains) and assessed for days of survival after infection and organ fungal burdens at end point. In both mouse backgrounds, male mice succumbed to disease faster than female mice (C57/BL6, $p = 0.0359$ and BALB/c, $p < 0.0001$). Fungal burdens in the brain, lung, kidney, and spleen did not differ between sexes at end point. From these data, we hypothesize that dissemination from the lung occurs earlier in males, leading to lethal fungal organ burdens earlier than in females. Future experiments will 1) determine timelines of lung dissemination in males vs. females, 2) identify any differences in lung immune cell interactions with spores between males and females, and 3) determine the roles of sex hormones in controlling time-to-disease. This study highlights a potential need for sex-specific therapeutic considerations when treating cryptococcosis.

P69 Investigating Culex Mosquito Populations in the Chicago's North Shore

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The mosquito species Culex pipiens pipiens is an important vector for disease, particularly West Nile Virus, but species within the Culex pipiens complex often hybridize, complicating identification. This project tests a set of primers to identify and assess the frequency of mosquito species in the Chicago area. Currently, no genome exists for the North American Cx. pipiens pipiens, and future work aims to assemble a reference genome from locally identified pure populations. By clarifying local species composition, this work will provide the foundation for genome assembly and advance understanding of mosquito genetics and future vector control strategies.

P70 Adiponectin Receptor Signaling Enhances Macrophage Antifungal Function During Invasive Aspergillosis

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Invasive aspergillosis (IA) is a life-threatening fungal infection in immunocompromised patients that remains associated with high mortality despite antifungal therapy. Previous studies from our laboratory demonstrated that adiponectin (APN) receptor signaling promotes alveolar macrophage antifungal activity through enhanced fungal killing and LC3-associated phagocytosis (LAP), while deficiency of APN signaling increases susceptibility to Aspergillus fumigatus infection. Pharmacologic stimulation with the adiponectin receptor agonist AdipoRon partially restores antifungal function in APN-deficient macrophages and reduces fungal burden in vivo.
Current studies are investigating whether activation of downstream metabolic pathways can recapitulate APN-dependent antifungal programming. Murine bone marrow-derived macrophages and human THP-1 macrophages are being treated with AdipoRon or the AMPK activator metformin to assess effects on fungal killing and macrophage activation. Ongoing experiments include microscopy-based killing assays and analysis of AMPK signaling by Western blot. These studies aim to define the contribution of AMPK-dependent metabolic programming to macrophage antifungal function and identify potential host-directed therapeutic strategies for invasive aspergillosis.

P71 Trehalose-6-Phosphate in Cryptococcus neoformans contributes to virulence and evasion of innate host defense including neutrophils

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As immunocompromised patient populations rise at an alarming rate, novel antifungals are more needed than ever before.One such potential target has been identified in the fungal pathogen Cryptococcus neoformans (C. neo), called Trehalose-6-Phosphate (TPS1). This pathway plays a distinct role in establishing virulence in murine models while being absent from humans, making it a compelling novel antifungal target. In previous work by our lab, it was observed that the loss of trehalose caused reduced capsule size surrounding yeast cells in the tps1Δ mutant leading to fungal susceptibility to resident and innate host immune defences. This study aims to investigate the exact role of the innate immune response's detection and response of the C. neo fungal pathogen using a murine model. Initial flow cytometry shows an increase in neutrophil recruitment in tps1Δ mutant, and in vitro assays revealed tps1Δ mutant cells vulnerability to killing by neutrophils compared to WT. This indicates that TPS1 must play a significant role specifically against detection and antimicrobial elements of neutrophil-mediated host defenses. To test the murine pulmonary infection model, Ly6G was depleted, an essential marker for neutrophil differentiation. These mice infected with tps1Δ showed higher fungal burdens 2-days post infection compared to mice with neutrophils present. Therefore, it seems that neutrophils appear to be the major innate immune cell that is contributing to avirulence of the tps1Δ mutant.

