Candida and Candidiasis

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#Candida2025

INVITED AND
OFFERED TALKS

MICROBIOLOGY SOCIETY

Invited talk: The commensal and pathogenic life styles of *Candida albicans*

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Abstract

Fungal infections are often underestimated as a significant cause of mortality. Most pathogenic fungi originate from the environment, but one of the most common and important fungal pathogens, *Candida albicans*, lives as a commensal within the vast majority of humans. The adaptation of this fungus to the human host is the result of an ancient, mostly commensal relationship which has led to the development of distinct fungal strategies to survive and proliferate in diverse host niches.

In its pathogenic phase, *C. albicans* relies on attachment to, invasion into, and damage of epithelial cells. The yeast-to-hypha transition is essential for these pathogenic events. Hyphae production is strongly linked to the expression of hypha-associated genes, a transcriptional pattern which represents a virulence program and anticipatory gene expression mechanisms. Filaments of *C. albicans* are more adhesive and more invasive than yeast cells. Invasion is accompanied by epithelial damage, however, it does not cause damage *per se*. In fact, most of the damage is due to the hypha-associated peptide toxin candidalysin, which is conserved among clinical isolates of *C. albicans*.

However, considering that *C. albicans* is predominantly a commensal within a normal microbiota we asked which selective pressures are responsible for the conservation of such a critical toxin. Only recently, we discovered that hypha formation and the expression of candidalysin is also critical for commensal growth. This led us to propose that the adaptation to a commensal lifestyle of *C. albicans* has primed the fungus to be such a successful pathogen.

Offered talk

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Establishing a Phenomic Atlas of *Candida albicans*

Austin Mottola[1], Nir Cohen[1], Shira Milo[2], Soyfa Rabinovich[2], Alexis García Avilés[1], Judith Berman[2], Markus Ralser[1]

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Abstract

The opportunistic pathogen *Candida albicans* is the most prominent cause of fungal bloodstream infections globally. Mortality rates for systemic candidiasis are extremely high, with conservative estimates of at least 20%. This is despite the observation that the vast majority of disease-causing isolates are susceptible to antifungal drugs *in vitro*. To better understand the factors that contribute to the success of *C. albicans* as a pathogen, we have compiled a collection of 1750 wild strains of *C. albicans* initially isolated from human, animal, and environmental sources worldwide. High throughput phenotyping across the collection has defined the scope of nutrient utilization, stress susceptibility, drug response, and filamentation capability at a species-wide scale. Resistance to antifungal azoles is rare, in line with previous observations; where resistance is observed, it is often associated with previously described mutations or aneuploidies. In contrast, tolerance to azole antifungals is frequent and varies widely across strains, with greater than 50% of strains exhibiting subpopulations that grow well in overall inhibitory drug concentrations. The capability and strength of filamentous growth also vary across strains; a surprisingly large proportion of strains – 17% – exhibited limited or no filamentation capability even in conditions considered to be potent inducers of filamentous growth in reference strains, suggesting that filamentation may be a less essential virulence factor than has been thought. Taken individually, these data are a useful resource; in conjugation with genome-wide association studies currently in-progress, they will expand our understanding of numerous biological processes.

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Unraveling the Pathogenicity of the Candida parapsilosis Species Complex through Comparative Genomics Analysis

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Abstract

Invasive fungal infections pose a life-threatening risk to millions of individuals each year. The *Candida parapsilosis* species complex (CPSC) is a major cause of human fungal disease and comprises three major species: *C. parapsilosis* (Cpara), *C. orthopsilosis* (Cortho), and *C. metapsilosis* (Cmeta). Despite their close phylogenetic relationship, Cpara alone accounts for over 90% of infectious cases within this complex. To elucidate the genomic basis underlying their distinct patterns of clinical prevalence, we performed comparative genomics assays on 1,051 genomes of CPSC clinical isolates collected from North America, Europe, and Asia. A pangenome analysis shows that Cpara possesses the largest core genome, while Cmeta exhibits the largest accessory genome. This is consistent with previous observations about high levels of homozygosity and low levels of variations across Cpara's genomes. We next explored the distribution of transposable elements (TEs) and found that most predicted TEs were fragmented, with only a few exceeding 1,000 bp in length. This suggests that TEs may not be the major contributors to the genomic differences within this complex. Interestingly, population structure and neighbor-net tree analyses suggested potential recombination events within Cpara species, which has been considered as largely clonal. However, these recombinations did not lead to substantial genomic variation among Cpara isolates. Together, our findings suggest that genomic streamlining may facilitate Cpara's successful adaptation during infection, potentially explaining its higher prevalence in clinic compared to its two sister species.

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The *Candida albicans* pangenome reveals novel structural variants that impact gene expression in diverse clinical isolates.

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Abstract

The genome of *Candida albicans* is variable in content and organization across phylogenetically diverse isolates. While some of this variation is important for *C. albicans* to adapt to the host environment, such as aneuploidy, loss of heterozygosity, and single nucleotide polymorphisms, studies assessing the role of structural variants (SVs) have been limited by the resolution of short-read sequencing. Transposable elements (TEs) are important SVs across biological kingdoms, having roles in speciation and tumorigenesis, yet the role of TEs in driving phenotypic diversity of *C. albicans* has remained elusive. We constructed a draft pangenome from 21 *de novo* long-read genome assemblies representing distinct and phenotypically diverse clinical isolates to comprehensively identify all SVs, including those that are missed with short-read sequencing. By looking across the pangenome, we identified novel transposable elements, including both ancient and recent mobile elements with varying copy number and insertion sites that do not directly correlate with phylogeny. TE insertions are found ubiquitously across the pangenome, including within genes and gene regulatory regions. To determine the impact of TE insertions on transcriptome differences between strains, we correlated insertion sites to publicly available RNA-Seq datasets. TEs impact both strain-specific expression of genes and we find that heterozygous insertions are involved in regulating differential expression of alleles within individual strains. We explore the impact of specific TEs on phenotypic differences between strains and discuss their role in adaptation to the host environment. This work assesses the functional impact of TEs in *C. albicans*, providing evidence for their evolutionary importance.

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Xenosiderophore transporter gene expression and clade-specific filamentation in *Candida auris* killifish infection

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Abstract

Candida auris is a World Health Organization (WHO) critical priority fungal pathogen with a ~45% associated crude mortality. A critical bottleneck in understanding virulence is the lack of gene expression profiling models during *in vivo* host tissue infection. We developed a fish embryo yolk-sac (*Aphanius dispar*; Arabian killifish; AK) microinjection model to decipher host-pathogen RNA-seq at 24 and 48 h post injection (HPI) at 37 °C across five major clades (I-V) of *C. auris*. Host responses featured heat shock, complement activation, and nutritional immunity, including haem oxygenase (*HMOX*) expression in response to clade IV infection. We identified an *in vivo* transcriptional signature across five clades of *C. auris* enriched for xenosiderophore transmembrane transporter candidates (*XTC*), a newly described seventeen-member expanded gene family, also including haem-transport related (*HTR*) proteins. The basal clade V isolate formed filaments during infection, coinciding with typical and atypical regulators of morphogenesis, including *UME6*, *HGC1*, and novel adhesin *SCF1*. Clades I and IV demonstrated increased virulence, coinciding with up-regulation of the mating-type locus (*MTL*) non-mating gene *PIKA* in both. Our study provides critical insights into the pathogenesis of *C. auris*, including species-wide up-regulation of *XTC* and *HTR* genes during clinically relevant *in vivo* host tissue infection.

Offered talk (12 minutes)

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CanID-PCR: A quick and low-cost PCR tool to identify *Candida* species on gDNA directly extracted from positive blood bottles

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Abstract

Candida bloodstream infection carries a high mortality. *Candida* species prone to antifungal resistance (e.g. *C. auris* and *C. glabrata*) are rising, and delay in species identification might adversely affect patients' outcomes. Current workflow by clinical microbiology laboratory requires ~24 hours for Candida speciation, time to allow for growth of isolates on the agar plates for testing. We established a simple and inexpensive PCR tool, named CanID-PCR, to speciate *Candida* directly from positive blood bottles. We selected *Candida* *ACT1* gene, which has an intron with varying length that enables the identification of 9 common *Candida* species. The tool was optimized for gDNA extracted directly from positive blood culture bottles. We showed, by testing positive blood cultures from 64 unique patients, that our tool detects the same species as the microbiology lab in 97% (62/64) of samples. The 2 samples with mismatched results were due to high similarity between *Candida metapsilosis* and *C. parapsilosis*, and failure of CanID-PCR to identify *C. tropicalis* in a patient with *C. albicans/C. tropicalis* fungemia. On the other hand, CanID-PCR identified a second species (*C. fabianii*) in a patient with *C. parapsilosis* fungemia that was missed by the microbiology laboratory. In conclusion, our inexpensive tool was accurate for rapid speciation of *Candida* directly from blood culture bottles, which could be valuable for clinical and/or research laboratories.

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A pilot study of digital retinal photography (DRP) and tele-ophthalmology of patients with candidemia

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Abstract

Background. There are contradictory recommendations on need for bedside retinal exams (BREs) of patients with candidemia.

Methods. Patients with candidemia underwent DRP by an ID physician. Images were interpreted remotely by an ophthalmologist. BREs were performed by ophthalmology residents.

Results. 41 patients with candidemia survived for examination (*C. glabrata*, 32%; *C. albicans*, 29%; *C. parapsilosis*, 12%; *C. dubliniensis*, 10%; *C. krusei*, 2.5%; *C. lusitaniae*, 2.5%; *C. glabrata*+*C. albicans*, 2.5%; others, 7.5%). Mean times from +BCs to DRP and BRE were 5 (0-45) and 3 (0-16) days, respectively. DRP image quality (per remote ophthalmologist) was high (54%), adequate (31%) or uninterpretable (14%). 24% and 4% of the first 17 and last 24 DRP images were uninterpretable (p=0.14). Images were interpreted as consistent with ocular candidiasis (OC) in 10% of patients (chorioretinitis, 7%; endophthalmitis, 3%), other eye diseases in 7%, and no disease in 80%. In 3%, the image was interpreted as abnormal but formal read was deferred to follow-up BRE. 32 patients had both DRP and BRE. All OC and other eye diseases identified by DRP were confirmed by BRE. In 3% of patients, DRP identified OC originally not identified by BRE. In another 3%, DRP did not detect OC originally identified by BRE; follow-up BRE did not identify OC, suggesting the original BRE was false+ or OC resolved. Overall, DRP findings impacted care in 22% of patients.

Conclusions. DRP with tele-ophthalmology is viable for screening patients with candidemia and a powerful tool for defining incidence and impact of OC.

Offered talk (14 minutes)

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A Global Resource for Candida albicans: Linking Genetic Diversity to Drug Resistance and Tolerance

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Abstract

The genetic diversity of Candida albicans is fundamental to understanding its physiology, virulence, and response to antifungal treatments. However, the field currently still lacks a joint, comprehensive global resource that broadly captures this diversity. To address this gap, the Berman and Ralser laboratories have collaborated in an ERC Synergy project term 'Fungal Tolerance' to build an extensive collection of over 1800+ C. albicans strains sourced globally. The strains come from different decades, environments, infections niches, are associated to different outcomes or commensalism, and for many we have extensive metadata. We systematically isogenized these strains, sequenced their genomes, conducted extensive phenotypic characterization, quantified antifungal drug resistance and tolerance, and profiled their proteomes using mass spectrometry. Utilizing this unprecedented dataset, we are dissecting the mechanistic distinctions between drug resistance and drug tolerance in C. albicans. Importantly, we will soon release this comprehensive resource publicly available, empowering the scientific community to significantly advance research into this clinically critical fungal pathogen.

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The *Candida* Corkscrew: A Morphological Strategy of Epithelial Invasion

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Abstract

The *Candida albicans* cell displays a high degree of morphological plasticity, with the ability to switch between at least 7 different forms, depending on its environment. Of these, the hyphal form is able to penetrate host mucosal surfaces and therefore plays an important role in the dissemination and development of disease. Under certain conditions, including physical confinement and nutrient restriction, hyphae grow helically- a little-studied morphology which may afford the cell both increased virulence and drug resistance. This study investigates the molecular basis behind helical growth via both a large-scale screen and targeted analysis, through which we have uncovered the cooperation of diverse cellular processes, including cell wall and plasma membrane dynamics, whole-cell polarisation and calcium homeostasis. Alongside these well-known processes, we found that the previously uncharacterised predicted rhomboid protease, Rbd1, has an integral role in the generation of helical hyphae. Furthermore, we have used a colon-on-chip model, which replicates the peristaltic stretch found in the human gut, to trigger 3D epithelial architecture, allowing the imaging and study of *C. albicans* hyphal invasion *in situ*. We have observed helical hyphal growth through gut epithelial layers, indicating for the first time that this morphology likely plays a role in tissue invasion. This work details a collaboration between diverse processes across the cell to produce a helical hypha morphology, which together aid human gut epithelial invasion.

