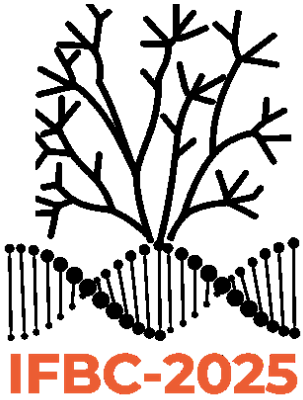

International Fungal Biology Conference: from Molecules to Communities

September 27-30, 2025 | Chania, Crete, Greece
Mediterranean Agronomic Institute of Chania, CIHEAM MAICH.

LIST OF ABSTRACTS

Honorary lecture

From fungi to microbial holozoa, a personal itinerary

Claudio Scazzocchio

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Opisthokonta include fungi, animals and a number of related unicellular organisms. Multicellularity developed independently in those two kingdoms. The study of unicellular holozoans, closely related to animals, is essential to understanding the crucial transition(s) to multicellularity in the animal kingdom. I will address how experience in fungal biology has contributed to this aim through collaboration with colleagues at https://multicellgenome.com/ in Barcelona. The study of nitrate assimilation was of foundational importance in the establishment of fungal gene regulation mechanisms and discovery of transcription factors. We unraveled its evolution throughout the eukaryotes, highlighting the role of horizontal gene transfer and the unsolved question of convergent recruitment of specific transcription factors. In *Creolimax fragrantissima* (Ichthyosporea, Ichthyphorida) nitrate presence results in a specific new morphology, while in *Corallochytrium limacisporum* (Teretosporea, Corallochytrea) a potentially novel nitrate assimilation pathway was uncovered. *C. limacisporum* shows a number of cell forms, with two paths of cell division, leading to coenocytes, uninuclear and binuclear cells, and uninucleate amoebas. Taking cues from previous fungal work, we developed a Crispr/Cas9 procedure for *C. limacisporum*, including gene inactivation and point mutation generation. Septins, GTP-binding proteins, involved crucially in cellular organization, have been thoroughly studied in model animals and fungi (e.g., *S. cerevisae*, *S. pombe*, *A. nidulans*, *M. oryzae, N. crassa*). Septin homologues are extant throughout the microbial holozoans. Only one canonical and a second non-canonical septin are encoded in the *C. limacisporum* genome, hopefully leading to the investigation of septin function at the root of the holozoans.

DAY 1 - Session 1: Fungal Metabolism, Homeostasis & Development

Coordination of fungal development, stress, pathogenicity and aging

Gerhard Braus

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Filamentous fungi as the mold *Aspergillus nidulans* as well as the vascular plant pathogen *Verticillium dahliae* form resting structures for overwintering in the soil. These are closed sexual fruiting bodies surrounded by Hülle cells (cleistothecia) for *A. nidulans* or asexual melanized microsclerotia for *V. dahliae*. Both have to be protected for survival against various other organisms in the soil. The plant pathogenic fungus has to grow through the soil to appropriate hosts, then has to colonize and penetrate the roots and additionally has to cope to the responses of the plant host in later developmental stages. During the past years, we have applied various approaches to dissect molecular mechanisms of these fungi, which provide the appropriate stress responses during fungal development, aging or pathogenesis. The current status of our work will be discussed.

Communication and cellular memory of fungal cells

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A fungal colony is a branched network of interconnected hyphal cells that exchange cytoplasm, organelles, nutrients and signals through septal pores. An interesting question is, if this cellular network also represents an information network for environmental signals, and if this information may be even stored as a sort of simple cellular "memory" in which a repeated stimulus is recognized quicker and the corresponding response can thus be stronger at subsequent exposures. We tried to answer these questions using the filamentous ascomycete *Aspergillus nidulans* and its capacity to produce antibacterial compounds as a model system. When we exposed colonies of *A. nidulans* on one side to a bacterial competitor (*Streptomyces*) and monitored the production of defense compounds we found a spatial restriction of the metabolic response. Only the confrontation area – but not other parts of the fungal colony - showed a reaction to the presence of Streptomyces and its starvation-triggering molecule Polaramycin-B. So even though an A. nidulans surface-grown colony represents a "cellular network", a systemic starvation response over the whole colony is missing. In contrast to the solid surface-grown colony, cells in a hyphal network of properly aerated submerged cultures are far more uniform. Using this uniform submerged culture system we asked if repeated exposure to starvation may evoke a sort of memory leading to a quicker and stronger response to a subsequent exposure. Indeed, we found such a transcriptional memory for about 800 genes that responded more vigorously to subsequent exposures compared to the initial signal. Analysis of the molecular events behind this memory effect identified transcription factor inheritance, certain epigenetic modifications and the production of "metabolic memory molecules" as triggers of *Aspergillus* cellular memory.

The fungal nicotinate degradation pathway

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Nicotinic acid (niacin, vitamin B3) is degraded in bacteria through different pathways. A different, unique pathway is extant in the Pezizomycotina. Notably, *Aspergillus nidulans* can utilize nicotinate as a nitrogen source. Eight enzymes, two transporters, and a transcription factor (HxnR) are encoded within three gene clusters; the cluster organization being variable within the phylum. The first step of the pathway is catalyzed by a paralogue of xanthine dehydrogenase, arising from a gene duplication at the root of dikarya. Only a few residues are involved in the shift in substrate specificity. The pathway includes previously undescribed metabolites, namely 5,6-dihydroxypiperidine-2-one and 3-hydroxypiperidine-2,6-dione. Differently from aerobic bacteria, the heterocyclic ring opening comprises an oxidative step followed by a hydrolytic step. HxnR is a Zn finger pathway-specific transcription factor, defined by both non-inducible and constitutive mutations, and responding specifically to the late metabolite 5,6-dihydroxypiperidine-2-one. While most enzymes of the pathway are localized in the cytosol, the early acting monooxygenase HxnX, localizes to the peroxisomal through a canonical PTS1 signal. HxnW, acting on 5,6-dihydroxypiperidine-2-one, co-localizes by piggy-backing HxnX. While the physiological roles of the putative transmembrane transporters, HxnP and HxnZ are unclear, it is interesting that they include plant-like non-canonical PTS1 motifs and localize to both the cell membrane and the peroxisome. The study of this pathway highlights the role of convergent evolution in the establishment of new pathways including the recruitment of novel genes, the occurrence of horizontal gene transmission events, the intracellular distribution of metabolites and enzymes and by comparison with bacterial pathways the different mechanisms involved in the opening of heterocyclic rings.

DAY 2 - Session 2: Fungal Mechanisms

EMBO Keynote Lecture

Compartmentalization beyond organelles

Anne Spang

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Eukaryotic cells contain organelles that perform specialized, essential functions. However, these organelles themselves are further compartmentalized, enabling the separation and regulation of the tasks they carry out. These subcompartments can be either static or dynamic, and their formation and dissolution may depend on factors such as the cell's metabolic state, cell cycle phase, stress levels, or other internal and external cues. Compartmentalization is not limited to membrane-bound organelles; both the cytoplasm and the nucleus also exhibit distinct, organized regions. There, membrane-less organelles carry out specific cellular functions. I will discuss examples of compartmentalization associated with membranes—particularly membrane contact sites—as well as membrane-less compartments such as processing bodies in the cytoplasm.