P72 Mitochondrial Polyamine Synthesis in Plasmodium falciparum

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The malaria parasite, Plasmodium falciparum (Pf), poses an increasing threat as drug-resistant strains emerge, requiring new control methods. Many antimalarial drugs target pathways supplying essential metabolites to the cell. One pathway of particular interest is polyamine biosynthesis, since its products, putrescine, spermidine, and spermine, are required for a variety of important functions such as post-translational modifications and free radical scavenging. Recent work from Plasmodium yoelii has shown that spermidine synthase (SPDS), which catalyzes the conversion of putrescine to spermidine, localizes to the parasite mitochondrion. The Pf mitochondrion is thought to have limited functionality during the asexual blood stage of the parasite life cycle, so this raises the possibility of an additional, previously unrecognized, role. To investigate this, we expressed a second, tagged copy of SPDS in Pf and confirmed that the protein localizes to the mitochondrion. We predict that mitochondrial targeting is mediated by an N-terminal extension not seen in homologs outside the Plasmodium genus. To test the functional importance of this localization, we are expressing a truncated version of PfSPDS lacking the putative signaling peptide. Additionally, using a conditional knockout line, we found that parasites die within one replicative cycle following PfSPDS loss, showing its essentiality for parasite growth and proliferation. This phenotype is not rescued with the addition of exogenous polyamines to the growth media, suggesting that Pf has limited or no polyamine salvage capabilities and relies on de novo synthesis. This finding is inconsistent with prior reports demonstrating that Plasmodium parasites can import polyamines to overcome drug-mediated inhibition of PfSPDS. Future work will determine if polyamine supplementation fails to rescue mitochondrial PfSPDS depletion due to an inability to transport polyamines across the parasite plasma membrane or the mitochondrial inner membrane. We will also determine why PfSPDS is required in this organelle using biochemical and metabolomic approaches.

P73 Evaluating Spatial Repellent Effects on Plasmodium falciparum Transmission Potential Through Gametocyte Quantification

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Malaria has continued to contribute to significant morbidity and mortality globally, with 94% of global cases occurring in the African sub-continent alone. Despite significant control efforts, malaria control has stalled highlighting the need for novel control strategies. In 2025, the World Health Organization (WHO) promoted the use of spatial repellents to control malaria, the first malaria control recommendation by the organization in 25 years. Spatial repellents act on mosquitoes by limiting their sensing abilities with the main goal being to limit vector-host contact. While studies on the effects of spatial repellents have focused on their effects on overall malaria incidence, little research has been completed to understand the effects these products have on parasite transmission through the quantification of gametocytes, the parasite stage transmitted to mosquitoes, within endemic regions. In this study, a digital PCR assay was developed to quantify transcripts for early-stage, female and male gametocytes. A cluster-randomized trial in Busia County, Kenya took place across a three-year study period with 20 clusters. A total of 854/924 from year 1, 1018/1044 from year 2 and 831/1091 from year 3 were analyzed using the dPCR assay. In year 1, 123/854 were positive for all three markers while 168/854 were positive for both the male and female target. 354/854 were positive for the early-stage marker. The male target had the highest positivity rate (405/854, 47.4%) while the female target had the lowest (186/854, 21.8%). Similar trends were seen in years 2 and 3 with the male target being the most prevalent at 327/1018 (32.1%) and 239/831 (28.8%) and the female target being least prevalent at 121/1018 (11.9%) and 98/831 (11.8%), respectively. Ongoing analysis will determine whether spatial repellent use significantly impacts gametocyte densities and, consequently, malaria transmission potential.

P74 A novel Plasmodium ER protein governs effector protein trafficking into the host cell

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The malaria parasite, Plasmodium falciparum, exports hundreds of effector proteins into the erythrocyte to create an intracellular niche for replication. Exported proteins are first secreted into the parasite vacuole (PV) before translocation into the host cell by PTEX, but it is unclear how cargo is identified by PTEX or whether specialized secretory pathway machinery governs exported protein trafficking. Many exported effectors possess a signal sequence that is proteolytically removed during ER entry to generate a mature N-terminus sufficient for translocation across the PV membrane. In contrast, some PEXEL-negative exported proteins (classic PNEPs) lack an N-terminal signal sequence and enter the secretory pathway via an internal hydrophobic region. Curiously, while this region ultimately constitutes a transmembrane domain at the terminal destination in the host cell, classic PNEPs appear to transit the early secretory pathway without being integrated in the ER membrane. Using a comparative proximity labeling approach to search for proteins involved in early export pathway events, we identified a novel, Plasmodium-specific ER protein (PF3D7_0625400). Knockdown of PF3D7_0625400 was lethal and resulted in a general block in protein export but did not impact trafficking of non-exported proteins, confirming a specific role in the export pathway. Remarkably, loss of PF3D7_0625400 resulted in differential trafficking defects with signal sequence-containing exported proteins accumulating in the PV while classic PNEPs were arrested in the ER. These results suggest PF3D7_0625400 is part of a Plasmodium-specific ER-exit pathway or chaperone system dedicated to classic PNEPs, possibly mediating their unique form of trafficking through the ER. Notably, the generalized block in export suggests PF3D7_0625400 is also required for PV trafficking of an unknown translocon component(s). Collectively, our work identifies new ER machinery required for Plasmodium effector trafficking, revealing mechanistic differences in exported cargo handling likely contingent on how proteins enter the secretory pathway.