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Structural Basis of the Fungal-Specific Potassium Channel TOK

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Abstract

In *Candida albicans*, potassium (K^+) channels likely fine-tune ionic balance under stressful environmental conditions, contributing to pathogenic growth within the human host. TOK potassium channels, uniquely found in fungi, remain insufficiently characterized despite early evidence implicating them in diverse intracellular processes essential for cellular growth and viability. Here, we describe the first atomic resolution structure of a TOK channel from *C. albicans* (CaTOK), revealing a previously unobserved architecture defined by eight transmembrane helices and a membrane topology distinct from all known K^+ channels. The first four transmembrane helices form a tetraspanin-like bundle with unexpected structural homology to mammalian auxiliary subunits, including transmembrane AMPA receptor regulatory protein (TARP) and calcium channel gamma subunits, which are known to modulate channel trafficking and gating. The selectivity filter exhibits atypical ion coordination under high K^+ conditions, consistent with CaTOK's permeability to both K^+ and sodium. Its C-terminal cytosolic region forms a structured domain that directly engages with the gating helices, establishing an intramolecular network likely essential for maintaining overall channel architecture and gating. These findings provide a structural framework for understanding TOK channel activity and lay the groundwork for future studies on fungal ion homeostasis, pathogenicity, and antifungal drug development.

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β-1,6-glucan, an unexplored and critical cell wall polymer of Candida albicans

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Abstract

The cell wall of human fungal pathogens plays critical roles as an architectural scaffold and as a target and modulator of the host immune response. Although the cell wall of the pathogenic yeast *Candida albicans* is intensively studied, one of the major fibrillar components in its cell wall, β-1,6-glucan, has been largely neglected, its biosynthetic pathway remains totally unknow. Our data show that β-1,6-glucan is essential for bilayered cell wall organization, cell wall integrity and filamentous growth. The set-up of a cell wall analytic approach allowed a comparative investigation of cell wall composition and β-1,6-glucan structure between growth conditions and cell wall mutants. For the first time, we show that β-1,6-glucan production compensates the defect in mannan elongation in the outer layer of the cell wall. In addition, β-1,6-glucan dynamics are also coordinated by host environmental stimuli and stresses with wall remodeling, where the regulation of β-1,6-glucan structure and chain length is a crucial process. As we point out that β-1,6-glucan is exposed at the yeast surface and modulates immune response, β-1,6-glucan must be considered a key factor in host-pathogen interactions.

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Elucidating the role of Gpd2 in *Candida albicans* pathogenicity

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Abstract

Candida albicans is a commensal fungus that naturally inhabits the urogenital tract. However, during periods of dysbiosis and immune suppression *C. albicans* can proliferate, outcompete our innate immune system and cause infection. Glycerol metabolism in *C. albicans* has been implicated in adhesion and biofilm formation, yet the precise role of glycerol production in infection remains unclear. In this study, we investigated the role of Gpd2, a key enzyme in glycerol production, in *C. albicans* innate immune recognition using a strain which overexpresses Gpd2. Phagocytosis assays revealed that overexpression of Gpd2 enhances immune evasion compared to the control strain. This enhanced innate immune evasion was independent of beta-glucan masking, chitin exposure and mannan biosynthesis. Gpd2 is a cytoplasmic protein but has been shown to interact with Factor H to inactivate the complement system, suggesting that Gpd2 can be localised in the cell wall. However, whether the enzymatic activity of Gpd2 and its role in glycerol biosynthesis is important for immune evasion is unknown. Work is ongoing to identify how Gpd2 is trafficked to the cell wall, whether the enzymatic activity of Gpd2 is required for immune evasion, and to investigate the impact of Gpd2 overexpression of the cell wall proteome. Together, these studies aim to elucidate the role of Gpd2 in *C. albicans* immune recognition and provide new insights into antifungal strategies.

Offered talk (15 minutes)

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TBK1 Phagosomal Recruitment Enhances Antifungal Immunity via Positive Feedback Regulation with SRC

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Abstract

TANK-binding kinase 1 (TBK1) is a versatile serine/threonine protein kinase, which is mainly recognized for its canonical role in antiviral immunity through interferon induction. Here, we report a previously uncharacterized function for TBK1 in antifungal defense. Using proteomic analysis, we identified TBK1 as a phagosome-directed protein after fungal infection, and biochemical analysis revealed that TBK1 is directly recruited to phagosome by SHP2 in a process driven by SRC-mediated phosphorylation. This recruitment facilitates TBK1 aggregation and trans-autophosphorylation at the phagosome. Activated TBK1 then phosphorylates SRC at serine 17, a prerequisite for full activation of SRC, thereby establishing a robust positive feedback loop among SRC, SHP2, and TBK1. Consistently, SRC-mediated anti-fungal signaling and production of proinflammatory cytokines and chemokines were significantly impaired in mouse bone-marrow derived macrophages (BMDMs) lacking TBK1. Myeloid *Tbk1*-deficient mice exhibited greater susceptibility to systemic *Candida albicans* infection. Overall, our findings reveal a critical role for TBK1 in antifungal immunity and highlight its potential as a therapeutic target for combating fungal pathogens.

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Increased spreading of the predominate azole-resistant *Candida tropicalis* genotype in the environment

Yin-Zhi Chen Chen[1], Kuo-Yun Tseng[1], Min-Nan Tseng[2], Jyh-Nong Tsai[3], Ching-Ching Hsu[4], Chih-Chao Lin[1], De-Jiun Tsai[1], Feng-Jui Chen[1], Yu-Chieh Liao[1], Li-Yun Hsieh[1], Chiao-Mei Lin[1], Chi-Jung Wu[1], Huey-Kang Sytwu[1], Hsiu-Jung Lo[1]

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Abstract

A predominant fluconazole-resistant *Candida tropicalis* clade 4 genotype causing candidemia in humans in several tropical countries was detected in the environment in a 2012 orchard survey in Taiwan. This follow-up study was to determine the distribution of the azole-resistant *C. tropicalis* and to monitor the trend of azole-resistant *C. tropicalis*, especially the clade 4 genotype, from 2012 to 2018 in orchard environments. Furthermore, the effects of azole fungicides use in orchards on the selection of azole-resistant *C. tropicalis* was investigated. This study compared *C. tropicalis* isolated from the same 53 orchards — including 23 wax apple, 17 grape, and 13 papaya orchards — in both the 2012 and 2018 surveys. The fluconazole-resistant rate of *C. tropicalis* was significantly higher in 2018 than in 2012 (27/55 vs. 9/46, $p = 0.003$). Furthermore, the use of azole fungicides significantly increased the rate of azole-resistant *C. tropicalis* detection. Like *C. tropicalis* causing infections in patients, azole-resistant isolates from the environment were genetically related. Approximately 77.8% (7/9) and 92.6% (25/27) of the azole-resistant isolates in 2012 and 2018, respectively, belonged to the clade 4 genotype. Our findings demonstrate that the rate of fluconazole-resistant *C. tropicalis* from orchards increased significantly and the clade 4 drug-resistant *C. tropicalis* spread widely in orchard environments, especially among grape ones. Hence, to identify which orchard environment has a priority to conduct intervention of the farmers' cultivation habits, especially on azole fungicide use is important.

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Elucidating the function of essential protein kinases of the pathogenic yeast *Candida albicans* by inducible gene deletion

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Abstract

Protein kinases are key components of many signaling pathways that regulate cellular activities and the responses of cells to external signals. The pathogenic yeast *Candida albicans* possesses 108 genes encoding known or predicted protein kinase catalytic subunits. We aim to establish which protein kinases are essential for the viability of *C. albicans* and to elucidate the cellular functions of uncharacterized kinases. We generated a comprehensive library of protein kinase deletion mutants of the wild-type *C. albicans* reference strain SC5314. For several possibly essential protein kinases, for which no homozygous mutants were obtained, we generated conditional mutants, in which a single copy of the corresponding gene could be excised by inducible, FLP-mediated site-specific recombination with high efficiency, resulting in an almost pure population of null mutants. Seven of 10 tested putative essential protein kinases turned out to be dispensable for viability, but in most cases the null mutants grew poorly under standard growth conditions, explaining previous failures to obtain homozygous mutants by traditional gene deletion methods. In contrast, three of the tested protein kinases were indeed essential for viability, as the mutants obtained after forced, FLP-mediated gene deletion were unable to form colonies even under optimal growth conditions. We focused on two protein kinases without a known function. When transferred to fresh medium after the induced gene deletion, the mutant cells displayed aberrant morphologies and collapsed after a few hours. We are currently using different approaches to elucidate the cellular functions of these protein kinases.

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Adaptation of *Candida albicans* to niche-simulating conditions influences immune recognition and responses

Arnab Pradhan[1], Ian Leaves[1], Mark Stappers[1], Sumita Roy[2], Alba Lozano[3], Benedetta Cerasuolo[4], Alistair J.P. Brown[1], Neil A.R. Gow[1]

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Abstract

Candida albicans has evolved immune evasion strategies that include cell wall remodelling via masking or shaving of pathogen-associated molecular pattern (PAMP) β-1,3-glucan. Previously we showed that *C. albicans* exploits a range of host-derived signals to induce the production of glucanases to clip off superficial β-1,3-glucan strands at the cell surface in an attempt to evade phagocytic uptake [*Nature Micro* 2, 16238; *mBio* 9, e01318-18; *Nature Comms* 10, 5315]. Here, we show that adaptation to niche-simulating conditions modulates the exposure of mannan-related PAMPs at the *C. albicans* cell surface.

Mannans are complex, comprising linear and branched polymers of mannose. Mannans are recognised by a range of C-lectin type receptors, including CRD4-7 (mannose receptor), DC-SIGN and dectin-2. However, the chemical nature of their mannan ligands and their location in the fungal cell wall are not accurately defined. In this study, we examined the impact of growing *C. albicans* in plasma-, vagina-, saliva- and gut-simulating media upon PAMP exposure and immune recognition. Standard laboratory media (SD and YPD) were used as controls. Using a combination of flow cytometry, fluorescent microscopy, TEM, cytokine assays, and neutrophil killing assays, we have shown that *C. albicans* cells exhibit significant differences in the exposure of mannan ligands on the cell surface depending on the growth conditions. These changes in PAMP exposure correlated with differential cytokine responses, as well as altered sensitivities to stresses and antifungals. Our data reinforce the view that adaptation to niche-simulating conditions makes *C. albicans* a moving target for the immune system.

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Targeting differences in fungal and human acetyl CoA synthesis to develop mechanistically novel antifungal molecules

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Abstract

One of the most direct approaches to improving antifungal therapy is to identify and develop drugs with new mechanisms of action. This approach is also the most likely to overcome the increasing resistance of medically important fungi to currently used drugs. In the nearly 100 years of the antibiotic era, only three mechanistically distinct antifungal drug classes have been developed and approved as primary therapies for life-threatening fungal infections: polyene ergosterol disrupters, azole ergosterol biosynthesis inhibitors, and 1,3-ß-glucan synthase inhibitors. Two novel classes, GPI-linked protein biosynthesis inhibitors and DHOH inhibitors, are currently in Phase II-III development. Here, we describe our efforts to take advantage of the distinct biochemistry used by human and fungal cells to synthesize the fundamental biological molecule, acetyl CoA. Under normal physiological conditions mammalian cells primarily use the enzyme ATP-Citrate Lyase to synthesize nuclear and cytosolic acetyl CoA from citric acid generated in mitochondria by the TCA cycle. In contrast, fungi as *Candida* spp. lack ATP-Citrate Lyase and, instead, are reliant on acetyl CoA synthetase to generate acetyl CoA in these compartments. We have identified two classes of small molecules that selectively inhibit fungal acetyl CoA synthetase; show antifungal activity in vitro; and have low toxicity against mammalian cells. The screening approaches used to identify these molecules; the characterization of their biochemical and microbiological activity, and the determination of their pharmacological properties will be presented.

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Oxidative stress potentiates cell wall stress in *Candida albicans*

Wanjun Qi, Udita Roy, Chunhui Cai, Liang Sun, Julia Köhler

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Abstract

Background. In immunocompetent hosts, *C. albicans* is a common commensal but is rarely able to cause invasive disease. Host phagocytes kill *C. albicans* cells with multiple stressors, oxidative stress prominent among them, while these cells deploy antioxidant defense systems. Cell wall stress can be applied pharmacologically to prevent or treat Candida infections by administration of echinocandins.

Methods. We generated conditional mutants in 2 crucial *C. albicans* antioxidant defense components, the thioredoxin and the glutathione systems. Genes encoding the single *C. albicans* thioredoxin reductase, Trr1, and the rate-limiting glutathione synthetic pathway enzyme, Gcs1, were placed under control of repressible *tetO*. Deletion mutants in *GCS1* and double mutants in *TRR1* and *GCS1* were generated. Stress signaling systems and -phenotypes were assayed. Metabolomic changes and activities of key enzymes were measured in the mutants.

Results. Hog1 signaling was inappropriately decreased in *trr1* cells. Additionally, cell wall stress hypersensitivity in *trr1* mutants appeared related to signaling systems increasing substrate flow through the pentose phosphate pathway (PPP) to generate NADPH. When *TRR1* is depleted, increased NADPH is not utilized and glucose-6-phosphate might be wasted in the PPP. It then may be lacking as a substrate for phosphoglucomutase for production of the cell wall biosynthetic precursor UDP-glucose. Lacking their substrate, beta-1,3- and beta-1,6-glucan synthases' decreased output may leave *C. albicans* hypersensitive to cell wall stress.