High-resolution structures of the UapA purine transporter reveal unprecedented aspects of the elevator-type transport mechanism

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Institute of Moleculr Biology and Biotechnology, FORTH-Hellas, Heraklion, Crete, Greece

UapA is an extensively studied elevator-type purine transporter from the model fungus *Aspergillus nidulans*. Determination of a 3.6Å inward-facing crystal structure lacking the cytoplasmic N-and C-tails, molecular dynamics (MD), and functional studies have led to speculative models of its transport mechanism and determination of substrate specificity. Here, we report full-length cryo-EM structures of UapA in new inward-facing apo- and substrate-loaded conformations at 2.05-3.5 Å in detergent and lipid nanodiscs. The structures reveal in an unprecedented level of detail the role of water molecules and lipids in substrate binding, specificity, dimerization, and activity, rationalizing accumulated functional data. Unexpectedly, the N-tail is structured and interacts with both the core and scaffold domains. This finding, combined with mutational and functional studies and MD, points out how N-tail interactions couple proper subcellular trafficking and transport activity by wrapping UapA in a conformation necessary for ER-exit and also critical for elevator-type conformational changes associated with substrate translocation once UapA has integrated into the plasma membrane. Our study provides detailed insights into important aspects of the elevator-type transport mechanism and opens novel issues on how the evolution of extended cytosolic tails in eukaryotic transporters, apparently needed for subcellular trafficking, might have been integrated into the transport mechanism.

Candida albicans morphogenesis: a tale of lipid asymmetry and cytoplasmic fluidity

Stephanie Bogliolo[1], Antonio Serrano [1], Chevalier Louis[1], Emily Plumb[1], Hayet Labbaoui[1], Johannes Elferich[2], Nikolaus Grigorieff[2], Robert Arkowitz[1], Martine Bassilana[1].

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The generation of polarized hyphal cells relies on different of processes, including the asymmetric distribution of lipids, both in the different cellular compartments and across the lipid bilayers. This asymmetric distribution is regulated in particular by lipid transporters, such as lipid transfer proteins (Osh, oxysterol binding proteins) and P4-ATPases (flippases). We investigated the role of these proteins in response to different stresses, e.g. filamentous growth inducers and antifungal drugs. Our comparative analyses indicate that the flippases Drs2 (1) and Neo1 regulate C. albicans invasive filamentous growth and stress response via distinct processes, with Drs2 particularly critical for plasma membrane organization and Neo1 for cell wall integrity. Both of these proteins localize to the Golgi, a hub for proteins and lipids destined for different locations, which move by Brownian motion in a dense and crowded cytoplasmic environment. To examine the interplay between the physical properties of the cytoplasm and morphogenesis, we analyzed the dynamics of a genetically encoded micro-rheological probe (2). We observed an increase in cytoplasmic fluidity, as a result of decreased ribosome concentration, upon filament elongation. Strikingly, repression of a gene encoding a protein required for ribosome biogenesis resulted in a substantial increase in cytoplasmic fluidity, as well as filamentous cells formation along with an induction of hyphal specific genes in the absence of external stimuli. Together, these results indicate that changes in cytoplasmic diffusion at the mesoscale, via altering ribosome concentration, are associated with filamentous growth, and suggest that inhibition of ribosome biogenesis is a trigger for the yeast to hyphal morphogenetic transition.

(1) Basante-Bedoya et al., PLoS Genetics, 2022, 18:e1010549; (2) Delarue et al., Cell, 2018, 174:338.

Uncovering the Hyphal Tip: New Insights into Fungal Cell Polarity and Secretion

Meritxell Riquelme, **Samantha V. González-Téllez, Juan Manuel Martínez-Andrade**

Centro de Investigación Científica y de Educación Superior de Ensenada CICESE, Ensenada, Mexico

The hyphal tip of filamentous fungi is a highly dynamic and specialized region that drives polarized growth through intense vesicle trafficking and cell wall remodeling, critical for hyphal elongation. Central to this process is the Spitzenkörper, a prominent, vesicle-rich structure composed of secretory vesicles, actin microfilaments, and associated regulatory proteins. Our findings in *Neurospora crassa* provide strong evidence that these secretory vesicles are not merely structural, but also serve as carriers of essential biosynthetic enzymes, including chitin synthases and β-1,3-glucan synthases. Over time, cumulative studies have revealed a complex protein network at the hyphal apex, comprising components of the polarisome, exocyst complex, motor proteins such as myosins, and Rab GTPases, and others. More recently, we have identified at or near the Spitzenkörper export chaperones and regulatory proteins specific to chitin synthases as well as smooth-tubular endoplasmic reticulum shaping proteins. By combining live-cell imaging, molecular genetics, and biochemical approaches, our research continues to uncover how these diverse elements orchestrate the targeted delivery and activation of cell-wall synthesizing enzymes. Together, these findings reinforce the concept of the Spitzenkörper as a multifunctional hub for vesicle sorting and signaling at the fungal apex.

Chytrid fungi and the evolution of fungal morphogenesis

Lillian Fritz-Laylin

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Cell morphogenesis is crucial for the physiology of animals and fungi alike. While animals directly shape their cells using a layer of actin under the plasma membrane, fungi control cell shape through polarized deposition of new cell wall material, inflated by intracellular osmotic "turgor" pressure. Understanding where and when these mechanisms evolve is essential for understanding the evolution of cell morphogenesis. Chytrid fungi, which have both a wall-less, motile zoospore stage and a walled, sessile sporangial stage, provide a powerful system for investigating this question. Here we show that, the zoospores of the chytrid *Batrachochytrium dendrobatidis* (Bd) use specialized vacuoles to keep internal pressure low and dynamic actin networks to shape the plasma membrane, reminiscent of animal cell morphogenesis. In contrast, Bd sporangia generate and maintain turgor pressure and shape themselves through polarized wall expansion, similar to morphogenic programs of yeast and other dikaryotic fungi. Genomic comparisons reveal that Bd retains homologs of actin regulators found in both animals and fungi. Moreover, homologs of animal regulators are expressed during the zoosporic stage while homologs of dikaryotic actin regulators are expressed in sporangia. Because chytrids diverge before the evolution of the Dikarya, these findings indicate that turgor pressure and actin-dependent fungal morphogenesis evolved early in the fungal lineage, and that stage-specific strategies for cell shape control may be ancestral. This developmental flexibility offers a window into how shifts in core cellular machinery can scaffold major evolutionary transitions.

DAY 3 - Session 3: Fungal Crosstalk with Hosts & Predatory Interactions

Plenary Lecture 1

Investigating the biology of effector-mediated invasive growth by the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice cultivation globally. To infect rice plants, *M. oryzae* forms a specialized infection structure called an appressorium. This cell forms in response to the hydrophobic leaf surface and requires a specific MAP kinase phosphorelay signaling pathway, coupled to cell cycle control and autophagic cell death of the fungal spore, and these signals collectively lead to appressorium morphogenesis. The appressorium generates enormous turgor, applied as mechanical force to breach the rice cuticle. We are studying the mechanisms by which appressoria develop and function and how septin-dependent re-polarization of the appressorium enables a rigid penetration peg to rupture the host cuticle. Invasive growth then requires differential expression and secretion of a large repertoire of more than 500 effector proteins that are delivered into plant cells using a specific secretory pathway, followed by uptake that appears to require clathrin-dependent endocytosis. These effectors suppress host immunity and target numerous cellular functions that impair defense and facilitate fungal growth and development in living plant tissue. Effectors are sequence-unrelated, often species-specific proteins, but fall into structurally conserved and transcriptionally co-regulated families, such as the Max and Zif effectors that target immunity components. The blast fungus also develops a transpressorium– a specific invasion structure used by the fungus to move from cell-to-cell using pit field sites, containing plasmodesmata, to facilitate its spread in plant tissue. This is controlled by the same Pmk1 MAP kinase signaling pathway as appressorium formation and also requires septin-dependent hyphal constriction. I will discuss recent progress into understanding the mechanisms of rice infection and immunity suppression by this devastating pathogen.