P75 IL-10 plays a crucial tissue-protective immunoregulatory role during Cryptococcal Meningoencephalitis.

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Cryptococcal meningoencephalitis (CM) is a highly lethal fungal disease caused by the pathogenic yeast Cryptococcus neoformans. Inflammatory immune responses during CM, driven by IFN-γ-producing CD4 T cells and iNos-producing inflammatory monocytes, play a role in fungal clearance but also induce brain damage resulting in severe neurological symptoms. The cytokine IL-10 is known to regulate inflammatory immune responses, protecting host tissue in various contexts, but its role in CM is unknown.

Using a mouse model of inflammatory CM, we investigated the role of IL-10 in immunoregulation and host protection during CM. We observe that IL-10 expression is induced in the brain during CM and that knockout of the IL-10 gene results in dramatically worsened disease outcomes. In IL-10 knockout mice, immune cell numbers were greatly increased along with heightened IFN-γ production in CD4 T cells and accelerated iNos expression in inflammatory monocytes. As a consequence of this exaggerated inflammatory response under IL-10 deficiency, synaptic Syt7 was strikingly reduced and neuronal Cleaved Capase-3 staining was greatly increased in IL-10 knockout mice compared to WT. Consistent with worsened brain damage, IL10 knockout led to significantly poorer neurological health as measured by a murine coma & behavioral scale (MCBS). Lastly, a major difference in survival between IL-10 knockout mice and WT mice indicates that hyperinflammation and resulting tissue damage translates to a substantially accelerated mortality.

Based on this data, we conclude that IL-10 plays a crucial protective role in CM, restraining inflammation-driven brain damage and mortality. Our future steps include determining whether Treg production of IL-10 is required for control of inflammation during CM using a Treg-specific IL-10 knockout mouse strain.

P76 Characterization of ATP Synthase during the Asexual Blood Stage of Plasmodium falciparum

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Plasmodium falciparum (Pf) contains a highly divergent mitochondrion, positioning it as an ideal drug target. Asexual-blood stage (ABS) Pf parasites rely minimally on oxidative phosphorylation, and the primary function of the mitochondrial electron chain (mETC) is thought to be the regeneration of oxidized ubiquinone by Complex III, which is required for de novo pyrimidine synthesis. Consistent with this model, inhibition of Complex III by the antimalarial drug atovaquone can be rescued by a heterologous pyrimidine salvage enzyme that bypasses the need for ubiquinone-dependent pyrimidine synthesis. Although atovaquone blocks the mETC, parasites still have an intact mitochondrial membrane potential (Δψm), suggesting that an alternative mechanism to maintain (Δψm) exists in Pf. One candidate is ATP synthase (Complex V), which can operate in reverse in other organisms, using ATP hydrolysis to maintain a proton gradient. To investigate its role in Pf, we generated conditional knockdown lines targeting the α and β subunits within the catalytic core of ATP synthase, and appended C-terminal HA tags to monitor protein abundance and complex assembly. Depletion of either subunit resulted in parasite death, demonstrating that ATP synthase is essential during ABS. Blue native PAGE and immunoblot analyses confirmed incorporation of both tagged proteins into ATP synthase complexes and verified efficient knockdown. Unexpectedly, parasites expressing HA-tagged α or β subunits displayed hypersensitivity to atovaquone despite active pyrimidine salvage. These findings suggest that the HA tags partially impair ATP synthase function, rendering parasites more dependent on residual mETC activity for maintenance of Δψm. Together, our results provide the first genetic evidence that ATP synthase is essential in Pf ABS and likely contributes to Δψm. Ongoing studies using untagged knockdown lines and a catalytically inactive ATP-β allele will determine the structural and catalytic contributions of ATP synthase to parasite survival.