Conclusion. During *TRR1* depletion, *C. albicans* cells may be maladaptively regulated by their oxidative stress signaling systems, leading to cell wall stress hypersensitivity and to growth arrest.

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The evolution of antifungal resistance evolvability in Candida auris

Darian Santana, Tracy Peters, Peijun Ma, P. David Rogers

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Abstract

The expansion of antimicrobial resistance has been called “one of the greatest threats we face as a global community” by the United Nations. While the mechanisms of resistance to available antifungals are well-studied, the process by which these mechanisms evolve is less so. One example of this: resistance in the “superbug” *Candida auris* is entirely driven by acquired resistance mutations, but these mutations occur substantially more frequently in *C. auris* than in other pathogenic *Candida* species. To interrogate the biology driving this evolutionary pattern, first, we observed widespread variability in the rates at which different lineages of *C. auris* acquire resistance mutations. Our findings suggest resistant lineages were more predisposed to develop resistance mutations than susceptible lineages. We developed a combinatorial-indexing single-cell transcriptomic approach for *C. auris* to trace the expansion of rare resistant subpopulations from an evolving population in order to characterize differences in the patterns of resistance signatures during evolution between lineages. Second, we asked how resistance mutations are stably maintained. A common resistance mechanism - overexpression of the efflux pump Cdr1 – carries no discernable fitness defect. However, its overexpression stimulates a substantial transcriptional response while conferring synthetic lethality to some small molecules, suggesting an essential physiological compensation carrying a hidden cost. We have developed and employed a genome-scale CRISPR knockout screen to probe the physiological dependency associated with this common resistance mechanism. Together, these data provide key insights into the mechanisms by which *C. auris* establishes and maintains resistance.

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Targeting Drug Resistance in *Candida glabrata*: Insights from Synthetic Genetic Array Screening

Catrin Cerian Williams, Jane Usher

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Abstract

Candida glabrata (*Nakaseomyces glabratus*) is the second most common cause of invasive candidiasis, with a mortality rate of ≥49%. It exhibits intrinsic low-level azole resistance and rapidly acquires high-level resistance. Resistance is primarily attributed to gain-of-function mutations in *CgPDR1,* leading to the upregulation of efflux pumps and reduced intracellular accumulation of azoles. Due to this, echinocandins are now the recommended first-line treatment. However, echinocandin resistance is increasingly documented in *C. glabrata* primarily due to hotspot mutations within *CgFKS1* and *CgFKS2.*

To identify novel therapeutic targets, we employed synthetic genetic array (SGA) screening in *Saccharomyces cerevisiae* to explore the genetic interaction networks driving drug resistance in *C. glabrata.* We have generated genome-wide double mutant strains which express either a *CgPDR1, CgFKS1* or *CgFKS2* allele and a single gene deletion. These combinations reveal synthetic sick or lethal interactions, offering insights into resistance mechanisms. Screens using both wild-type *CgPDR1* and the clinically derived *CgPDR1*^{R592S} allele highlighted the role of chromatin remodelling and transcription pathways in resistance, identifying *CgGCN5* a component of the SAGA complex, as a promising therapeutic target. *In silico* screening identified the Methotrexate as a potential inhibitor of *CgGCN5. In vitro* testing demonstrated synergistic effects between Methotrexate and Fluconazole against *C. glabrata* and other *Candida* species. Additionally, this study is the first to map the genetic interaction networks associated with *CgFKS1* and *CgFKS2* mediated echinocandin resistance using SGA. Collectively, these findings demonstrate the utility of unbiased SGA screening for uncovering novel antifungal targets.

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Loss of Putative Cell Wall Protein Fgr41 in *Candida albicans* Increases Proinflammatory Immune Response and Attenuates Virulence in a Dectin-1 Independent but β(1,3)-glucan-dependent Manner

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Abstract

Candida albicans causes life-threatening invasive infections, and an important virulence factor is evasion of host immune responses. *C. albicans* cell wall β(1,3)-glucan triggers a robust pro-inflammatory response, but an outer layer of mannosylated proteins covers, or masks, these β-glucans. This hinders recognition by immune receptors such as dectin-1. Prior work showed that putative cell wall protein Fgr41 is downregulated in conditions that trigger unmasking, such as caspofungin treatment or activation of the Cek1 MAP kinase pathway. Disruption of *FGR41* also increases *C. albicans* β(1,3)-glucan exposure and reduces kidney fungal burden in murine systemic infections in an immune-dependent manner. We tested the impact of *FGR41* on immune evasion by measuring TNF-α elicitation from cultured mouse macrophages. The *fgr41ΔΔ* mutant elicited ten-times more TNF-α than wild-type. Antibody neutralization of dectin-1 did not significantly reduce TNF-α released from macrophages by *fgr41ΔΔ*, however soluble β-glucan fully eliminated *fgr41ΔΔ*-induced stimulation, indicating that macrophage recognition of *fgr41ΔΔ* is driven by detection of exposed β(1,3)-glucan, but not by dectin-1. Moreover, the *fgr41ΔΔ* mutation similarly attenuates virulence in both wild-type and dectin-$1^{-/-}$ mice, suggesting a non-dectin-1 mechanism controls virulence. Genetic data implicates the cell wall β-glucanase *ENG1* as interacting with *FGR41.* Overexpression of *ENG1* moderately suppresses *fgr41ΔΔ* unmasking, but the opposite is not true. Furthermore, an *eng1ΔΔ* mutant elicits 3-fold more TNF-α than wild-type, but is partially dectin-1 dependent. Ongoing work is exploring macrophage signaling pathways triggered by *fgr41ΔΔ* to discover how it drives TNF-α signaling and compromises virulence in mice.

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Fungal glycogen contributes to *Candida albicans* β-(1→3)-glucan masking, immune activation, and oral commensalism

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Abstract

Background: Complex carbohydrates are major components of the fungal cell wall and serve as molecular patterns to potentiate innate immunity. We recently reported that glycogen and β-(1→3,1→6)-glucan form a covalently linked macromolecular complex at the *Candida albicans* cell wall.

Methods and Results: Using a combination of biochemical and genetic approaches, we confirmed that loss of *GSY1* (*i.e.*, glycogen synthase) ablated cell wall glycogen content. Challenge of macrophages with fixed or live *gsy1*Δ/Δ led to exacerbated pro-inflammatory cytokine secretion (*e.g.*, IL-1β) or neutrophil swarming. Analysis of cell wall components by fluorescence staining revealed that levels of total glucan, mannan and chitin remained similar, while reduced glycogen content correlated with significantly increased β-(1→3)-glucan exposure. Antibody-mediated blockade confirmed that exacerbated cytokine release observed in *gsy1*Δ/Δ (and glucan-glycogen particles) was partially dependent on Dectin-1 signaling. To establish translational impact of our findings, a collection of *C. albicans* clinical isolates was screened for glycogen content, which revealed significant intra-species heterogeneity. Remarkably, overexpression of *GSY1* in reduced glycogen accumulation isolates reversed their hyperinflammatory phenotype and deletion of *GSY1* in WT-like glycogen accumulation isolates induced a hyperinflammatory phenotype during human macrophage challenge. Using an immunocompetent model of oropharyngeal candidiasis, mice inoculated with *gsy1*Δ/Δ constructed in multiple isolate backgrounds showed markedly decreased early fungal burdens.

Conclusions: Collectively, our data demonstrate that the glucan-glycogen macromolecular complex may be a novel cell wall determinant important for governing the host-*Candida* interaction and that fungal glycogen content contributes to commensalism in the oral cavity.

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Translational Regulation of *Candida albicans* Virulence Properties by the Asc1 Phospho-signaling Molecule and eIF4E-binding Proteins

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Abstract

While transcriptional mechanisms that control virulence properties of the major human fungal pathogen *Candida albicans* are well-studied, considerably less is known about the role of translational mechanisms. *C. albicans* Asc1 is a non-essential 40S ribosome component important for morphogenesis and virulence whose *S. cerevisiae* ortholog functions as a key phospho-signaling molecule. *S. cerevisiae* Asc1 and the Eap1 eIF4E-binding protein (eIF4E-BP) are components of the SESA complex, which directs gene-specific translational repression. Eap1 regulates cap-dependent translation by disrupting the eIF4F complex. Mutants for the *C. albicans* eIF4E-BP orf19.7034 (Eap1 ortholog) are defective for morphogenesis and sensitive to various host environmental stress conditions. We demonstrate that *C. albicans asc1* mutants show reduced P-body formation under multiple stress conditions and are resistant to cell wall stresses and rapamycin but sensitive to cell membrane stress. We also show that several highly conserved phosphosites of Asc1 are important for differential responses to multiple environmental stress and filament-inducing conditions. In addition, we demonstrate genetic interactions among *C. albicans ASC1*, *orf19.7034*, and the *CAF20* eIF4E-BP under both environmental stress and morphogenesis conditions. A preliminary mass spectrometry analysis identified 662 interacting partners of Asc1, including SESA complex components, Caf20, and other proteins involved in stress responses, virulence, and translation. Interestingly, Asc1 is also important for expression of *UME6*, a key regulator of hyphal extension, under filament-inducing conditions. Gaining a better understanding of Asc1, eIF4E-binding proteins, and their interacting partners/target genes could lead to the identification of new targets for development of novel and more effective antifungal therapies.

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Candida point prevalence survey in Mwanza, Tanzania

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Abstract

Background

Candida auris is an emerging fungal pathogen and cause of worldwide nosocomial outbreaks. A challenging species identification often aggravates clinical management. However, little is known about *Candida* colonization -including *C. auris*- in East Africa. Thus, we evaluated current predictors and assessed the performance of selective-chromogenic media at a tertiary care hospital in Tanzania.

Methods and Materials

We conducted a point-prevalence study based on WHO guidelines. After informed consent, all eligible patients were noninvasively swabbed at ≥2 body sites and potential predictors were assessed using a standardized questionnaire. *Candida* was detected using CHROMagar-*Candida*-Plus (CA+) and confirmed by MALDI-TOF-MS. Predictors were analysed using logistic regression. Institutional and national ethical clearance was granted by Tanzanian authorities.

Results

We processed 1082 samples from 448 eligible patients with a yield of 596 suspected *Candida* isolates classified by CA+. Of these, 541 were confirmed by MALDI-TOF showing good comparability for *C. albicans* (92% (207/225)) and moderate for *C. parapsilosis* (83% (57/69)), *C. tropicalis* (80% (58/73)), *C. krusei* (68,8% (11/16)) and *C. glabrata* (56% (53/94)). Importantly, in 9 cases *C. auris*-like growth phenotypes on CA+ could not be confirmed by MALDI-TOF (4x *C. orthopsilosis,* 4x *C. parapsilosis,* 1x *C. lusitaniae*), indicating initial misclassification. Predictors of *Candida* colonization include prior hospital admission

(OR=1,73, p=0,023), prior antibiotic use (OR=1,74, p=0,054) and low age (OR=2,88, p=0,033). Resistance and genomic analysis are currently ongoing.

Conclusion

For the first time we systematically assessed *Candida* prevalence in Tanzania, could identify weaknesses in chromogenic-agar-specificity and found no *C. auris* strains to this date.

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Identifying novel regulators of azole resistance in *Candida auris* and their impact on extracellular vesicle production

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Abstract

The rise of antifungal resistance and the emergence of multidrug-resistant fungi, such as *Candidozyma (Candida) auris*, pose a clear and growing threat to global health. While numerous resistance mechanisms have been identified, the role of extracellular vesicle (EV) production in mediating resistance remains underexplored. Notably, no direct link has yet been established between resistance to azoles, a major class of antifungal drugs, and EV production in *C. auris*. To investigate this potential association, we employed experimental evolution by serially passaging fluconazole-susceptible *C. auris* isolates in subinhibitory azole concentrations until resistance emerged. Evolved isolates exhibited both significantly increased azole resistance and tolerance as well as alterations in EV production, suggesting a possible correlation between these phenotypes. Whole-genome sequencing of evolved lineages revealed both known resistance-associated mutations as well as novel variants in signalling pathways not previously linked to EV regulation. These findings point to a potential mechanistic overlap or co-regulation between azole resistance and EV production in *C. auris*. Elucidating this relationship could provide critical insight into the adaptive strategies of this pathogen in human infection and inform the development of more effective antifungal interventions.

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Candida albicans induces immune-metabolic reprogramming of intestinal epithelial cells

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Abstract

Background

Candida albicans (*Candida*) is a potentially life-threatening pathogen, yet it colonizes the gut of most healthy individuals without causing disease. We hypothesized that intestinal epithelial cells support this commensal state.

Methods

We employed translating ribosome affinity purification (TRAP) to identify transcripts which are translated by ileal epithelial cells *in vivo* during *Candida* colonization. Using an intestinal epithelial cell line co-cultured with live *Candida*, we examined epithelial-intrinsic responses and identified fungal metabolites which influence these responses. Immune response was assessed in γδ T cell-deficient mice, and human biopsies matched with donor *Candida* load allowed us to validate the epithelial responses observed in mice in humans.