Small-secreted proteins as fungal virulence factors in the predatory interaction of *Arthrobotrys flagrans* with *Caenorhabditis elegans*

Reinhard Fischer

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A very intriguing fungal-host interaction is the predatory lifestyle of nematode-trapping fungi. Nematodes are the most numerous animals on earth and live predominantly in soil. Nematode-trapping fungi are also common members of the soil microbiome and live saprotrophically on organic material. However, when nutrients are limited and nematodes are present, they switch to a predatory lifestyle. They form special trapping devices, catch a nematode, penetrate it and colonize the entire body. They have a potential for being used as biocontrol agents (1). We are interested in the molecular biology of the interaction and discovered in *Arthrobotrys flagrans* that it recognizes *Caenorhabditis elegans* through nematode-specific pheromones. In other words the fungus "smells" the nematodes. The pheromones trigger a signaling cascade in the fungus which leads to the induction of the formation of adhesive traps. We identified a 7TM GPCR receptor responsible for ascaroside “smelling” and found that it signals from the cytoplasmic membrane but also localizes to mitochondria where it stimulates respiration (2). Another interesting question concerns the overcome of the defense reactions of the nematode. It was thought that the fungus secretes lytic enzymes and digests the nematode (3). However, we discovered that the fungus secretes in addition different small proteins or peptides, and those peptides are used to penetrate the nematode, paralyze it or reprogram nematode cells (4, 5). This shows that the interaction is a very sophisticated system which reflects more than 400 Million years of co-evolution. I am going to present results for several new effector proteins which target different processes in *C. elegans*.

(1) Wernet, V., and Fischer, R. (2023) Establishment of Arthrobotrys flagrans as biocontrol agent against the root pathogenic nematode Xiphinema index. Environ. Microbiol. 25: 283-293. (2) Hu, X., Hoffmann, D., Wang, M., Schuhmacher, L., Stroe, M.C., Schreckenberger, B., Elstner, M., and Fischer, R. (2024) A dual-function G-protein coupled receptor activates mitochondria and reprograms fungal cells to form adhesive traps for nematode hunting. Nat. Microbiol., 9:1752-1763. (3) Emser, J., Seidler, L., Kovacevic, E., Yu, K., Rudolf, T., Wohlmann, E., and Fischer, R. (2025) The Egh16-like virulence factor TrsA of the nematode-trapping fungus Arthrobotrys flagrans facilitates intrusion into its host Caenorhabditis elegans. PLoS Pathog, in press. (4) Fischer, R., and Requena, N. (2022) Small secreted proteins as virulence factors in nematode-trapping fungi. T. Microbiol. 30: 616-617. (5) Emser, J., Wernet, N., Hetzer, B., Wohlmann, E. & Fischer, R. (2024) The cysteine-rich virulence factor NipA of Arthrobotrys flagran interferes with cuticle integrity of Caenorhabditis elegans. Nat. Commun., 15:5795.

Mechanisms involved in fungal hijacking of insect host behaviors

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The evolutionary arms race between *Ophiocordyceps* fungi and their carpenter ant hosts has culminated in extended behavioral phenotypes that benefit fungal survival, spore development and dispersal. The precise mechanisms involved in these and other behavioral manipulations remain unknown. To unravel these mechanisms, we have developed the *Ophiocordyceps*-ant interaction into an integrative model system to study parasitic behavioral manipulation in greater detail. By combining fungal culturing and lab infections with quantitative behavioral assays and multi-omics approaches, we have proposed several comprehensive mechanistic hypotheses about the fungal effectors and ant targets involved. To test these hypotheses, we are currently, for the first time in this model, integrating functional genetics assays to determine the function of presumed fungal "manipulation" effectors, the host behaviors they elicit, and the targeted host pathways underlying these phenotypes. Our efforts include the development of novel *Ophiocordyceps* genetics tools and the adoption of established model organisms to test our hypotheses and produce candidate effectors heterologously. In this lecture, I will specifically focus on our most recent interdisciplinary efforts to unravel the function of a novel secreted cysteine-rich peptide, which is highly upregulated by *Ophiocordyceps* during the final, most striking stages of host manipulation. Using the powerful genetics toolbox of *C. elegans*, we found that this peptide binds to a well-conserved scramblase protein that is involved in neurotransmitter release at neuromuscular junctions. Introducing the scramblase-binding peptide as well as knock-downs of scramblase in nematodes and ants both resulted in measurably altered odor-related behaviors. These behaviors are important for normal ant functioning, communication and social immune detection. Taken together, this study is among the first to advance our mechanistic understanding of fungal hijacking of insect host behavior through effector production.

Mechanosensation in predator-prey interactions between trapping fungi and nematodes

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Predator-prey interactions drive evolutionary arms races across the Tree of Life, with animals evolving diverse behavioral adaptations to evade predation. Using *Caenorhabditis elegans* and the nematode-trapping fungus *Arthrobotrys oligospora* as genetic models, we investigated the role of mechanosensation in shaping behavioral and physiological responses during predator-prey encounters. Upon contact with fungal traps, *C. elegans* rapidly enters a quiescent state characterized by an immediate cessation of pharyngeal pumping and locomotion. This response is mediated by the activation of the sleep-promoting neurons ALA (Anterior Lateral neuron A) and RIS (Ring Interneuron S). We further show that mechanosensory input and epidermal growth factor receptor (EGFR) signaling are essential for these behavioral changes, revealing how prey neurophysiology is tuned to detect and respond to mechanical stress induced by fungal attack. Conversely, successful prey capture by *A. oligospora* triggers a distinct infection program: the fungus forms a penetration tube at the trap-nematode interface, followed by development of an infection bulb from which invasive hyphae emerge to colonize and digest the nematode. To test whether mechanosensation also regulates this fungal transition, we used live-cell imaging to monitor infection bulb formation in traps capturing either motile or non-motile nematodes. Infection bulbs consistently formed when the fungus captured live, motile prey, but were rarely observed following capture of non-motile nematodes, suggesting that mechanical stimulation is required to initiate fungal infection structures. We have identified candidate mechanosensitive channels in the *A. oligospora* genome and are currently investigating their roles in fungal mechanosensation and predation.

Unraveling cell cycle regulation to understand *Magnaporthe oryzae* pathogenesis

Miriam Oses-Ruiz

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The filamentous fungus *Magnaporthe oryzae* causes a devastating disease in cultivated rice that destroys enough rice to feed 60 million people in the world. *M. oryzae* it is widely known for being highly variable, undergoing host jumps and causing new outbreaks, constituting a threat to global food security. *M. oryzae* causes infection thanks to the formation of a specialized cell called the appressorium. The appressorium develops from a three-celled spore upon contact with the surface of a leaf. During appressorium development the apical cell of the spore undergoes a round of mitosis, whilst the other two undergo autophagy-mediated cell death. It is unknown how the mitotic cell cycle operates coordinated with appressorium development. In the lab we are dissecting the molecular mechanisms associated to cell cycle- to understand how it is intertwined with other pathways to drive appressorium development and infection. We use a combinatory approach of phosphoproteomics, cell biology, transcriptomics and genetics for it.