P77 Defining the Roles of NRG1 and ECE1 in Candida albicans Filamentation and Damage Across Pathogenic and Commensal Forms

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Candida albicans is a fungal pathogen that is a crucial component of normal human microbiota. C. albicans exists in both commensal (non-harmful) and pathogenic (disease-causing) forms mediated by its dynamic transition between the immunogenic hyphal form and the stable yeast form. Two strains of C. albicans: SC5314 (genome reference strain) and 529L (oral infection strain) provide an important framework in this study to understand the genetic factors underlying phenotypic variation between strains of C. albicans. SC5314 is classified as “pathogen” as it readily filaments, releases the toxin candidalysin, and is lethal to mice. 529L is classified as “commensal like” due to the formation of incomplete hyphae causing low host immune response and allowing it to colonize oral, gut, and vaginal niches long-term. Two genes identified due to their essential roles in damage and filamentation in C. albicans are NRG1 and ECE1. NRG1 is a transcription factor which negatively regulates hyphal formation in C. albicans, while ECE1 is an essential part of C. albicans virulence as it encodes candidalysin. CRISPR/Cas9-mediated complete single and double gene knockouts will be performed in both SC5314 and 529L before allele swapped strains are constructed. Filamentation and LDH damage assays will be performed to measure the impact of gene loss and allele contribution in each strain. Understanding the role of diverse alleles in virulence will allow for greater understanding of how C. albicans is able to cause disease in hosts as well as possible medical advancements.

P78 Quantifying Acid Phosphatase Production Across C. glabrata Strains

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Nakaseomyces glabratus, also known as Candida glabrata, is an opportunistic fungal pathogen. Candida species are the most commonly isolated invasive human fungal pathogens, and amongst these species Candida glabrata is the second most commonly isolated one associated with invasive candidiasis. Fungal pathogens must adapt to and overcome a variety of defense mechanisms and environmental stressors whilst invading a host, such as attack or engulfment from host immune cells, increased temperature, varying pH, and reduced nutrient availability. One limited nutrient is phosphorus, which is an important element in a variety of components of the cell, such as ATP production. Our lab showed that C. glabrata exhibit classic phosphate starvation response after being engulfed by macrophage cells, suggesting that they experience phosphate limitation when encountering host immune cells. Genome plasticity is a mechanism used by human-fungal pathogens in order to rapidly adapt to hostile environments. C. glabrata has been shown to be able to switch between haploid and diploid, and this switch promotes both the creation of mutations and a diversity of phenotypes. This results in clinical isolates of C. glabrata differing in several biological aspects. Understanding how and where these differences arise from could provide insight for the development of new clinical treatments. The primary goal of this research is to examine the production of acid phosphatases, which are a secreted enzyme that cleaves inorganic phosphate from its substrate. These allow the cell to be able to grow in environments where there is limited access to inorganic phosphate, and the production acid phosphatases are a downstream effect of C. glabrata undergoing a phosphate starvation response. We will show results quantifying this production as well as the growth in phosphate limiting conditions in order to better understand the variation in C. glabrata strains in regards to survival and nutrient acquisition strategies.

P79 Fatty Acid Synthase Targeted Inhibition of Growth in Candida albicans and Candida auris