Results

Candida colonization induced both metabolic and immune reprogramming in the intestinal epithelium. We found that long-chain fatty acids produced by *Candida* activate epithelial Hnf4a and upregulate fatty acid metabolism genes in a cell-intrinsic manner, both *in vitro* and *in vivo*. *Candida* upregulated the translation of Hnf4a target genes Btnl1 and Btnl6, immune-regulatory proteins that modulate intraepithelial γδ T cells, which were essential for intestinal fungal clearance. In humans, *Candida* levels were associated with Hnf4a activation and reduced inflammatory responses in the gut. However, these effects were lost in inflammatory bowel disease patients.

Conclusion

Our findings reveal a novel epithelial response to *Candida* colonization, linking metabolic sensing and immune modulation.

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Pan-Candida monoclonal antibodies as novel immunotherapies to treat drug resistant life-threatening invasive candidiasis

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Abstract

Effective treatment of invasive candidiasis is challenging due to increasing prevalence of drug resistant infections, drug toxicities and drug:drug interactions.

We have identified novel, surface-exposed peptide targets that are more abundant in drug-resistant *Candida albicans* and become upregulated in pathogenic *Candida* species when exposed to current antifungals. Our targets are cell wall proteins that are critical for cell wall remodelling and pathogenicity and are expressed during infection. One target Pga31 is pan-*Candida,* and the second, Utr2 is pan-fungal belonging to a chitin:glucan crosslinking enzyme family.

A panel of Pga31 and Utr2- specific binders was isolated from a phage display antibody library and reformatted into human IgG1 anti-Pga31 and anti-Utr2 monoclonal antibodies (mAbs). All mAbs had a strong affinity to their target with EC50 values reaching around 300 pmol. The mAbs were cross-reactive to fungal cells across all major *Candida* pathogens with enhanced binding when cells were stressed with caspofungin and fluconazole. Preferential binding to *C. albicans* hyphae was observed and after antifungal challenge the anti-Pga31 antibody re-localized to hyphal tips. Enhanced antibody-mediated opsonisation was detected, the binding of antibodies significantly induced phagocytosis of *C. albicans* by murine J774.1 macrophages. Importantly the mAbs have proven in vivo efficacy in murine invasive candidiasis models that represent the immunocompetent and immunosuppressed status of patients. There is huge potential for unique, fungal-specific cell wall targeting mAbs with a novel mechanism of action as monotherapy or co-therapy with existing antifungals to improve clinical outcomes in complex patients and combat the AMR crisis.

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Emergence of Fluconazole-Resistant *Candida glabrata* Small Colony Variants in Clinical isolates

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Abstract

The CandiRes study investigates the emergence of antifungal resistance in intensive care unit (ICU) patients with suspected or confirmed candidiasis, with a particular focus on the impact of antifungal exposure.

In this context, we have identified multiple series of fluconazole-resistant *Candida glabrata* small colony variants (SCVs) exhibiting the petite phenotype, an under-recognised mitochondrial defect. These SCVs were isolated from oral and perianal swabs, as well as abdominal fluid samples from patients with invasive candidiasis, and were found in mixed populations alongside fluconazole-susceptible wild-type *C. glabrata*.

The SCVs displayed classic features of the petite phenotype: markedly elevated fluconazole minimum inhibitory concentrations (>128 μg/mL), reduced colony size and growth rate, and an inability to grow on non-fermentable media, consistent with mitochondrial dysfunction.

One SCV population emerged within seven days of initiating fluconazole therapy and persisted beyond treatment cessation, highlighting their rapid development and sustained resistance under antifungal pressure *in vivo*. *In vitro* serial passaging experiments confirmed the stability of this petite phenotype, with SCVs maintaining high-level resistance in the absence of fluconazole across multiple passages. Ongoing molecular analysis is being conducted to characterise mitochondrial dysfunction and elucidate the molecular mechanisms underpinning this resistance.

These findings underscore the profound impact of antifungal therapy on colonising *Candida* populations *in vivo,* driving the emergence of subpopulations of petite mutants capable of persisting both in the presence and absence of drug pressure. Our findings suggest that petite *C. glabrata* represents a clinically relevant yet underrecognised resistance phenotype, likely shaped by antifungal selective pressure.

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Regulation of Population Heterogeneity: Persister Cells and Heteroresistant Cells of *Candida glabrata*

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Abstract

Decades of research on antibiotics has uncovered multiple independent processes that can contribute to diminished susceptibility *in vitro* and therapeutic failure *in vivo*: resistance, tolerance, heteroresistance, and heterotolerance (or persistence). Heteroresistance and heterotolerance occur transiently and reversibly in small subpopulations of otherwise susceptible cells. To shed light on all these processes in fungi, we have adapted powerful Tn-seq methods to perform genome-wide genetic screens in *C. glabrata* exposed to diverse antifungals. Using Tn-seq in different micafungin treatment conditions, we find almost no overlap between the gene sets that regulate resistance and tolerance. Drugs that increase (manogepix) or decrease (FK506) calcineurin signaling strongly alter micafungin tolerance. The number of persister cells that develop in response to antifungal stress strongly depends on tolerance but may be uncoupled from calcineurin signaling. We also utilized Tn-seq to explore echinocandin heteroresistance in *C. glabrata*. Though wild-type strains of *C. glabrata* do not exhibit such heteroresistance, introduction of a *FKS2-F659Δ* resistance mutation causes a dramatic increase of heteroresistance that was not replicated by introduction of the homologous *FKS1-F625Δ*mutation. We hypothesize that heteroresistance generally requires differential sensitivity of *FKS* gene products to echinocandins, mutual antagonism between *FKS* genes, and perhaps differential expression of *FKS* genes in response to cell wall stress. Forms of heteroresistance that are specific to a particular echinocandin may incorporate additional regulatory inputs such as a “lipid code” that is beginning to emerge. A deeper understanding of all these processes may lead to new strategies for combating the root causes of echinocandin failure.

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Regulation of *Candida albicans* cell wall homeostasis by Cdc14 phosphatase

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Abstract

Infections by *Candida* species are still associated with high mortality rates, underscoring the need for improved therapeutic strategies and understanding of fungal virulence mechanisms. The fungal cell wall is an essential structure and validated antifungal drug target, providing a barrier against environmental stress and mediating host–pathogen interactions. We recently reported that Cdc14 phosphatase is a critical virulence factor in *C. albicans.* Reduced Cdc14 activity causes hyper-susceptibility to cell wall stress and constitutive activation of the cell wall integrity (CWI) pathway. Here, we report evidence supporting that Cdc14 is a key regulator of cell wall homeostasis in actively growing cells. Transcriptomic profiling of Cdc14-deficient strains revealed extensive dysregulation of cell wall–related genes, closely resembling transcriptional responses triggered by caspofungin-induced cell wall stress. Cytological analyses further confirmed structural defects, including increased cell wall thickness and elevated chitin deposition, suggesting impaired regulation of cell wall assembly or remodeling in the absence of functional Cdc14. Current investigations using auxin-inducible degradation (AID)-coupled RNA-seq and phosphoproteomics are underway to identify direct Cdc14 substrates and further elucidate the molecular mechanisms governing cell wall regulation. Improper cell wall remodeling in the absence of functional Cdc14 may alter recognition by host immune cells, potentially contributing to reduced virulence and we are currently testing this hypothesis in several ways. These studies will provide valuable insights into fungal cell wall regulation mechanisms, potentially guiding the development of novel antifungal therapies.

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Genomic super-enhancer-like features regulate cell fate in *Candida albicans*

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Abstract

Candida albicans, an opportunistic pathogen, is one of the few fungal species that undergoes reversible, heritable switching between white and opaque morphologies which is hypothesized to contribute to its pathogenesis. In higher-eukaryotes, cell fate transitions are regulated by non-coding elements called “super-enhancers”, characterized by the presence of high densities of transcription factors, histone acetylation marks and occupancy by core transcriptional machinery.

We examined whether these features are associated with cell fate in *C. albicans* using an integrative approach, combining genome-wide binding of core white-opaque regulators and transcriptional mediators, acetylation of lysine 27 on histone H3 (H3K27ac), chromatin accessibility and gene expression. We identified super-enhancer-like (SE) elements using Mediator complex occupancy and show that they are associated with increased RNA polymerase II occupancy, H3K27ac, and neighboring gene expression.

We found that six of the eight core white-opaque regulators are regulated by SE elements and 80% of these SE elements are occupied by at least one of these regulators. SE elements are more accessible with increased binding of core white-opaque regulators. Even though we identified SE elements in both cell types, they regulate cell-type specific functions, *i.e.* phenotypic switching in opaque cells, and housekeeping functions in white cells. Additionally, 70% of genes regulated by SE elements are highly conserved.

Overall, our findings support a role for SE elements in regulating cell fate in *C. albicans* and indicate that white-opaque regulators drive cell fate switching in a concerted manner through co-localization in regulatory regions.

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Novel inositol pyrophosphates in *Candida albicans*

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Abstract

Inositol pyrophosphates (PP-InsPs) are derived from fully phosphorylated *myo*-inositol carrying one or more β-phosphates. These high-energy signaling molecules are found across eukaryotes and they regulate important cellular processes such as energy metabolism, phosphate homeostasis, stress responses, glucose metabolism, intracellular trafficking, and gene expression. They exhibit multiple mechanisms to regulate these processes including allosteric regulation through SPX domains, protein pyrophosphorylation without a kinase, and serve structural elements in proteins. PP-InsPs are well studied in the model yeasts (*Saccharomyces cerevisiae, Schizosaccharomyces pombe*) and they have been linked to virulence in some pathogenic fungi, such as *Cryptococcus neoformans*; however, they are understudied in *Candida albicans*. We have constructed deletion mutations in the genes encoding the kinases and phosphatases that metabolize PP-InsPs, and analyzed changes to the metabolic pools using mass spectrometry. Unexpectedly, we found novel isoforms of the PP-InsPs that have not been described in *S. cerevisiae* or *S. pombe*. We report on the discovery of these new isoforms and our work to identify the kinase(s) important for their synthesis. We found increased aneuploidy and loss-of-heterozygosity in the *vip1*-null mutant but not in the *siw14*-null mutant, suggesting a role for PP-InsPs in genome stability. Our results indicate that inositol pyrophosphates – both the isoforms and metabolism – are different in pathogenic fungi relative to model yeasts, and they may play novel physiological roles.

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The Antifungal Peptide EntV Inhibits Virulence by Reducing Extracellular Vesicle Release

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Abstract

New antifungal agents with novel mechanisms of action are desperately needed. We have previously reported that a bacteriocin produced by *Enterococcus faecalis*, EntV, is a potent antivirulence agent that blocks adhesion and biofilm formation in *Candida albicans* in invertebrate and murine infection models. Structural studies and systematic peptide library screening have identified a 10 amino acid synthetic peptide, P4D, that is effective at reducing tissue fungal burden in oral and systemic models in mice, and prolongs survival after intravenous inoculation. P4D has activity against *C. auri*s in mice, and against *Cryptococcus* and *Coccidioides* species in invertebrate models. Moreover, it is orally bioavailable in mice and is not toxic to mammalian cells. It is neither static nor cidal to *Candida* species, nor does it synergize with other stresses. Instead, it binds to the cell surface in dynamic punctae without specific stoichiometry. It colocalizes with dyes that preferentially stain extracellular vesicles (EVs), which are immunostimulatory and contain cargo important for extracellular matrix production. We screened a published panel of mutants that are known to reduce EV production in nematodes, mostly components of the ESCRT pathway, finding that these mutants generally were less sensitive to the peptide, but also attenuated for virulence. Direct treatment of *C. albicans* with EntV peptides reduces EV production ~8-fold, and the vesicles are more heterogeneous than those from untreated cells. Thus, the anti-virulence mechanism of this unusual peptide is associated with a reduction in EV release.

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Into the void: towards a new understanding of how heritable phenotypic switching is regulated in Candida albicans

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Abstract

Candida albicans can undergo heritable switching between two pathogenic cell types, white and opaque, which can persist across hundreds of generations. This transition is governed by the opaque master regulator Wor1, which initiates a highly intertwined transcriptional regulatory network resembling those that control cellular differentiation in higher eukaryotes. We find that Wor1 and additional white-opaque regulators are associated with the formation of opaque-specific super-enhancer-like elements that promote robust expression of target genes. These regulatory elements coincide with extensive chromatin remodeling, including eviction of “fragile” nucleosomes, which appears linked to the epigenetic “memory” of the opaque state. Notably, the white-to-opaque switch is correlated with large, macromolecular condensates of Wor1 in the nuclei of opaque cells, likely formed via liquid-liquid phase separation. Together, our findings suggest that Wor1-driven super-enhancers may assemble into transcriptional hubs that stabilize the opaque program, paralleling mechanisms that control lineage specification and cancer in higher eukaryotes.