Transposons drive rapid adaptation in a clonally evolving fungal pathogen

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Many filamentous pathogens reproduce predominantly as clones. Genomes of asexually reproducing fungal pathogens are often compartmentalized into conserved core and lineage specific accessory regions (ARs), which are enriched in transposable elements (TEs). ARs and TEs are thought to drive pathogen adaptation, but direct experimental evidence is sparse. Using an evolve and re-sequence approach, we found that serial passaging of the cross-kingdom fungal pathogen *Fusarium oxysporum* through different conditions rapidly increased fitness under the selection condition. TE insertions were the predominant type of mutations detected in the evolved lines, with a single non-autonomous hAT-type TE accounting for 63% of the total events. TEs inserted preferentially at sites of histone H3 lysine 27 trimethylation, a hallmark of ARs. We found that recurrent evolutionary trajectories selected during plate adaptation led to increased proliferation at the cost of reduced virulence. Unexpectedly, adaptive mutations in genes located on ARs strongly impacted core functions such as growth, development, quorum sensing and virulence. Together, these results show that TEs and ARs function as drivers of rapid adaptation in this important fungal pathogen.

Session 4: Fungal Societal Interactions-Microbiomes

Shaping of bacterial-fungal interactions and the composition of microbiomes by natural products

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An important scientific question concerns the identification of widespread universal communication molecules in nature that govern bacterial-fungal interactions across kingdoms and the structure and composition of microbiomes. Recently, we have discovered such molecules that are represented by microbial natural products, specifically ubiquitous bacterial arginine-derived polyketides. This was concluded from the discovery of an unprecedented tripartite interkingdom microbial consortium consisting of the bacterium *Streptomyces rapamycinicus* (or *S. iranensis*), the fungus Aspergillus nidulans and the green alga *Chlamydomonas reinhardtii* involving NPs. The streptomycete produces the arginoketide azalomycin F that triggers the expression of the otherwise silent ors gene cluster of A. nidulans resulting in the production of orsellinic acid and derivatives. In this way, the bacterium re-programs the epigenetic machinery of the fungus leading to acetylation of histones located in the ors gene promoters. Azalomycin F is also released in presence of *C. reinhardtii*. As a response, the alga swims to the mycelia of the fungus and is thereby protected from the toxic activity of azalomycin F. Furthermore, sublethal concentrations of azalomycin F trigger the formation of a protective multicellular structure by *C. reinhardtii*, which we named gloeocapsoid, suggesting that NPs may have contributed to the evolution of multicellularity. Together, the algae survive lethal NPs by forming a multicellular structure and of an alliance with a fungus. The ubiquitous distribution of biosynthesis gene clusters for the biosynthesis of arginine-derived polyketides in bacteria on all continents on earth except Antarctica and the ease with which we were able to isolate both arginoketide producers and fungal responders to the signal underlines the universality of this communication system. Arginoketides impact surrounding microorganisms both directly and indirectly, by inducing the production of fungal NPs that further influence the composition of microbial consortia.

PNAS 2009; 2011; eLife 2018; 2020; ISME J 2020; PNAS 2021; Nature Microbiology 2023; microLife 2024

Cell and Network Dynamics in Arbuscular Mycorrhizal Fungi

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Arbuscular mycorrhizal fungi (AMF) form extensive hyphal networks that play a critical role in ecosystem function. Despite their ecological importance and growing application in crop systems, the cellular and network-level processes underlying AMF development remain poorly understood. Here we explore network morphogenesis, highlighting the spatiotemporal dynamics that enable efficient exploration and trade with plant hosts. We then examine cytoplasmic flow patterns, lipid dynamics, nuclear behavior, and the spatial regulation of gene expression, which together support long-distance transport and metabolic coordination across the fungal network. Together, these novel views offer a systems-level perspective on AMF biology, linking intracellular processes to network functions advancing our fundamental understanding of these fungi.

The holobiont concept in arbuscular mycorrhizal fungi

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Since 400 million years ago Arbuscular Mycorrhizal Fungi (AMF) have accompanied the majority of land plants with a symbiotic alliance centered on nutrient exchanges. By forming an intimate association with plant roots, AMF provide several benefits to host plants including an improved mineral nutrition (especially phosphorus, P) and an increased tolerance to biotic and abiotic stresses. However, beside plants, AMF can also establish interactions with other organisms, a feature that can integrate and expand their functional traits (Duan et al. 2024). For example, bacterial communities living on the surface of extraradical hyphae of AMF can mineralize organic P (Wang et al. 2023), which is an activity not present in AMF, and thus significantly contribute to P acquisition and transfer across the soil bacteria-AMF-roots continuum. In addition to their external microbiota, many AMF possess endobacteria living inside hyphae and spores which modulate not only the fungal gene expression but also the plant metabolism (Venice et al. 2021). Lastly, several viral sequences were also described inside AMF, but their functional role is still undeciphered (Turina et al. 2018). All these additional genomes raise the level of the genetic complexity of AMF and of their regulatory processes. The case of the AMF *Gigaspora margarita* (BEG 34), that has been investigated in more detail so far, will be presented to discuss the holobiont concept in AMF.

Duan et al. 2024. Nat Rev Microbiol 22(12): 773-790. doi: 10.1038/s41579-024-01073-7 Turina et al. 2018. Environ Microbiol 20(6): 2012-2025. doi: 10.1111/1462-2920.14060 Venice et al. 2021. Plant J. 108(6): 1547-1564. doi: 10.1111/tpj.15578 Wang et al. 2023. New Phytol 238(2): 859-873. doi: 10.1111/nph.18642

Reductive and divergent evolution of the DNA replication and repair machinery in AM fungi

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The molecular machinery for replicating and repairing DNA accurately is critically important and highly conserved across the Tree of Life. In organisms involved in evolutionary arms races, mutations can however provide genetic diversity that is advantageous for survival. Here, we report the unexpected loss of components of DNA replication in the Glomeromycotina subphylum. DNA polymerase genes typically essential for genome stability are lost in a lineage-specific manner which strikingly mirrors the speciation pattern of AM fungi. We find that catalytic and non-catalytic subunits of replicative and translesion synthesis polymerases are co-eliminated, reflecting the physical and functional interactions described in other eukaryotes. For up to 360 My, the Glomeraceae family has lived with the most reduced replisome, which correlates with their higher speciation rate, range expansion and host benefit [1, 2]. We detect lineage-specific variation in genome-wide mutation rates, showing that DNA polymerase gene losses correlate with increased genetic variation. We uncover a highly divergent, yet structurally conserved cell cycle checkpoint in AM fungi, which could reflect mechanistic adjustments to replisome reduction. Our findings suggest the existence of unconventional replication and replication-associated DNA repair mechanisms, and prompts to comparatively examine cell cycle activity and regulation across AM fungal taxa. We propose that the evolvability of DNA replication fidelity and alternative modes of cell cycle regulation promoted adaptive evolution of these intracellular obligate symbionts.

[1] Sale, V., et al., Ancient lineages of arbuscular mycorrhizal fungi provide little plant benefit. Mycorrhiza, 2021. 31(5): p. 559-576. [2] Perez-Lamarque, B., et al., Analysing diversification dynamics using barcoding data: The case of an obligate mycorrhizal symbiont. Mol Ecol, 2022. 31(12): p. 3496-3512.

The secret life of nematode-trapping fungi

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Nematode trapping fungi are soil-borne microbes that play an important ecological role in controlling nematode numbers in soils and thus reducing infection in animals and plants. The biology of some of these fungi and the molecular mechanisms behind trap formation and nematode killing have received significant attention in the last decade, and key advances in the interaction process and on the sophisticated methods used to lure and control nematodes have been made. Here we show a novel aspect in the life cycle of the model NTF *Arthrobotrys flagrans* and *A. oligospora*, which grow as endophytes in the roots of many plants inducing important changes in root development. Furthermore, they are able to form traps on the root surface and catch nematodes while living on plants. This tripartite interaction opens new avenues of research and represents a potential strategy not only to control populations of plant parasitic nematodes in soil but to avoid plant penetration.