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Antifungal resistance in Candida albicans and Candida auris highlights the need for novel therapeutic strategies and new drug candidates. This study investigated the antifungal activity of several candidate compounds targeting metabolic pathways associated with fatty acid synthase, which is crucial for biofilm and membrane formation. Initial measurements identified 4,5, -dichloro-3H -1,2-dithiol-3-one (DCT) as the strongest inhibitor among the tested compounds with concentration-dependent suppression of fungal growth for Candida albicans and Candida auris. An analog of DCT was created, 7-15, and inhibited growth of C. albicans at 50 µM. Strains of C. auris and C. albicans reacted differently to the tested compounds, demonstrating that different metabolic pathways may be targeted. Serial dilution assays were performed to estimate inhibitory ranges and compare activity against amphotericin B, with a minimum inhibitory concentration (MIC) between 0.78 and 0.39 µM. DCT demonstrated strong antifungal activity with a MIC between 41.67 and 50 µM, supporting its potential as a lead antifungal candidate. Certain phenylthiazoles also exhibit promising fungicidal activity in both C. albicans and C. auris, with antibiofilm properties. To determine the toxicity of DCT and 7-15, the two compounds were tested against the human cell line HCT-8 where the MIC values were 202.4 and 124 µM respectively. Future studies will further investigate mechanistic interactions of DCT/7-15 with fatty acid synthase and different metabolic pathways.

P80 The thioredoxin-related protein 1 (Trxl-1) of the malaria parasite Plasmodium.

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Thioredoxins (Trx) play central roles in the redox networks of organisms across all domains of life, including the protozoan parasite Plasmodium. Cellular redox signaling via these Trx systems is often mediated by downstream transducer proteins that act as effectors or links to diverse cellular processes. Here, we characterize a novel thioredoxin-like protein (TrxL-1) from the rodent malaria parasite Plasmodium berghei (PbTrxL-1). The pbtrxl-1 gene encodes a 208-amino-acid protein with a central thioredoxin domain that lacks a classic CXXC active-site motif. Conservation of the trxL-1 gene appears to be restricted to the phylum Apicomplexa. Quantitative real-time PCR analysis revealed that pbtrxl-1 is strongly and stage-specifically upregulated during P. berghei ookinete development. Deletion of the trxl-1 gene using CRISPR/Cas9 showed that trxl-1 is dispensable for the asexual development of Plasmodium in the host's red blood cells. However, ookinete maturation is significantly delayed and, in some cases, leads to severe malformation of the parasite. Using anti-TrxL-1 antibodies, we investigate a potential role for TrxL-1 in regulating parasite microtubules.

P81 Determining IFNγ-induced restriction factors against Toxoplasma gondii infection in rat macrophages

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Toxoplasma gondii (T. gondii) is a globally distributed intracellular parasite that infects virtually all nucleated cells of warm-blooded animals. In immunocompromised individuals, T. gondii infection causes diseases like encephalitis, pneumonitis, and myocarditis, and outcomes are determined by both the parasite genotype and the host's genetic background. Studies have shown that mice succumb to T. gondii infection in a dose- and type-dependent manner during the acute stage. In contrast, rats, like humans, are resistant to acute T. gondii infection and typically develop chronic infections marked by cyst formation in the brain tissues. IFNγ-mediated immune responses have been known to control T. gondii infection in various host species. In murine models, IFNγ induces the expression of immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs), which mediate disruption of the parasitophorous vacuole, T. gondii's intracellular replicative niche. Given the differing infection outcomes between mice and rats, it is possible that rat macrophages mount a different IFNγ-mediated response to control T. gondii infection.
Here, to identify IFNγ-induced restriction mechanisms in rat macrophages against T. gondii
infection, we infected rat and murine BMDMs following IFNγ stimulation with type I RH, type II ME49, and type III CEP parasites following IFNγ stimulation. We observed a strain-dependent inhibition of T. gondii growth in murine infected BMDMs but not in rat BMDMs. This suggests that rat macrophages may deploy potentially distinct IFNγ-induced effector pathways that are effective against diverse T. gondii strains. We have further identified 4 significantly upregulated IFNγ-induced rat genes – Irgm, Igtp, Gbp2 and Gbp7, that might be contributing to rat macrophage's broad growth inhibition of T. gondii strains. Gene Ontology (GO) and Gene Set enrichment analysis (GSEA) of upregulated genes revealed these genes are enriched in biological processes such as response to type II interferon, activation of immune response and cell killing, indicating the transcriptional reprogramming of rat macrophages towards an activated antimicrobial state. To functionally validate and determine the roles these genes play in rat BMDMs, we will employ Cas9-RNP electroporation to knock out these genes and perform downstream assays to assess the effects of the gene's disruption on parasite growth.