Offered talk (16 minutes)

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A Copper Nexus of Mutualism in *Candida albicans* and*Staphylococcus aureus* Mixed Biofilms

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Abstract

Although fungi and bacteria commonly coexist within polymicrobial communities, the molecular mechanisms underlying their interactions are still not well understood. Here, we show that the fungus *Candida albicans* forms biofilms with the bacterium *Staphylococcus aureus* along a nutritional axis of mutualism and propose that “a copper economy” shapes fungal-bacterial biofilm interactions.

Using *in vitro* biofilms formed on plastic, we found that dual species biofilms are consistently larger than single-species counterparts, indicating a cooperative interaction. Dual species proteomic analysis revealed non-reciprocal copper handling: *C. albicans* increased copper uptake via Ctr1, as well as the CuZn superoxide dismutase Sod1 and its copper chaperone partner Ccs1, and copper binding protein Sco1. On the other hand, *S. aureus* enhanced its copper export machinery. Dual species biofilms exhibited specific sensitivity to both copper depletion and supplementation, with corresponding reductions in biomass. We identified fungal copper import as the crucial element in this mutualistic interaction and extend this finding to multiple Gram-positive bacteria. Moreover, fungal hyphae served as a critical scaffold for biofilm architecture, a role that was compromised under copper-replete conditions. Notably, copper nanoparticles disrupted these dual species biofilms, highlighting a potential therapeutic avenue for treating clinically challenging mixed biofilms.

We posit that copper is traded between *C. albicans* and *S. aureus* in a manner that supports mutualistic interaction within biofilms. This work establishes copper as a central mediator of *C. albicans* and *S. aureus* cooperative interactions and suggests that a "copper economy" underpins mutualistic interactions in biofilms.

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Exploring the antibacterial activity of candidalysin

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Abstract

Candida albicans causes superficial infections and severe systemic infections under certain predisposing conditions. Recently it was demonstrated that hypha formation plays a crucial role in facilitating *C. albicans* gut colonization in the presence of an undisturbed commensal microbiota. In this niche, hypha-competent *C. albicans* outcompeted yeast-locked mutants. This fitness advantage was primarily attributed to the expression of *ECE1*, encoding candidalysin (CaL) – the first ribosomal peptide toxin discovered in a human pathogenic fungus.

We explored the susceptibility of selected co-colonizing bacteria from different body sites to Ece1 peptides. Our screening revealed that CaL influences growth of several members of the microbiota. To further understand the mechanism of CaL-mediated antibacterial activity, we focused on the model organism *Escherichia coli*. With fluorescent labeling of CaL and protoplastation experiments, we showed that the peptide binds the surface of *E. coli* and that bacterial cell membranes are susceptible to CaL-mediated lysis, respectively. Using genetic tools, we identified the lipopolysaccharide (LPS) component of the bacterial membrane as important for the defense of these bacteria against CaL. By analyzing the transcriptional response of wild-type *E. coli* and an LPS-defective mutant strain by RNA-seq, we found a specific transcriptional stress response of the bacteria upon CaL-treatment.

Our study provides mechanistic insights into the antibacterial activity of CaL. Further, our results demonstrate the importance of this multifunctional fungal factor that likely evolved in *C. albicans* to improve fungal fitness during gut commensalism or polymicrobial infections through inter-kingdom competition.

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The lipase family of *C. albicans* in fungal commensalism, microbiome interaction, and host metabolic health

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Abstract

Candida albicans is among the most important fungal pathogens of humans, and one of very few fungi that are permanently associated with humans. As a commensal of the gut, its niche is defined by the host, the diet, the microbiota, and the interplay between all those.

A striking characteristic of its genome is the presence of many large gene families, including a family of ten lipase genes. The inherent redundancy has made it technically challenging to ascribe biological functions to these genes. With CRISPR-Cas technology, we were now finally able to delete all lipase genes, individually and as a complete *lip1-10*Δ mutant.

We found that the extracellular lipases support *C. albicans* growth with a plethora of dietary oils and fats. They are required for commensal growth in a murine gut colonization model, and the *lip1-10*Δ strain is quickly outcompeted by the wild type – especially with a high fat diet resembling the typical human diet. The presence of the *Candida* lipases strongly affected serum triglyceride levels and even murine weight gain. In i*n vitro* co-culture with typical gut-resident microbiome members, the *C. albicans* lipases positively and negatively affect bacterial growth in dependence of fat sources. Importantly, the lipases also affect *C. albicans* morphology within the murine gut, with the lipase-deficient mutant forming much more hyphae than the wild type, again depending on dietary fats.

The lipases of *C. albicans* can therefore be considered a *bona fide* commensalism factor that directly and indirectly affect the interplay between fungus, host, microbiome, and diet.

Offered talk (18 minutes)

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The evolution of antifungal drug tolerance in clinical isolates of *Candida albicans*

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Abstract

Infections caused by *Candida* species remain a major source of morbidity and mortality despite available antifungal drugs. Treatment failure in persistent or recurrent fungal infections may be driven by antifungal tolerance, a phenomenon arising from phenotypic heterogeneity that enables a subpopulation of cells to persist and grow slowly under drug exposure. Prolonged antifungal treatment may further promote resistance evolution, as highly tolerant strains divide more frequently under drug pressure, increasing the likelihood of acquiring mutations. To investigate the factors driving tolerance development and its link to resistance, we conducted selective evolution experiments using fluconazole disk diffusion assays in clinical *Candida albicans* isolates with varying baseline tolerance levels. Multi-colonies were serially passaged from tolerant (inside inhibition zone) and sensitive (outside) regions. Across all strains with different baseline tolerance levels, tolerance gradually increased from passage 2, while resistance significantly increased after passages 4–6 but rapidly declined. No evolutionary changes occurred in sensitive populations. Notably, heteroresistant cells emerged at different passages in certain strains, with some colonies within the inhibition zone growing larger after 48 hours. Consistent trends among biological replicates suggest common evolutionary mechanisms, while differences in their extent indicate the possibility of different strategies at play. Further evolution experiments with different antifungals, combined with whole-genome sequencing and proteomics, will determine whether stress-induced chromosomal instability, mutagenic factors, or metabolic shifts drive tolerance evolution. These findings will elucidate the patterns and dynamics of tolerance evolution, identifying novel therapeutic targets to improve long-term antifungal treatment efficacy.

5

An Integrated Fluxomics Workflow Reveals Metabolic Rewiring during the Yeast-to-Hyphae Transition in *Candida albicans*

Chen Liao[1,2], Heesoo Jeong[1], Ajinkya Kulkarni[3], Margot Delavy[1], Tobias Hohl[1,4], Richard Bennett[3], Joao Xavier[1]

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Abstract

Background A critical factor enabling *Candida albicans* to transition from a harmless commensal organism to a virulent pathogen is its ability to switch from the budding yeast form to the filamentous hyphal form. While the genetic regulators of the hyphae formation are well characterized, the metabolic changes that support and drive this transition remains incompletely understood.

Objective The metabolite abundance and metabolic flux together provide a complete view of cellular metabolism. Conventional metabolite profiling measures changes in metabolite abundance but falls short of capturing the dynamic processes driving these changes. To bridge this gap, we aim to provide a holistic view of "metabolic rewiring" by analyzing both aspects during the yeast-to-hyphae transition.

Method We developed an integrated experimental and computational workflow to profile untargeted metabolomics and fluxomics within a single experiment. This workflow combines isotope tracing experiments for tracking labeled metabolites, Gas Chromatography-Mass Spectrometry (GC-MS) for measuring metabolite abundance and isotope labeling patterns, Computational metabolomics for analyzing tracer enrichment dynamics, and Metabolic Flux Analysis for metabolic flux quantification.

Results We applied this workflow to compare metabolism in *C. albicans* and its yeast-locked *(*Δ*efg1*) and hyphae-locked (Δ*nrg1*) mutants grown on universally-labeled glucose as the sole carbon source. TCA cycle labeling was similar across strains but the hyphae-locked mutant showed delayed and lower amino acid labeling, suggesting increased amino acid recycling via macromolecular degradation. We also showed a systems-level discrepancy between metabolite abundance and flux, highlighting the importance of considering both aspects when studying metabolic rewiring.

7

Postoperative Infections Regulated by Candida Strain Diversity through ALS9

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Abstract

Postoperative infection is a major cause of poor prognosis and mortality, yet our understanding of the role of mycobiome underlying postoperative infection is still limited. Here, using MK-SpikeSeq for absolute quantification, we determined the dynamic changes in fungal communities in 691 fecal samples of 129 longitudinal liver transplant recipients (82 with infections). We found that fungal communities exhibited high diversity during both pre-operative and early post-operative stages, with late post-operative clustering predominantly characterized by *Candida* species. By fecal fungal culturomics, we isolated 2,497 *C. albicans* isolates in 296 fecal samples of 60 PLT recipients and further corroborated that *Candida* species being the predominant cultivated gut fungi (CGFs). Through whole genome sequencing (WGS) analysis, we revealed ten distinct groups with SNPs more than 100,000 by phylogenetic analysis. Intriguingly, we uncovered that all the C. albicans isolates can be devided into three strain types (ST1, ST2 and ST3) based on the SNPs of *ALS9* gene, among which ST2 strains were specifically enriched in patients with postoperative infections with high filamentation. Mechanistically, we found that co-culture of ST2 strains and epithelial cells can trigger the upregulation of *GIMAP2* compared with ST1/ST3/WT. This upregulation can induce the chronic inflammation or immune tolerance which promotes postoperative infection. Together, these findings reveal the strain-specific nature of Candida species in immunomodulation and highlight new diagnostic and therapeutic targets against postoperative infection.

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"Should I stay or should I go?" – A strategy of *Candida glabrata* to delay its escape from macrophages

Theresa Lange[1], Luisa Fischer[1], Julia Mantke[1], Johannes Sonnberger[1], Raghav Vij[1], Nadja Jablonowski[1], Colin Clairet[2], Christophe d'Enfert[2], Lydia Kasper[1], Sascha Brunke[1], Bernhard Hube[1,3,4]

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Abstract

Candida glabrata can cause life-threatening systemic infections in humans. During infection, the fungus faces different types of immune cells, including macrophages, which are crucial for initiation of antifungal immune responses and fungal clearance. Macrophages can efficiently internalize *C. glabrata* cells, however, the fungus has evolved strategies to survive and proliferate inside the phagosome. Unlike *C. albicans*, which quickly forms intracellular filaments to escape from macrophages, *C. glabrata* replicates in the yeast morphology and can persist inside these phagocytes for several days. It has been suggested that *C. glabrata* may even use macrophages as a reservoir to evade immune detection and disseminate throughout the body.

We observed that C. glabrata cells escape from macrophages after two to three days *in vitro*. This exit is delayed in comparison to *C. albicans* and occurs by bursting of host cells. Host transcriptome analyses revealed that this is a fungal-driven process, rather than a host activity, and it requires more than simple replication of yeasts. We identified fungal protein kinases that affect the exit kinetics, especially the Ksp1 kinase: A *ksp1*Δ mutant shows accelerated macrophage cell lysis, increased mitophagy, and a higher formation rate of *petites*. This respiration-deficient phenotype is associated with resistance toward antifungals and, more importantly, toward phagocytic killing. Moreover, deletion of *KSP1* enhances resistance to multiple antifungals, suggesting a role in the broader survival strategy in clinical scenarios. Collectively, our findings indicate that *C. glabrata* may actively prolong its intramacrophagal phase, which could contribute to immune evasion, antifungal resistance, and potential re-infections.

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Defining the antifungal mode of action of miltefosine to unlock its potential in targeting eukaryotic pathogens

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Abstract

The rise of antimicrobial resistance (AMR) is a critical global health concern. While much of the focus has been on antibiotic resistance, antifungal drug resistance is an equally pressing threat, exacerbated by the slow development of new antifungal agents. This challenge is further fuelled by the emergence of multidrug-resistant fungal pathogens such as *Candida auris*. A key obstacle in antifungal drug development is the close evolutionary relationship between fungi and humans, which limits the availability of selective drug targets. Miltefosine, an FDA- and WHO-approved drug for visceral leishmaniasis, has recently been approved and repurposed for systemic candidiasis treatment. Despite its potential, significant knowledge gaps remain regarding its precise mode of action and broad-spectrum antifungal efficacy. Recent studies suggest that miltefosine targets ergosterol and inositolphosphorylceramide (IPC)-based sphingolipids, similar to its mechanism in protozoan parasites.

Our study aims to elucidate miltefosine's antifungal mode of action in greater detail. Using fluorescent-tagged *Saccharomyces cerevisiae* reporter strains, we are holistically defining the impact of miltefosine on the plasma membrane and membrane-bound organelles. This is complimented with systematic screening of fungal deletion libraries for mutants that display altered resistance, and in-depth omics profiling to capture the fungal response to miltefosine. Microevolution experiments are also ongoing to determine whether *Candida albicans* can develop resistance to miltefosine and if such resistance confers cross-resistance to other antifungals. Keys findings will be presented showcasing insights into miltefosine’s mode of action and therapeutic potential, and its implications for antifungal resistance.