DAY 4 - Session 5: Fungal Virulence and Evolution

Antifungal resistance: A one health challenge

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The azole fungicides are used extensively as plant protection products (PPPs) with over 100 million tones being applied to crops in the EU over the last 10 years. This sustained use has driven a cross-continent expansion of azole resistant Aspergillus fumigatus that is impacting clinical outcomes. Mortality rates for individuals infected with a drug-resistant isolate increase from 40% to around 65% with some patients fairing even worse. Recently a new class of clinical antifungal has been developed that can treat azole resistant infections. However, just as this compound, olorofim, is about to emerge on the market, a fungicide, ipflufenoquin, that has the same mechanism of action, has been approved for use in the USA. We have shown that ipflufenoquin can drive olorofim resistance in A. fumigatus raising concerns for the sustainability of this new clinical treatment. There is a critical need for policy makers to act. The Environmental Protection Agency in the USA have developed a framework that considers the risk posed by novel pesticides on clinical fungicides and antibacterials and an EU report on the impact of azole use has led the development of a roadmap for action. Here i discuss our latest data and how it may inform policy change.

Novel molecular insights in immunopathogenesis of invasive mold infections

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Opportunistic molds, mainly *Aspergillus* spp. and the Mucorales, are emerging human pathogens. Physiologically, professional phagocytes, mainly alveolar macrophages (AMs) and recruited neutrophils, confer sterilizing immunity against molds. A rapidly expanding group of immunocompromised patients with incompletely understood immunometabolic defects in phagocytes develop invasive mold infections (IMIs). IMIs are associated with inappropriately high mortality rates of 50%, limited therapeutic options and incompletely understood pathogenesis. There is an unmet need to dissect the early molecular events in host-fungal interplay shaping the infection outcome in order to design novel host-directed therapeutic strategies and improve IMIs outcome. Phagocytosis is a fundamental host defense mechanism employed by AMs to eliminate inhaled fungal spores on a daily basis. The molecular pathways regulating phagosome biogenesis and killing of phagocytosed fungal spores following their uptake by AMs are incompletely characterized at the molecular level. Of interest, AMs mount specialized host defense pathways against Aspergillus vs Mucorales, which are shaped by the dynamic changes in cell wall composition of the fungal cell wall surface inside the phagosome. Furthermore, metabolic reprogramming of AMs in response to changes of fungal cell wall composition shapes unique effector mechanism to resolve the infection. During my talk I will highlight how the removal of cell wall melanin on *Aspergillus* conidia activates LC3 associated phagocytosis (LAP), a specialized autophagy pathway that orchestrates a balanced inflammatory response and promotes phagolysosome fusion and fungal killing by AMs. I will also highlight novel pathogenetic mechanisms of LAP blockade as a result of immune deactivation in sepsis-induced aspergillosis. Furthermore, I will demonstrate how prolonged LAP blockade induced by surface retention of melanin in phagocytosed Mucorales spores activates a unique immunometabolic host defense mechanism to inhibit fungal growth inside AMs. Overall, harnessing metabolic host defense pathways can be exploited as a novel therapeutic strategy against IMIs

Discovery of the molecular bases for cryptococcal tolerance to host CO_2

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Cryptococcus neoformans is a ubiquitous free-living soil yeast and opportunistic pathogen that causes ~223,100 cases of cryptococcal meningitis per year, killing over 180,000 people. This fungus is responsible for 19% of deaths in AIDS patients. The pathogenicity of this environmental fungus relies on its adaptation to the host conditions. An important difference between its natural environment and the mammalian host is the concentration of CO_2. CO_2 levels in the host fluctuate around 5%, which is ~125-fold higher than in ambient air (~0.04%). We recently found that while clinical isolates are tolerant to host levels of CO_2, many environmental isolates are CO_2-sensitive and virulence-attenuated in animal models. The molecular basis responsible for cryptococcal adaptation to high levels of CO_2 was unknown. In the past few years, we utilized multiple independent approaches to identify the genetic bases responsible for CO_2 tolerance, including quantitative trait loci (QTL) mapping of progeny derived from a cross between a clinical and an environmental isolate, experimental evolution of CO_2-sensitive natural isolates, and genetic screen of gene deletion mutants in a reference strain. Our findings indicate that (1) CO_2 tolerance is critical for cryptococcal pathogenesis, (2) CO_2 tolerance can be evolved in vitro and during infection in animals and humans, (3) the regulation of thermo-tolerance and CO_2 tolerance is intertwined but distinct, and (4) post-transcriptional regulation is critical for cryptococcal adaption to CO_2. These findings highlight the underappreciated role of *C. neoformans* tolerance to host CO_2 levels and its importance in the ability of an opportunistic environmental pathogen to cause disease.

Quantitative dynamics of the vaginal mycobiome reveal fungal burden and cross-kingdom interactions underlying mucosal infections in pregnancy

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Fungi are common colonizers in the vaginal tract and may lead to vaginitis under certain predisposing conditions, such as pregnancy. However, little is known about the vaginal fungal community (i.e., the mycobiome) and its dynamics in fungal vaginitis. To address this knowledge gap, we conducted a longitudinal study of the vaginal mycobiome in 715 pregnant women. Using ITS2 amplicon sequencing, we classified the vaginal mycobiome into six distinct community state types. We showed that transitions between asymptomatic colonization and symptomatic vaginitis caused by *Candida albicans*—but not by other fungal species—were marked by a significant shift in absolute fungal burden. Whole-genome analyses of fungal isolates from paired vaginal and anal samples further revealed that in some cases, fungal vaginitis could arise from anal-to-vaginal transmission of the pathogen. Interestingly, we also found that women with vaginal fungal colonization in the first trimester had a higher risk of developing bacterial vaginosis in the second or third trimester, even when their initial vaginal microbiota was dominated by *Lactobacillus* species. Collectively, our study highlights the critical role of the vaginal mycobiome in mucosal infections during pregnancy.

Plenary Lecture 2

Evolution of sexual reproduction: a view from the fungal kingdom

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Our studies have revealed two novel modes of sexual reproduction. During unisexual reproduction isolates can undergo sexual reproduction either entirely on their own without a mating partner or following fusion with an isolate of the same mating type. Unisexual reproduction enables production of either clonal or admixed F1 progeny and also illustrates the capacity for sexual reproduction to generate diversity de novo. During pseudosexual reproduction, two isolates of opposite mating type fuse, but one of the two nuclei is lost at hyphal branches of the dikaryon, and the solo remaining nucleus goes on to undergo meiosis, resulting in F1 progeny that are clonal with the nuclear genotype of one parent yet can inherit the mitochondrial genome from the opposite parent. These findings on fungal sexual reproduction reveal parallels in modes of selfing sexual reproduction shared with plants and animals. Our studies also provide insights into the evolutionary trajectory of sexual reproduction in eukaryotes and may provide insights into how sex first evolved. Our findings on unisexual reproduction suggest that there may have been an evolutionary epoche in which there was sexual reproduction before there were mating types or sexes.

Giant transposons as a natural mechanism of eukaryotic horizontal gene transfer

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Horizontal gene transfer (HGT) disseminates genetic information between species and is a powerful mechanism of adaptation. Yet, we know little about its underlying drivers in eukaryotes. Giant Starship transposons have been implicated as agents of fungal HGT, providing an unprecedented opportunity to reveal the evolutionary parameters behind this process. These elements are unique in that they not only incorporate the genetic machinery for their own movement, but also mobilize a vast diversity of fungal genes. Through a combination of comparative genomic and molecular biology approaches, we have demonstrated that Starships are mobile within and between fungal genomes. We observe the recurrent transfer of Starships with adaptive cargo, such as genes for heavy metal resistance, between species including those in distinct taxonomic orders, showing how these elements frequently mediate rapid adaptation. Furthermore, we now have experimental validation that Starships can transfer between species under laboratory conditions. Our results demonstrate the key role Starships play in mediating HGT in fungi, elevating the importance of this process in eukaryotic biology.