P82 Characterizing Human Pathogenic Atypical Toxoplasma gondii Strains in the Laboratory Rat Model

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Toxoplasma gondii is an obligate intracellular parasite that can cause severe disease in immunocompromised individuals and developing fetuses. While infection with clonal lineages (e.g., Type I, II, III) commonly found in North America and Europe is typically asymptomatic in immunocompetent individuals, genetically distinct atypical strains from South America, particularly the Amazonian region, are associated with severe toxoplasmosis outbreaks in otherwise healthy people. Current pathogenesis studies primarily rely on laboratory mouse models that are highly susceptible to acute infection by both clonal lineages and human-pathogenic Amazonian strains, limiting our understanding of the strain-specific pathogenicity observed in humans. In this study, we are investigating inbred rats as a laboratory host model to characterize the hypervirulent phenotype of Amazonian Toxoplasma strains, as rats are naturally resistant to clonal lineages.

We found that rats infected with several Amazonian strains (e.g., VAND and TgH18008) exhibited severe acute disease characterized by rapid weight loss and acute mortality, indicating that Amazonian strain infection in rats better recapitulates the virulence patterns seen in humans. Surprisingly, we found that deletion of rhoptry proteins ROP5 and ROP18 attenuated the virulence of the Amazonian VAND strain in rats. Because ROP5 and ROP18 are well known to counteract IFNγ-mediated parasite restriction by immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs) in mice, their importance in rat virulence may suggest that atypical ROP5 and ROP18 alleles, but not clonal alleles, can also counteract rat IRGs and GBPs, despite rats possessing a potentially different set of immune effectors. To identify these rat immune effectors, we performed transcriptomic profiling of IFNγ-stimulated rat macrophages and identified several IRG and GBP orthologs that are upregulated following IFNγ activation. Current work is being done to validate whether these IRG and GBP-coding genes mediate the restriction of Toxoplasma clonal lineages in rat macrophages and whether Amazonian strain hypervirulence stems from specific ROP5/18 alleles that antagonize these interferon-stimulated genes.

P83 In Situ Cryo-ET Reveals that the Ciliopathy Protein RP2 Coordinates IFT Organization and Central Pair Biogenesis in Trypanosoma brucei

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Retinitis pigmentosa 2 (RP2) is a conserved transition-fiber protein at the ciliary base that is essential for cilia and flagellum assembly and is implicated in human ciliopathies. Despite its clinical importance, the mechanistic role of RP2 in organizing ciliary assembly remains incompletely understood. Trypanosoma brucei, a unicellular flagellated parasite and a powerful model for studying cilia and flagella, offers key advantages for addressing this question as it assembles a single flagellum with conserved intraflagellar transport (IFT) machinery in a highly ordered and accessible cellular context. In T. brucei, RP2 is hypothesized to be a transition-fiber protein at the flagellum base. RP2 depletion causes severe flagellar assembly defects, including disruption of intraflagellar transport (IFT) at the flagellar base and abnormal central pair orientation, but how these defects arise structurally remains unclear.

Here, we used in situ cryo-electron tomography to investigate the ultrastructural consequences of RP2 depletion in enucleated T. brucei cells (“zoids”), an established cryo-ET-compatible system for visualizing the trypanosome flagellum in a native cellular context. This approach allowed us to examine the basal body, transition zone, and proximal axoneme, as well as IFT organization and docking behavior at the flagellar base in situ. In RP2-depleted cells, we observed an elongated, widened IFT structure on average, although the overall docking pattern and multi-landing events appeared preserved. Strikingly, we identified recurrent defects in central pair biogenesis, including abnormalities in microtubule formation and evidence of misalignment of the central pair relative to the axoneme, which arose from the earliest stages of central pair initiation rather than solely as a secondary consequence of defective flagellar beating. We also observed abnormalities in the paraflagellar rod (PFR), an extra-axonemal structure, including a more proximal initiation site and altered orientation relative to the cell body.

Together, these findings provide in situ ultrastructural evidence that RP2 has a broader role than previously appreciated in coordinating flagellar assembly. Our results suggest that RP2 not only contributes to IFT organization at the flagellar base, but also to the proper initiation and spatial organization of axonemal and extra-axonemal structures. This work establishes T. brucei as a powerful in situ model for dissecting the conserved cellular functions of ciliopathy proteins and suggests that defects in early biogenesis, rather than motility alone, may underlie central pair disorganization.