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The pathogenesis of CNS candidiasis is governed by transcriptional regulators with organ specific functions

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Abstract

Candida infection of the central nervous system (CNS) is a devastating disease. It most commonly affects premature infants and causes higher rates of neuro-developmental disability than bacterial CNS infections. In the standard mouse model of disseminated candidiasis, the brain is initially infected but is cleared of *C. albicans* within 3-4 days. Infection of IL1-receptor knockout mice results in high, persistent fungal burden in the brain but similar kidney burden relative to WT. We took advantage of this infection model to carry out a concomitant competitive fitness screen in the brain and kidney using pools of barcoded *C. albicans* transcription factor (TF) mutants. Here, we present the results of this screen as well as in-depth studies of two TFs that affect fitness in the CNS but not in the kidney: the carbon metabolism regulator Tye7 and the adhesion and morphology regulator Ahr1. In the kidney, Tye7 and Gal4 redundantly regulate central carbon metabolism whereas only Tye7 is required in the brain. Ahr1 is a negative regulator of brain, but not kidney, clearance. The toxin candidalysin (Ece1) activates the CARD9-IL1 axis in the brain, leading to fungal clearance. Although the *ahr1*Δ/Δ mutant fails to fully activate IL1 signaling and, consequently, is not cleared from the brain of immunocompetent BL6 mice, it expresses *ECE1* at WT levels in the brain. These data indicate that additional Ahr1-regulated genes contribute to CNS clearance. The details of these experiments as well as progress toward identifying Ahr1 targets required for CNS clearance will be presented

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Investigating Micafungin Heteroresistance in Candida parapsilosis.

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Abstract

Candida parapsilosis is a common cause of fungemia in hematopoietic cell transplant recipients on micafungin prophylaxis. Most breakthrough infections are caused by micafungin-heteroresistant strains, in which a small proportion of cells display a higher minimal inhibitory concentration (MIC) than the rest of the population. The stability and underlying cause of this phenotype remain unclear.

We investigated the stability of micafungin heteroresistance in clinical isolates. During serial passage in micafungin-free conditions, heteroresistance persisted for at least 10 days without a reduction in the frequency of the resistant subpopulation. In ampicillin-treated mice colonized with a heteroresistant *C. parapsilosis* strain, micafungin treatment did not increase the frequency of the resistant subpopulation.

However, the frequency of resistant cells in a heteroresistant strain was higher in the murine gut compared to the frequency of the resistant cells in the inoculum. A pilot experiment indicated that stopping ampicillin in *C. parapsilosis*-colonized mice led to a further increase in the frequency of resistant cells, consistent with the idea that micafungin heteroresistance may confer a fitness advantage when intestinal bacteria expand in the intestine after ampicillin discontinuation.

We further showed that the micafungin-resistant subpopulation was less susceptible to cell wall stressors than micafungin-susceptible cells in the same heteroresistant isolate. The resistant subpopulation had a distinct transcriptomic profile, with differences in the transcription of glycosylphosphatidylinositol (GPI)-anchored proteins and GlcNAc-induced genes compared to micafungin-susceptible cells. These observations raise the possibility that micafungin heteroresistance in *C. parapsilosis* may represent a phenotypic adaptation to the intestinal niche.

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Candida auris Metabolism and Growth Preferences in Physiologically Relevant Skin-like Conditions

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Abstract

Candida auris is an opportunistic, multidrug-resistant yeast with high capacity of skin colonization in healthcare settings, which can lead to subsequent infections with high mortality rates. Given the recent emergence of at least four distinct clades at the global scale, little remains known about how *C. auris* is so adept at growing on skin and the key genes and pathways it utilizes to metabolize the scarce nutrients available. Here, I identify the roles that conventional and alternative carbon metabolism genes and metabolic pathways have in facilitating *C. auris* growth through laboratory-based experiments and bioinformatics analyses. In artificial skin-like media, all four clades of *C. auris* were more capable of growing than *C. albicans* SC5314, a clinically relevant counterpart. By investigating the differential regulation of *C. auris* when growing in skin-like media as compared to rich fungal media, I uncovered hundreds of genes in multiple metabolic pathways. To further test the mechanisms of these metabolic pathways, I deleted several non-essential gene candidates including *FOX2* (B9J08_002847), *CAT2* (B9J08_000010), and *ICL1* (B9J08_003374). The mutant strains all exhibited abrogated growth in skin-like media and demonstrated nutrient preferences that differed from the wild type. Thus, I propose a model of how *C. auris* has the capacity to metabolize nutrients that are naturally available on skin by changing its metabolic profile. Targeting these metabolic pathways to mitigate *C. auris* growth on skin is a potential avenue to explore in controlling the spread of this emerging human fungal pathogen.

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"Polygenetic Determinants of Azole Resistance, Tolerance, and Heteroresistance in *Candida albicans*"

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Abstract

Azole antifungals are widely used to treat *Candida albican*s infections, yet resistance mechanisms remain incompletely understood. In addition to resistance, fungal populations can exhibit tolerance (the ability of subpopulations to grow slowly above the minimum inhibitory concentration) and heteroresistance (the presence of rare resistant subpopulations within an otherwise susceptible population), both of which can contribute to treatment failure. To uncover genetic determinants of these complex drug responses, we performed a genome-wide association study (GWAS) on over 550 clinical and environmental *C. albicans* isolates, phenotyped for fluconazole susceptibility, tolerance, and heteroresistance. We identified multiple loci significantly associated with each phenotype, including novel candidate genes implicated in ploidy and telomere maintenance. Our findings highlight the polygenic nature of antifungal adaptation and expand our understanding of drug resistance mechanisms in *C. albicans*.

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Stress-Tested: Decoding Genomic Adaptation and Combinatorial Stress Resistance in Candida glabrata

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Abstract

Candida glabrata is an emerging human fungal pathogen notable for its exceptional resistance to antifungal drugs and innate immune attack. Unlike other *Candida* species, *C. glabrata* thrives under combinatorial stresses—such as those encountered within macrophage phagolysosomes—by deploying unique genomic adaptations. In this work we have combined, forward genetics, comparative genomics, and transcriptomic profiling to uncover the genomic basis of this stress resilience.

We have developed and exploited a functional mating system in *C. glabrata* to dissect heritable resistance traits through tetrad analysis of combinatorial stress-resistant mutants. Bulk segregant sequencing and SNP mapping of over 400 dissected tetrads have identified single-locus variants conferring high-level resistance to oxidative and osmotic stress combinations. Parallel time-resolved RNA-seq during combinatorial stress exposure reveals a distinct transcriptional program that is not simply additive, suggesting emergent regulatory mechanisms unique to stress synergy.

These genomic insights are further contextualized by *ex vivo* and *in vivo* assays demonstrating how combinatorial stress adaptation enhances immune evasion and virulence. Together, this data uncovers a combinatorial stress response (CSR) network in *C. glabrata*—a potential reservoir of therapeutic and diagnostic targets.

Highlighting the power of integrative genomics to illuminate pathogen evolution and genome plasticity under host-imposed pressures. It provides a model for how genomic innovation can drive clinical persistence and resistance, with broad implications for antifungal strategy development and understanding genome dynamics in fungal pathogens.

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Candidalysin Activates Ion Channel-Dependent Calcium Signalling to Modulate Mitochondrial Function and Innate Immune Responses

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Abstract

Background: Candidalysin damages epithelial cells and induces calcium influx which triggers innate immune signalling pathways via EGFR and c-Fos activation. However, whether calcium influx is governed by specific epithelial ion channels or results from candidalysin-induced membrane damage is unclear.

Methods: To investigate the role of ion channels in candidalysin-induced calcium influx and innate immune responses, TR146 epithelial cells were treated with a panel of ion channel inhibitors, and candidalysin-induced calcium influx, extracellular ATP (eATP), MTT, LDH, and cytokine secretion were quantified. Mitochondrial calcium dynamics were assessed using a matrix-localised fluorescent calcium reporter, and the effect of candidalysin on mitochondrial respiration was evaluated using the Seahorse Mito Stress Test.

Results: Candidalysin promoted the efflux of ATP through connexin hemichannels and pannexin channels, and calcium influx, as well as innate immune responses and membrane damage, were linked to P2X purinergic receptors. Candidalysin also induced rapid mitochondrial calcium uptake and transient, concentration-dependent changes in mitochondrial oxygen consumption. Interestingly, low concentrations of candidalysin enhanced mitochondrial spare respiratory capacity, an indicator of the mitochondria's ability to produce extra energy during stress, suggesting an adaptive metabolic response under mild stress conditions.

Conclusion: Calcium influx following candidalysin exposure is facilitated by specific epithelial ion channels rather than membrane damage, which drives mitochondrial calcium loading and metabolic dysfunction. These findings provide new insights into candidalysin biology and identify ion channels as potential therapeutic targets, whereby modulating these pathways may offer new strategies for mitigating mucosal inflammation and tissue damage during *Candida albicans* infection.

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Clostridioides difficile interacts with fungal colonizers of the gastrointestinal tract

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Abstract

Introduction: We previously demonstrated that fungal colonizers of the gastrointestinal (GI) tract modulate *Clostridioides difficile* infection (CDI) severity in murine models. *Candida albicans* protects against lethal CDI, whereas *Nakaseomyces glabratus* exacerbates disease by increasing *C. difficile* burden and toxin production. We propose that specific fungal species influence CDI outcomes through effects on *C. difficile* colonization, toxin output, and persistence via biofilm interactions and metabolic signaling. We aim to determine the molecular mechanisms involved in these interactions.

Hypothesis: We hypothesize that by defining the molecular mechanisms governing *C. difficile*-fungal interactions, we can modulate these interactions and mitigate CDI.

Methods: *C. albicans* (SC5314) biofilms were formed anaerobically for 24 hours in DMEM supplemented with serum and amino acids. *C. difficile* (UK1) was introduced in fresh media, and spent media was collected, filter sterilized, and diluted 1:1 with fresh media for downstream assays.

Results: *C. difficile* disrupts mature *C. albicans* biofilms without affecting viability of either organism. Disruption also occurs with a *p*-cresol-deficient mutant (Δ*hpd*), indicating the effect is independent of *p*-cresol. Biofilm disruption only occurs when the organisms are co-cultured with direct contact; spent media from isolated cultures or physically separated (0.22 μm filter) co-cultures lacks activity. Boiling or protease inhibitor treatment abolishes activity, while EDTA does not—suggesting a heat-labile, secreted protease is responsible. Ongoing mass spectrometry aims to identify this factor.

Conclusions: These findings support a contact-dependent mechanism by which *C. difficile* disrupts *C. albicans* biofilms via a secreted protease. Understanding these interactions will inform microbiome-guided therapies for CDI.

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Accumulation of Trehalose-6-Phosphate in *Candida auris* results in Decreased Echinocandin Resistance and Tolerance by Affecting Cell Wall Chitin Synthesis

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Abstract

Echinocandins, targeting β-glucan synthesis, are the first-line therapy for invasive *C. auris* infections. However, the resistance to this drug class is increasing, underscoring the urgent need for new antifungal targets. This study investigates the role of trehalose biosynthesis in *C. auris* by either blocking biosynthesis of the intermediate molecule trehalose-6-phosphate (T6P), by disrupting the trehalose-phosphate synthase Tps1, or blocking biosynthesis of trehalose, by disrupting the trehalose-6-phosphate phosphatase Tps2. The *tps2*Δ strain demonstrated increased susceptibility to echinocandins, while the *tps1*Δ and *tps1*Δ *tps2*Δ strains maintained resistance and tolerance levels comparable to the wild type (WT) strain. Subsequent analysis revealed a link between chitin biosynthesis and T6P levels. In the absence of T6P (in the *tps1Δ* or *tps1Δ tps2Δ* strains) there was a strong increase in hexokinase activity and accelerated glycolysis, with no significant impact on chitin biosynthesis. However, the *tps2*Δ strain accumulated high levels of T6P, resulting in the inhibition of hexokinase and a reduced flux of glucose 6-phosphate into the chitin biosynthesis pathway, thereby significantly decreasing cell wall chitin content. The inability to compensate the reduction in β-glucan levels with increased chitin production during echinocandin treatment in the *tps2*Δ strain, rendered this strain highly susceptible to these drugs. Furthermore, an *in vivo* systemic infection model demonstrated that the *tps2*Δ strain exhibited a reduced fungal burden in tissues, with infected mice showing marked improvement during caspofungin treatment. This suggests that tps2 is a putative target for improving echinocandin treatment and reducing virulence in *C. auris.*

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Differences between *Candida albicans* strains in gut commensalism and host immunity

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Abstract

Genotypic and phenotypic C. *albicans* strain diversity during commensal and pathogenic states has been well documented. A comparative analysis of the oral isolate *C. albicans* 101 and blood isolate SC5314 in the oral cavity revealed notable differences in virulence and niche colonization. SC5314 caused more damage, triggered a stronger inflammatory response, and was subsequently cleared, whereas strain 101 was able to persist in the oral cavity. Here, we characterised gut commensalism and colonization-mediated host protective immunity.