Factors facilitating *Candida albicans* pathogenicity

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Candida albicans is one of four "critical priority" fungal pathogens defined by the World Health Organization (WHO). Intriguingly, C. albicans is a natural commensal organism thriving in symbiosis with other components of the human microflora. However, in immunocompromised individuals, *C. albicans* is capable of causing a spectrum of pathologies ranging from superficial mycoses to life-threatening systemic infections. The ability of *C. albicans* cells to engage the genetic programs resulting in filamentous hyphal growth is critical for virulence. Hyphal growth facilitates invasion of host tissues and escape from phagosomes of engulfing macrophages. Interestingly, the latter occurs in a microenvironment with a restricted nutrient content, which represents a considerable bioenergetic challenge. We have recently found that proline catabolism provides the metabolic energy to initiate and maintain hyphal growth of phagocytized *C. albicans* cells. Proline-induced filamentous growth is coupled to its catabolism in mitochondria; a process linked to the generation of ATP. Accordingly, filamentation is sensitive to glucose availability in a manner consistent with glucose repressed mitochondrial gene expression. Using a combination of time lapse microscopy, immunocytochemistry, and biochemistry, we have defined key temporal aspects of proline catabolism in *C. albicans* that are requisite for invasive growth on collagen and escape from macrophages. Applying intravital two-photon microscopy (IV2PM), we have successfully visualized *C. albicans* colonizing kidneys in living hosts. Our findings reveal that single fungal cells colonize peritubular sites and form invasive hyphae. Notably, proline catabolism is required for hyphal morphogenesis in situ, as mutant cells unable to metabolize proline fail to form hyphae in kidneys. These results provide insights regarding nutrient availability and bioenergetics underlying filamentous growth within infected hosts. Given that *Candida* infections are common among immune compromised individuals, proline derived from host-driven degradative processes may be key to understanding fungal virulence.

Session 6: New Tools Final Questions and Conclusions

Tracing hyphal scale metabolic dynamics in soil fungi via Raman microspectroscopy

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Metabolic processes in soil fungi are essential determinants in terrestrial biogeochemical cycles. Better understanding of the fungal metabolic dynamics and fitness under changing environmental conditions is thus crucial for predicting their response and effect on overall ecosystem functioning. While studies on fungal growth and respiration are performed in bulk, in filamentous fungi in particular, individual hyphae in a mycelium are heterogeneous in their function and metabolic activity levels, responding to the conditions in their immediate microenvironment that may trigger specific biochemical reactions. Therefore, fundamental mechanisms behind can only be fully understood if we also study them at microscale. With lack of currently available approaches for single-cell scale real time measurements, we aim to develop new protocols for the use of advanced microspectroscopy, often in combination with microfluidic technology-based soil chips, for in situ monitoring of nutrient substrate uptake for estimating fungal growth and metabolic activity rates and tracing of secreted metabolites produced in response to biotic (e.g. microbial interactions) and abiotic (e.g. temperature, pollutants) environmental stimuli in both laboratory-grown soil fungi and microbial communities from real soil. Specifically, we show that stable-isotope probing using Raman microspectroscopy can be used to trace glucose metabolism rates and response to excess copper stress in hyphae of laboratory grown soil fungus *Psilocybe subviscida*. Investigation of biochemical signatures in the Raman spectra also indicate copper-impaired mitochondrial function. We further show that the approach can be used for analyzing both fungal and bacterial fitness under varying frequencies of freeze-thaw cycles in natural microbial community from arctic biological soil crusts. Finally, we are exploring the use of surface-enhanced Raman spectroscopy (SERS) for detection and characterization of secondary metabolites at single-cell scale for in vivo monitoring of chemical signaling agents between interacting soil fungi.

Selected Talks

Regulation of mitochondrial and actin dynamics by reactive oxygen species

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Our research has advanced the understanding of reactive oxygen species (ROS) as critical regulators in developmental processes and cellular physiology. Focusing on p38-type SakA stress MAPK pathway in *Aspergillus nidulans*, we uncovered multifaceted roles for H2O2 in governing mitochondrial division. Specifically, H_2O_2 induces widespread mitochondrial division, dependent on the dynamin-like protein DnmA and its receptor FisA (analogous to Drp1 and Fis1 in animal cells) (1, 2). Absence of mitochondrial division has moderate effects on respiration but severely disrupts polar growth and development while elevating mitochondrial ROS levels. H_2O_2 triggers mitochondrial constriction before division, involving progressive depolarization, Ca^{2+} involvement, and endoplasmic reticulum-mitochondria interactions. H_2O_2 promotes DnmA aggregation (indicative of oligomerization), as well as actin depolymerization and reorganization, suggesting redox-mediated regulation of actin dynamics (3, 4). Substitutions at DnmA C450S and C776S critically impair mitochondrial and peroxisomal division without altering DnmA distribution. These mutations exert opposing effects on oligomerization in FisA-deficient contexts. Molecular dynamics simulations reveal that C450S/C776S substitutions and C450 oxidation alter DnmA's signaling element-stalk domain angle, solvent accessibility, and salt-bridge networks. The high probability of C450 oxidation by H_2O_2 suggests this modification primes DnmA's multimeric assembly (4). We posit that H_2O_2 coordinates mitochondrial constriction and DnmA oligomerization to regulate division. Here, C450 oxidation would act as a critical priming event enabling productive self-assembly for mitochondrial scission. Current work investigates dynamin VpsA's role in mitochondrial and peroxisomal division.

Our current work is supported by PAPIIT-UNAM grant IN205125 and Wellcome Trust grant 323477/Z/24/Z.
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The Unfolded Protein Response is involved in antibacterial defense in fungi.

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Saprophytic mushroom *Coprinopsis cinerea* responds to antagonistic bacteria with the significant overexpression of genes encoding antibacterial effectors. One of the most highly induced molecules upon bacterial confrontation is the secreted lysozyme LYS1, which cleaves peptidoglycans in the bacterial cell wall (Kombrink et al., 2019). We recently discovered that the lys1 gene is also strongly induced by dithiothreitol (DTT), a reducing agent and inhibitor of protein folding in the endoplasmic reticulum (ER). DTT is a well-established elicitor of the unfolded protein response (UPR), a central signalling network required for ER homeostasis and conserved across eukaryotes. In fungi, the UPR is known to overlap with regulatory pathways controlling thermotolerance, antifungal drug resistance, virulence, and pathogenic development (Hartig and Heimel, 2020). Comparative transcriptomic analysis further revealed that DTT does not only induce lys1 but the entire set of genes previously identified as the core antibacterial defense response in C. cinerea (Kombrink et al., 2019). Our findings indicate a functional connection between antibacterial defense and the UPR, thus suggesting an additional role of the UPR in fungi besides those already characterized. To elucidate this connection, we first monitored the induction of both the UPR and antibacterial defense upon DTT or bacterial treatment at different time points. All treatments induce both responses 2 hours post inoculation. However, the UPR subsides in bacterial-treated *C. cinerea* following overnight incubation, pointing towards stimulus-specific temporal dynamics. We then inoculated C. cinerea with bacteria or DTT and the known UPR inhibitor 4μ8C to investigate the effect of the latter on lys1 expression. The significant downregulation of lys1 in the presence of 4μ8C suggests that the UPR might not only overlap with but also be required for antibacterial defense. We are now confirming this hypothesis by monitoring the induction of antibacterial defense genes in UPR-null mutants of *C. cinerea*.

Harting, R., & Heimel, K. (2020). Genetics of the unfolded protein response in fungi. Genetics and Biotechnology, 49-76. Kombrink, A., Tayyrov, A., Essig, A., Stöckli, M., Micheller, S., Hintze, J., ... & Künzler, M. (2019). Induction of antibacterial proteins and peptides in the coprophilous mushroom Coprinopsis cinerea in response to bacteria. The ISME journal, 13(3), 588-602.

A novel peptide-GPCR system senses plant entry to drive fungal infection

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Pathogen-host interactions often depend on the ability to sense host-derived signals, frequently mediated by nutrients or dedicated signaling molecules. One major way in which eukaryotic cells sense the environment is via G-Protein Coupled Receptors (GPCRs), which share a seven-transmembrane architecture and G-protein-mediated signaling. While mammalian GPCRs are well-characterized and widely targeted in pharmacology, fungal GPCRs remain poorly understood. To address this gap, we performed a structure-guided screen for putative GPCRs in the maize pathogen *Ustilago maydis*. This led to the identification of Gpe1, a previously uncharacterized GPCR essential for establishing a successful plant infection. Strikingly, Gpe1 is activated by a peptide encoded by the adjacent gene pit2. Upon host entry, the secreted Pit2 protein is processed by plant-derived proteases, releasing a mature peptide ligand that activates Gpe1. GPCR activation orchestrates infection-related gene expression and strongly promotes fungal proliferation. We show that the Gpe1/Pit2 pair is conserved across related fungi but exhibits co-evolutionary signatures preserving receptor-ligand specificity. While this GPCR/peptide system appears to be exclusive to smut fungi, our findings uncover a novel concept in GPCR biology - a host-triggered, peptide-based activation mechanism - with potential parallels in other fungal systems. This work provides a new framework for understanding host-responsive fungal adaptation and expands the landscape of GPCR-based sensing mechanisms.

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

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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) [1]. 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 diff use 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 [2] 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 using cryo-electron microscopy [3] 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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Molecular circuit between *Aspergillus nidulans* transcription factors MsnA and VelB to coordinate fungal stress and developmental responses

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Development and secondary metabolism of the filamentous fungus *Aspergillus nidulans* are tightly controlled by concerted actions of several master regulator transcription factors (TFs). The connection between fungal development and cellular stress response programs is often elusive. Here we show that the zinc finger TF MsnA, which controls salt-stress response, is a novel major regulator of fungal development. A molecular circuit among MsnA and the velvet domain regulator VelB was discovered, which mutually fosters the actions of both regulatory proteins during development. Chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq) and gene expression studies have revealed that MsnA controls the expression of several genes encoding key transcriptional regulators of asexual as well as sexual development. The double mutant of msnA with velB showed that both genes share an additive genetic relationship, under normal and salt stress conditions, with each protein to control distinct phenotypical features. In addition, MsnA directly and indirectly affects the synthesis of specific secondary metabolites relevant for fungal defense against other organisms and growth, in addition to salt-stress responses. Moreover, the expression of genes encoding the epigenetic regulators VapA, VipC and LaeA are also directly controlled by MsnA. The VapA-VipC-VapB methyltransferase signal transduction complex promotes asexual differentiation, while the VeA-VelB-LaeA complex balances light response, development and the secondary metabolism of the fungus. MsnA is therefore placed at a novel prominent position of the central regulatory network, which coordinates stress responses with the developmental and metabolic fate of the fungus.

Mechanisms of RNA interference in *Cryptococcus*

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RNA interference is a conserved mechanism of genome regulation in eukaryotes. It is mediated by small RNAs, which are typically generated from long double-stranded RNA by Dicer enzymes, and associate with Argonaute effector proteins to direct down-regulation of target RNAs based on sequence complementarity. In the human fungal pathogen *Cryptococcus*, RNAi plays an important role in silencing transposable elements, with loss of RNAi resulting in elevated rates of transposon-induced mutation that can contribute to adaptation and drug resistance. However, beyond the involvement of core RNAi pathway components (Dicer, Argonaute and RNA-dependent RNA polymerase), relatively little is known about the factors and mechanisms that underpin RNAi function and regulation in *Cryptococcus*. We have set out to dissect the molecular mechanisms of RNAi in *Cryptococcus*, focusing on *C. deneoformans*, which (in contrast to some other species) retains the full ancestral complement of core RNAi machinery including two Dicers and two Argonautes. We first explored the relative contributions of these core proteins, with sequencing of endogenous sRNAs in various mutant backgrounds revealing that Dcr1 and Dcr2 function largely redundantly to one another in sRNA production, while Ago1, but not Ago2, is also generally required for stable sRNA accumulation. Subsequent IP-MS analysis allowed us to identify several novel Argonaute interactors that represent candidate RNAi accessory proteins, including putative RNA helicase and methyltransferase enzymes. We are currently further characterizing the functions of these factors to understand their potential roles in RNAi function and/or regulation.

Quantitative single-molecule FISH reveals subcellular localization of flb family mRNA in the filamentous fungus *Aspergillus niger*

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Aspergillus niger is a filamentous fungus important in carbon cycling due to its ability to degrade diverse organic materials. During growth, it forms colonies with distinct regions, where peripheral zones show enhanced growth and protein secretion. While colony heterogeneity is well described, it is unclear whether spatial organization arises from localized gene expression at the hyphal level. To investigate this, we analyzed the spatial distribution of Flb proteins and their mRNAs in *A. niger* using reporter proteins and single-molecule FISH. The flb gene family (flbA–flbE) regulates growth, sporulation, and secretion. FlbA and its mRNA were evenly distributed, but FlbB–E showed distinct spatial patterns. Notably, flbD mRNA localized at hyphal tips, while its protein was nuclear throughout the hyphae. This mRNA localization was disrupted in ΔflbE and Δrrm4, reducing nuclear FlbD levels. These findings suggest that spatial mRNA localization affects protein distribution, possibly contributing to A. niger's growth and colony organization.

Diversity and regulation of carbon metabolism in filamentous fungi

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Research into central carbon metabolism in fungi has a long history and resulted in the identification of genes for most pathways. However, in recent years omics-driven studies together with modern genetic methodologies have revealed a level of complexity and diversity that challenges the relatively simple pathway organization that was previously assumed. Detailed studies in the ascomycete *Aspergillus niger* revealed the involvement of multiple enzymes in many of the steps of sugar metabolism. However, these enzymes have distinct biochemical properties while their corresponding genes have distinct expression profiles. Expansion of these studies to other fungi revealed a high diversity of the number of paralogs of these metabolic genes as well as the recruitment of unrelated genes for specific pathways, suggesting diverse evolution of these pathways in fungi. These evolutionary differences can also be observed at the level of regulation of the expression of these metabolic genes. The set of regulators present in ascomycete genomes is only partially conserved, as is the set of genes each regulator controls. Interestingly, a clear case of parallel evolution was observed for the L-arabinose responsive transcriptional activators AraR (Eurotiomycetes) and Ara1 (Sordariomycetes). Their low sequence similarity indicates that they have not evolved from a common ancestor, yet their roles in the carbon regulatory network are highly similar. The presentation will provide an update on the current knowledge of central carbon metabolism using recent data to exemplify the complexity and diversity among ascomycete fungi.