C. albicans 101 reached higher colonization densities in SPF mice in a diet-based colonization model, but not in dysbiotic and germ-free mice. It was outcompeted by SC5314 *in vivo,* especially in the absence of bacterial microbiota. We tested if colonization of SPF, dysbiosed, and germ-free mice with either strain protects against intravenous challenge with a low dose of SC5314. Colonization with *C. albicans* 101 conferred less protection than SC5314, and the lack of protection was more pronounced in dysbiotic and germ-free mice. To test if these differences are due to limited cross-protection, we performed colonization and systemic infection with strain 101. While low-dose infection was non-lethal in non-colonized controls, doubling the dose resulted in lethal candidiasis with no significant difference in disease severity between groups. Both strains significantly induced IgG, but the amount was higher with SC5314. Conversely, 101 induced a stronger Th17 response. Colonization with either strain led to distinct shifts in the bacterial microbiota. Overall, these findings emphasize how *C. albicans* strain variation shapes host immunity, microbiota dynamics, and infection outcomes.

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Intraspecies diversity, metabolic specialism, and niche fitness in *Nakaseomyces glabratus*

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Abstract

"Metabolic generalism" is the ability of an organism to utilise a wide range of nutrients and flexibly adapt to dynamic environmental conditions. For most *Candida* spp., this versatility is crucial for their survival and virulence in diverse niches. In contrast, metabolic specialists follow a strict hierarchy of carbon source utilisation. While this hierarchy does not preclude specialists from potentially utilising a wide range of nutrients, it could delay carbon source switching during transitions between niches. A large-scale metabolic analysis of 853 fungal strains suggests that only ~10% of yeasts are metabolic specialists. Of these, *Nakaseomyces glabratus* (formerly *Candida glabrata*) was the only identified pathogenic metabolic specialist. But does *N. glabratus* display other aspects of metabolic flexibility that facilitate its fitness *in vivo*? Work on *N. glabratus* has primarily focused on two sequenced strains, CBS138 (ATCC 2001) and BG2. BG2 utilizes a broader range of nitrogen sources compared to CBS138. However, deletion of *SNF3*, a major nutrient sensor, significantly enhanced *Galleria mellonella* killing in the CBS138 background compared to the parent strain, but not in BG2. Interestingly, our recent analysis of >90 clinical isolates identified only strict metabolic specialists but revealed heterogeneous mitochondrial activity and carbon utilisation profiles. This is striking, given our previous work found that ~67% of *Saccharomyces cerevisiae* clinical isolates were metabolic generalists, whereas the type strains are strict specialists. Our research is plugging a vital gap in understanding how a metabolic specialist, *N. glabratus*, leverages its metabolism to support pathogenesis and host fitness.

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Functional profiling of fungal pathogens in host-mimicking metabolic conditions

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Abstract

Fungal pathogenicity depends on both intrinsic and extrinsic factors. In this context, metabolism plays a central role. As a primary interface between host and pathogen, fungal metabolic flexibility is linked to pathogenicity. Similarly, metabolic environments can reshape fungal cellular physiology, leading to unexpected phenotypic outcomes. However, a functional and quantitative understanding of fungal metabolism at infection sites remains limited. Here, we designed and phenotyped fungal pathogens against a humanized metabolite library recapitulating human plasma. Different yeast pathogens exhibit distinct metabolic capabilities in host-associated environments. Alongside mass spectrometry-based analyses, we identified key metabolic host-pathogen interactions. These interactions have direct functional consequences: host-derived metabolites can interact with antifungal drugs and modulate fungal susceptibility. For example, we find that the antifungal drug flucytosine is inhibited by host-derived metabolites, which compete for uptake and reduce its intracellular accumulation. In biofluids such as urine, physiological metabolite concentrations raise the minimal inhibitory concentration of flucytosine in otherwise susceptible strains, surpassing the drug's epidemiological cutoff. As pathogenic yeasts such as Candida albicans and Nakaseomyces glabratus share similar uptake systems for flucytosine, the effect is conserved. These findings underscore the importance of considering host-specific conditions when developing strategies to combat fungal infections and reveal potential factors contributing to treatment failure.

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The Rising Threat of *C. auris* as a Urinary Pathogen

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Abstract

Candida auris (recently renamed *Candidozyma auris*), a rapidly emerging and highly concerning multidrug-resistant fungus, is increasingly detected in urine, raising alarms about its role in urinary tract infections (UTIs), particularly catheter-associated UTIs (CAUTIs). To further understand its prevalence in UTIs, we performed an analysis of 12,996 *C. auris* strains from NCBI's Pathogen Database 2,996 identifies urine as a major global isolation source, second only to blood in the US and third worldwide, often associated with urinary catheters and high mortality rates, highlighting the urgent need for in-depth investigation. Due to *C. auris* genetic diversity, we initiated to analyze whether phylogroups are associated with uropathogenesis and infection severity. We evaluated clinical isolates from urine, blood, and skin for the ability to form biofilms in urine. From these initial screening, we further characterized the uropathogenesis in our mouse model of CAUTI of three clinical isolates: 1) Clade I urine isolate, 2) Clade IV urine isolate, and 3) Clade IV skin isolate. We found that all strains were able to infected the catheterized bladder; however, only the Clade I urine isolate exhibited the alarming ability to disseminate systemically, leading to mortality. Our novel slippery urinary catheter, previously proven effective against bacterial and *C. albicans*CAUTIs in mice, also significantly reduced *C. auris* infections and prevented systemic dissemination in our model. These findings underscore the urgent understand *C. auris* clade-specific uropathogenesis and highlight the promising potential of slippery catheter technology in mitigating severe CAUTIs caused by this emerging threat.

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Tolerance to the antifungal drug fluconazole is mediated by tuning cytoplasmic fluidity

Emily Plumb[1], Antonio Serrano[1,2], Louis Chevalier[1], Johannes Elferich[3,4], Ludwig Sinn[5], Nikolas Grigorieff[3,4], Markus Ralser[5], Judith Berman[6], Martine Bassilana[1], Robert Arkowitz[1]

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Abstract

The efficacy in treating infections with the most widely used antifungal drug fluconazole, which is fungistatic, is impacted by the emergence of tolerance, *i.e.* the ability of strains to grow at drug concentrations above the minimal inhibitory concentration (MIC). Drug tolerance is thought to be a manifestation of phenotypic heterogeneity within a population of cells. A range of critical cellular functions take place in the cytoplasm, including protein folding, enzymatic catalysis, intracellular signaling, intracellular transport *etc.* In this dense, crowded and heterogenous milieu, diffusion is reduced,- in contrast to an aqueous phase where molecules diffuse freely-, which can negatively affect diffusion-limited reactions. The physical properties of the cytoplasm have been shown to change in response to external perturbation, hence we have been using a genetically encoded micro-rheological probe to investigate how prolonged exposure to high antifungal concentration affects the cytoplasm in *Candida albicans*. Our results reveal a dramatic decrease in cytoplasmic fluidity associated with fluconazole tolerance. This striking reduction in diffusion at the mesoscale is observed upon antifungal drug or genetic inhibition of the sterol biosynthesis pathway and is reversible upon antifungal drug removal. Reducing the concentration of a major cytoplasmic crowder, *i.e.* ribosomes, restores the antifungal drug-induced decrease in cytoplasmic fluidity and reduces tolerance. We have begun to quantitate cytoplasmic ribosome levels in cells grown in fluconazole, as well as investigate other means by which cytoplasmic fluidity is regulated. Our results suggest that tuning cytoplasmic fluidity may enable growth and survival in presence of high levels of antifungal drug.

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CRISPR-Induced Genome Rearrangements Drive Adaptive Evolution in *Candida albicans*

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Abstract

Candida albicans is a common member of the healthy human microbiome and an opportunistic pathogen capable of causing superficial to systemic infections. Its ability to rapidly adapt to diverse host microenvironments and acquire drug resistance during systemic infection is associated with genome instability. This instability generates genetic diversity, facilitating the selection of fitter genotypes. As such, clinical isolates of *C. albicans* are karyotypically diverse, often with chromosomal rearrangements around repetitive elements including the Major Repeat Sequence (MRS).

We hypothesise that repetitive elements including the MRS serve as instability hotspots, facilitating genomic rearrangements and rapid evolution. This project aims to establish a cause-and-effect relationship between repeat-associated chromosomal rearrangements and generation of fitter genotypes. To this end, we have targeted repetitive elements with CRISPR-Cas9, inducing chromosome rearrangements. Rearranged strains have been phenotyped, and evolved in clinically relevant stresses, including antifungal drugs.

We have implemented long-read, short-read and RNA sequencing to characterise the novel genomes and transcriptomes. This has shown that CRISPR-Cas9 can be used to generate different classes of chromosomal rearrangement in *C. albicans*. Strains bearing rearrangements have morphological and fitness changes, as well as reduced pathogenicity during *in vivo* infection models. This indicates that rearrangements at repeat loci are sufficient to generate phenotypic diversity. Exposure of rearranged strains to antifungal drugs results in the rapid acquisition of resistance, associated with additional karyotype changes. I will present this unpublished analysis, providing evidence that genome instability can contribute to antifungal drug resistance.

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Candida albicans genes and biologic processes that contribute to pathogenesis of intra-abdominal candidiasis (IAC)

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Abstract

Background. Pathogenesis of intra-abdominal candidiasis (IAC) is poorly understood.

Methods. We used RNA-Seq to measure *C. albicans*SC5314 gene expression during early peritonitis (30-min), late peritonitis (24-hr) and abscesses (IAA; 48-hr) of mice. ≥2-fold differences were significant (false discovery ≤0.01). We screened duplicate signature-tagged, homozygous deletion libraries for 165 *C. albicans* transcription factors (TFs) in 72-hr IAA.

Results. The 50 genes most highly expressed during early peritonitis were associated with pH (e.g., RIM101, PHR1), oxidative stress responses (e.g., SOD4-6), and adhesion/hyphal growth (e.g., ALS3, HWP1, ECM331, SAP6). Corresponding 50 late peritonitis genes were associated with phagocyte responses and nutrient acquisition (glyoxylate cycle, fatty acid β-oxidation, iron homeostasis). Responses within IAA included DNA damage and iron metabolism. Null mutants for genes involved in adhesion (ALS1, ALS3), transport (OPT8, SGE11), biofilm (ZCF23), DNA damage responses (RFX1, RFX2, DDI1), cell wall responses (DAP2) and copper metabolism (CCC2) were attenuated during peritonitis and/or IAA. Biologic processes over-represented were regulation of pH responses, biofilm, hyphal formation, echinocandin responses, and copper metabolism. 9 pH response regulators were confirmed to contribute to virulence, including RIM101, STP2 (alkaline), ASH1, SFL1, SFL2 (neutral), MNL1, SKO1, PHO4 (weak acid), and CSR1 (acid). Over-expression of aspartyl protease SAP5 in rim101 restored virulence during IAA. SKO1 or PHO4 over-expression in mnl1 restored weak acid responses in vitro and virulence.

Conclusions. Numerous *C. albicans* environmental response genes make temporal-spatial contributions to IAC, including pH response regulators adhesion, transport, biofilm, DNA damage responses, cell wall responses and copper metabolism.

Offered talk (20 minutes)

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Host albumin unlocks alternative pathogenicity mechanisms in *Candida albicans*

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Abstract

During infection, activation of inflammatory responses leads to vascular permeability and influx of plasma proteins into tissues. With this in mind, we investigated how abundant plasma proteins affect *Candida albicans* interactions with host tissues and immune cells. Albumin, by far the most abundant plasma protein, has a multifactorial role in human physiology. While this protein is often excluded from the in vitro models used to dissect *C. albicans* pathogenicity mechanisms and immune interactions, we found that its introduction into these models dramatically alters host-pathogen interactions. The presence of albumin neutralises the efficiency with which candidalysin causes host cell damage, but upon interaction with this protein, *C. albicans* undergoes transcriptional and metabolic changes that allow it to bypass candidalysin to cause host cell cytotoxicity and resist killing by innate immune cells. Taken together, this is a clear example of host-pathogen co-evolution and helps to explain why clinical *C. albicans* isolates that do not form extensive hyphae still successfully cause infections.

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Cross kingdom warfare – investigations into the mode of action of the antifungal effector Tfe1 elicited by the *Serratia marcescens* Type VI Secretion System

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Abstract

Recent studies have revealed that gram-negative bacteria can deploy their Type VI Secretion System (T6SS) as a potent antifungal weapon via the secretion of specific antifungal toxins. This secretion system is a contractile nano-syringe which rapidly punctures neighbouring cells releasing effector toxin proteins. Although primarily considered to function in interbacterial competition through the secretion of antibacterial effectors, the identification of antifungal effectors illustrates an additional role for the T6SS in shaping polymicrobial communities and we are interested in the role of the T6SS in modulating the fungal composition of polymicrobial communities such as the gut microbiome.

We have amassed evidence that the use of the T6SS against fungal competitors is widespread. The *Serratia marcescens*T6SS secretes two potent antifungal effectors Tfe1 and Tfe2, and homologues of such effectors are found in a range of gram-negative bacterial species. Tfe1 displays potent activity against *Candida albicans* and *Candida auris* yeasts, and high resolution microscopy revealed that this effector accumulates at distinct loci at the plasma membrane. Our structure-function analyses, together with non-biased approaches, have provided a global overview of the impact of Tfe1 on yeast cell biology, with recent biochemical approaches defining the mode of action of this potent antifungal effector. As fungal pathogens cause an estimated 1.5 million deaths per year, can we exploit the mode of action of these potent antifungal effectors to identify new strategies to combat pathogenic fungi?

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Developing novel microbiome-based interventions to prevent life-threatening invasive *Candida albicans* infections

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Abstract

We propose that products secreted by in the human gastrointestinal (GI) tract could be used as a microbiome-based intervention to clear colonising *Candida albicans* from the GI tract and prevent invasive infections from occurring.

To identify human GI bacteria that kill *C. albicans*, faecal samples from 27 healthy anonymous donors (UNSW HC210301) were collected. Statistical associations between the *in vitro* killing of *C. albicans* and the presence of specific bacterial amplicon sequence variants (ASVs) in the faecal microbiotas were identified. GI bacterial isolates (n=39) representing these ASVs were obtained from the Australian Microbiome Culture Collection. Culture supernatants from 10 bacterial isolates inhibited the growth of the *C. albicans* lab strain (SC5314) as well as a *C. albicans* strain isolated from a faecal sample from a healthy anonymous donor. Culture supernatants from at least three bacterial isolates also killed both *C. albicans* strains.

To identify when the inhibitory products secreted by one bacterial species were produced, *C. albicans* was anaerobically co-cultured with the bacteria while physically separated by a semi-permeable membrane. *C. albicans* growth inhibition was observed when the bacteria were in late-log/early stationary phase. GC-MS was used to quantify the production of short chain fatty acids and related metabolites by the bacteria at different growth phases. Four key metabolites were identified whose concentration in the growth media increased through the phases of growth, peaking when maximal *C. albicans* growth inhibition was observed. These metabolites may form the basis of a microbiome-based intervention capable of preventing invasive *C. albicans* infections.

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Candida albicans epithelial invasion induces host membrane rupture at distinct subcellular niches following priming by the fungal toxin candidalysin

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Abstract

Candida albicans is an opportunistic fungal pathogen normally found as a commensal in human mucosa. In susceptible patients, its hyphal filamentous form can invade and damage host epithelium leading to local or systemic infection. The secreted fungal toxin candidalysin is required for epithelial damage, although both hyphal extension and candidalysin are required for inducing maximal infection damage, for reasons that are not well understood. Here we study the interplay between hyphal extension and host damage *in vitro* using live cell imaging combined with damage-sensitive reporters followed by single-cell level quantitative analysis. We show that fungal load is inversely proportional to single-cell damage potential, and we quantify candidalysin-induced damage. We then determine the three-dimensional architecture of invasion and show that the spatial organization of host membrane rupture is regulated by candidalysin and is predictive of host cell death. Finally, we demonstrate that membrane rupture occurs at two distinct host subcellular niches: the juxtanuclear region and the cell-cell boundary, with the latter the main driver of subsequent host cell death. Based on these results, we propose that *C. albicans* invasion damages epithelia in a sequential process in which candidalysin secretion acts to prime host membranes enveloping invading hyphae for subsequent rupture. Hyphal traversal of two distinct host subcellular niches then triggers membrane rupture, resulting in a niche-specific host response that is predictive of host cell survival or death.

Reference to preprint: https://doi.org/10.1101/2025.05.13.653632

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Macrophages mediating the Trojan invasion of Candida auris Cross Blood-brain barrier Triggering Neurological Defects in Drosophila

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Abstract

Candida auris, a recently characterized invasive fungal pathogen, exhibits resistance to a broad spectrum of antifungal medications. Designated by the WHO as one of the pathogenic fungi with a substantial impact on human health, Candida auris has the potential to infect multiple organs, including the brain, through local infiltration and the bloodstream. The specific mechanism by which it breaches the blood-brain barrier remains unclear. Following infection by Candida auris, the host displays a range of neurological and behavioral abnormalities, suggesting its capacity to infiltrate the brain tissue. This study aims to investigate the transgression of Candida auris across the blood-brain barrier into the brain's parenchyma, explore whether this process relies on a Trojan horse mechanism involving peripheral blood macrophages, clarify the underlying molecular pathways, and provide insights into the mechanisms leading to neurological and behavioral defects as well as brain damage.

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GENETIC PLASTICITY AND EVOLUTION OF DRUG RESISTANCE IN *CANDIDA GLABRATA* WITHIN HOST MACROPHAGES

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Abstract

Candida glabrata is an opportunistic human pathogen and the second leading cause of invasive *Candida* infections after *C. albicans*. *C. glabrata* can rapidly evolve genetic resistance to frontline antifungal drugs and displays remarkable genetic diversity, manifested by a variety of karyotypes (chromosomal rearrangements) and high levels of short nucleotide polymorphisms (SNPs). Although this evidence suggests that this organism can generate and tolerate high levels of genetic change, the mechanisms facilitating genetic instability in *C. glabrata* are not understood. Our previous study showed that the DNA damage checkpoint is attenuated in *C. glabrata* compared to its close relative *S. cerevisiae*, suggesting that if *C. glabrata* were to experience DNA damage in the host, this damage may trigger significant genetic instability. Although it has been hypothesized that pathogens taken up by macrophages experience DNA damage due to bursts of reactive oxygen species (ROS), this has never been directly demonstrated. Thus, we have asked whether *C. glabrata* experiences DNA damage and instability while persisting within macrophages, one of its host niches. Indeed, comet assays showed that *C. glabrata* cells engulfed by a human-derived monocyte/macrophage cell line have increased levels of DNA breaks relative to *C. glabrata* grown in culture. Pulse-field gel electrophoresis and next-generation sequencing showed that *C. glabrata* persisting with macrophages accumulate large-scale chromosomal changes. Finally, we found that *C. glabrata* persisting within human macrophages developed drug-resistant mutations. Together, these results strongly suggest that *C. glabrata* experiences DNA damage and genetic instability within host macrophages, which facilitates evolution of drug-resistant mutations.

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Using intravital imaging in zebrafish to understand the role CXCR2 receptor-signaling plays in phagocyte activity during the innate immune response to *C. albicans* infection.

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Abstract

Candida albicans is a commensal fungus affecting immunocompromised patients due to their impaired innate immune response which is integral in preventing lethal invasive candidiasis. Innate immune cells maintain immunity by early recruitment to the site of infection and clearing and containment of infection through phagocytosis or production of extracellular traps. However, defects in this recruitment cascade increase susceptibility to invasive candidiasis. Although, it is understood the molecular defects that lead to these recurrent infections, it is unclear how those defects translate to altered phagocyte recruitment, phagocytosis, and fungal killing. Intravital imaging of inhibited immune cells in the context of infection could shed some light into how specific genes and pathways control immune cells' functional responses. To quantify defects in recruitment, containment, and clearance, we monitored immune cell recruitment in larval zebrafish during hindbrain injection of *C. albicans* which models a systemic infection. Our preliminary results indicate that the CXCR2 chemokine receptor is important for recruitment, as expected. However, quantitative imaging suggests that this decreased phagocyte recruitment early in infection doesn't lead to lower phagocytosis efficiency. Instead, the receptor seems to limit *C. albicans* fungal burden growth between 4 and 24 hours post-infection through other mechanisms. Current time-lapse intravital imaging experiments seek to understand these mechanisms by identifying differences in clearance through tracking *C. albicans* containment and killing at the single-cell level over 24 hours. A cellular understanding of the roles of these pathways in candidiasis may lead to targeted treatments for increasing survival in immunosuppressed patients.

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Metabolic imprinting regulates protective epithelial memory during mucosal *Candida albicans* infection

Marc Swidergall

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Abstract

Trained immunity, a form of innate immune memory driven by epigenetic and metabolic reprogramming, has been well-studied in innate immune cells during invasive fungal infections. However, it remains unknown whether innate memory confers resistance during mucosal *Candida albicans* infection. Here, we demonstrate that oral mucosal priming with *C. albicans* enhances protection during reinfection, independent of adaptive immunity. In contrast to invasive candidiasis, this enhanced protection occurs independent of monocytes and macrophages. To elucidate the underlying mechanisms, we trained human oral epithelial cells with β-glucan and found that β-glucan priming enhances production of proinflammatory cytokines during *C. albicans* infection. Using metabolic approaches, we identified that exposure to β-glucan induces proline catabolism via proline dehydrogenase (Prodh), supporting the TCA cycle and mitochondrial oxidative functions. Consistent with our in vitro findings, oral mucosal *C. albicans* infection similarly induces proline catabolism and increases TCA cycle intermediates in epithelial-enriched tissues. Proline catabolism was partially required for the establishment of epithelial memory, as inhibition of Prodh during β-glucan stimulation reduced the expression of specific cytokines during subsequent *C. albicans* infection. Notably, protective epithelial memory occurred independently of glycolysis. Moreover, Prodh-deficient mice exhibited increased susceptibility and diminished proinflammatory responses during reinfection with *C. albicans*. Collectively, these findings reveal that β-glucan-induced proline catabolism is a critical determinant of epithelial memory and plays a vital role in host defense during mucosal fungal infections.

Offered talk (25 minutes)

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Longitudinal profiling of the vaginal microbiome reveals distinct fungal-bacterial interactions underpinning mucosal infections in pregnancy

Yani Fan[1,2], Feiran Jia[2], Feiling Wang[1], Yuanyuan Tang[2], Manchun Li[1], Miao Peng[2], Xiaoyu Yan[2], Rui Dong[1], Xiaoyue Liu[1], Qiumei Li[2], Bo Sun[1], Chen Liao[3], Lijuan Wu[1], Yuanfang Zhu[1], Bing Zhai[2]

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Abstract

The vaginal microbiota comprises bacteria, fungi, virus, and protists and plays a vital role in female reproductive health, yet the non-bacterial constituents remain largely understudied. Fungi are commonly isolated from vaginal samples and their colonization can lead to vulvovaginal candidiasis (VVC), the second most prevalent form of vaginitis. Due to the limited data resolution, previous studies have failed to establish clear associations between the vaginal fungal colonization and infections. To address this gap, we recruited 715 pregnant women and characterized the dynamics of vaginal fungi and bacteria and quantified the cross-kingdom interactions. Culturomics data revealed persistent fungal colonization throughout pregnancy, regardless of topical antifungal therapies. Quantification of the microbial community showed that only VVC caused by *Candida albicans* – but not non-*albicans* species – is coupled with elevated fungal loads. By high-resolution amplicon sequencing data, we identified six fungal Community State Types (fCSTs) and observed a strong negative correlation between *Lactobacillus crispatus* and *Candida* species colonization. A Markov chain model analysis suggested that VVC may promote the progression of bacterial vaginosis (BV) during pregnancy. Collectively, these findings highlight the importance of a comprehensive view of fungal and bacterial communities in improving the prognosis and management of vaginal infections.

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---The emerging fungal pathogen *Candida auris* induces IFNg to colonize mammalian hair follicles

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Abstract

Public health alarm concerning the emerging fungus *Candida auris* is fueled by its antifungal drug resistance and propensity to cause deadly outbreaks. Persistent skin colonization drives transmission and lethal sepsis although its basis remains mysterious. We compared the skin colonization dynamics of *C. auris* with its relative *C. albicans*, quantifying skin fungal persistence and distribution and immune composition and positioning. *C. auris* displayed a higher propensity to colonize hair follicles and avidly bound to human hair. While *C. albicans* triggered an effective sterilizing type 3/17 antifungal immune response driven by IL-17A/F-producing lymphocytes, *C. auris* triggered a type 1, IFNg-driven immune response targeting hair follicles. Rather than promoting fungal clearance, IFNg enhanced *C. auris* skin colonization by acting directly on keratinocytes to impair epithelial barrier integrity and repress antifungal defense programs. *C. auris* exploits focal skin immune responses to create a niche for persistence in hair follicles.

Offered talk (6 minutes)

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Coding of Candida infections in Electronic Health Records: Curiouser and Curiouser

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Abstract

Electronic Health Records (EHR) are now used in medical facilities ubiquitously worldwide. ICD codes (International Classification of Diseases) are standardized codes used to record diseases and diagnoses within EHR. New versions of these ICD codes are regularly revised and released. ICD9 codes were used up until ~2016 and ICD10 codes have been used since then, although not all health systems adopt the new version at the same time. When analyzing EHR for disease prevalence and incidence over time, it is important to look for both ICD9 and ICD10 codes for the disease of interest. An ICD11 version has been proposed and may be released in the near future.

When looking at the ICD codes for Candida infections, several curious issues have been identified. First, there is no ICD code for systemic candidiasis, only septic candidiasis. Second, there is no ICD code specific to a particular Candida species, so there are no unique codes for *Candida auris*, *Candida glabrata, Candida krusei* or *Candida parapsilosis.* This means the ICD codes do not allow physicians to distinguish *Candida auris* from *Candida albicans* in the electronic record, and can lead to problems with diagnosis,treatment, research, and public health monitoring. Issues with ICD codes and Candida (and other fungal diseases) will be presented.

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