Endosomal mRNA transport and cell wall remodeling

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Long-distance transport of mRNAs on motile endosomes is a conserved strategy used by highly polarized cells, from fungal hyphae to neurons, to control gene expression in time and space (1). An open key question is how cargo mRNAs are assembled into defined RNA regulons by the interplay of RNA-binding proteins forming higher-order transport mRNPs. Among the best-studied model systems are infectious hyphae of the plant pathogenic fungus Ustilago maydis. The key endosomal transporter is the RNA-binding protein Rrm4, which contains three N-terminal RNA recognition motifs (RRMs) for RNA binding (2) and C-terminal MademoiseLLE domains for endosomal attachment (3). Prominent cargo mRNAs include all four septin mRNAs that are translated on the cytoplasmic surface of endosomes during transit. Translation products are assembled into heteromeric complexes for the formation of defined septin filaments at the growth pole. Other prominent mRNA cargos encode cell wall synthetic enzymes such as chitin synthases and glucan synthases for local cell wall synthesis at the growth apex (2,4) Progress on the molecular link between endosomal mRNA transport and cell wall remodeling will be presented.

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VapA/Scs2 sustains polarized growth in *Aspergillus nidulans* by maintaining AP-2-mediated apical endocytosis

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Growth of filamentous fungi is highly polarized requiring the coordinated apical delivery of cell wall components and plasma membrane (PM) material, primarily lipids and proteins, to hyphal tips via conventional vesicular secretion. Fungal growth also requires the tight coordination of exocytosis (secretion) with endocytosis and recycling of proteins and lipids, which occurs in a defined region behind the growing tip known as the endocytic collar. Here, we genetically characterized proteins tentatively implicated in the formation of endoplasmic reticulum–plasma membrane (ER–PM) contact sites, including Scs2/VAP, tricalbins and Ist2 homologues, in Aspergillus nidulans. We showed that among these proteins, only the single Scs2/VapA homologue is essential for normal fungal growth, and this requirement is due to the critical role of VapA in maintaining the polarized localization of apical cargoes, such as the lipid flippases DnfA and DnfB or the SNARE protein SynA. In ΔvapA mutants, these cargoes lose their polarized localization, a phenotype that correlates with the mislocalization of the AP-2 cargo adaptor complex, which is essential for the endocytosis and recycling of apical membrane components. Further analysis provides evidence linking the defect in apical cargo endocytosis observed in ΔvapA mutants to altered membrane lipid partitioning, suggesting that VapA contributes to lipid domain organization critical for cargo recycling. Strikingly, deletion of VapA does not impair the localization or endocytosis of non-polarized (subapical) plasma membrane transporters, indicating that the trafficking and biogenesis of polarized (apical) versus non-polarized (subapical) cargoes are differentially dependent on membrane lipid composition and domain-specific organization.

Elucidation of genetic diversity and pathogenicity mechanisms of the opportunistic human pathogen *Fusarium oxysporum*

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Fungi of the genus *Fusarium* can cause life-threatening infections in immunocompromised patients and serious, poorly treatable infections of the cornea, called keratitis, which primarily affect otherwise healthy contact lens wearers. *Fusarium* species (spp.) are extraordinary trans-kingdom pathogens, which can infect multiple hosts, i.e., plants, animals and humans. Recently, the World Health Organization published the fungal priority pathogens list with *Fusarium* spp. in the high priority group. Little is known about how this important group of pathogens is able to proliferate in the mammalian host and which virulence factors contribute to this. In our laboratory, we use *Fusarium oxysporum* as a model. For plant-pathogenic isolates of *F. oxysporum*, remarkable host specificity has been demonstrated. This host-pathogen interaction is mediated by specific virulence genes, which are found on accessory and mobile 'pathogenicity chromosomes'. Recent genome sequencing of two clinical *F. oxysporum* isolates revealed accessory chromosomes – not found in any of the plant pathogens – which we now demonstrated are mobile and which could provide an advantage in the mammalian host. In order to assess the species diversity in clinical, indoor and environmental samples, to be able to identify species with greater pathogenic potential, we are collecting and strain typing isolates of the *F. oxysporum* species complex, with a subset being subjected to whole-genome sequencing. Analyzing the presence of the above indicated accessory chromosomes, we could detect conserved shared sequences in clinical and indoor *F. oxysporum* isolates. Additionally, we are elucidating the contribution of these accessory chromosomes and therein encoded putative virulence factors to *F. oxysporum* pathogenicity, via loss-of-function mutants and in vitro infection models. Preliminary results reveal greater resistance to increased temperatures and macrophage-mediated acidification, i.e., conidial inactivation, of the clinical isolates compared to the plant-associated strains.

Chitosan, biocontrol fungi and plants: stress can be good to you

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Introduction. Chitosan inhibits and kills important human as well as plant fungal pathogens (ii) increasing intracellular reactive oxygen species (ROS) and permeabilizing their high-fluidity plasma membrane (1). Conversely, chitosan enhances some biocontrol fungi with low fluidity membrane and ß-1,3 glucan enriched cell wall. Fungi are an emerging alternative source from crustacean shell wastes for chitosan production (iii). Fungal chitosan production re-uses waste, is non-seasonal, low ash, no demineralization required and free from animal protein (2). CROPSAFE, a Horizon CBE-JU project, tests fungal chitosan for sustainable plant pest management. Knowledge of chitosan mode of action on fungi and their hosts will help chitosan development for agrobiotechnological and medical applications (1). Questions addressed. Exogenously applied chitosan enhances conidiation and virulence of fungal parasites of invertebrates (iv), such as (1) *Purpureocillium lilacinus*. This fungus showed the highest chitosan promotion of conidiation (6,000% vs. control) of all fungal cultures tested (3). Chitosan coacervates reduce naturally occurring plant pathogenic fungi and increase Purpureocillium in field soil (v) (4). The nematophagous fungus *Pochonia chlamydosporia* (Pc) is also chitosan resistant (1). Pc synthesizes and degrades chitosan when infecting nematode (RKN) eggs (1). Chitosan and RKN modifies the expression of Pc genes encoding glycoproteins, involved in ROS, carbohydrate, lipid metabolisms and transcription factors (1). Chitosan induces carbohydrate and protein hydrolases involved in egg-penetration. They are targets for improving Pc as a biocontrol agent. Pc also colonizes crop roots endophytically. Plants recognize Microbe Associated Molecular Patterns (MAMPs). Chitin is main a fungal MAMP. We hypothesize that Pc avoids plant recognition by chitin deacetylation (CDAs) and shielding (vi) (LysM effectors), both expressed during root colonization. Pc produces mostly hydrocarbon VOCs, some repel insect and nematode (vii) pests. Chitosan modulates Pc VOCs production (5). VOCs are part of externally Induced Plant Resistance (plant recruitment of pest parasitoids and predators). This “cry out for help” hypothesis should be tested in nematophagous fungi such as Pc Major conclusions (In bold (i), ii), iii), iv), v), vi), vii)).

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New solid-state NMR approaches to decipher the molecular organization of intact fungal and yeast cell wall at atomic resolution

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Most microbes possess a "cell surface", i.e. a critical surface architecture which can have several purposes: protection against the external environment, a mimicry strategy to evade host defenses, or a pathogenic weapon to damage host membranes. The ability to study such cell surfaces at a molecular level is of prime interest, to understand how pathogens can survive, proliferate and carry out their harmful action. Current state-of-the-art analytical approaches to study cell surface have two main drawbacks: (i) they are destructive or rely on the extraction of weakly-bound molecules, requiring chemical methods prior to the analysis. (ii) Because each component is quantified in isolation, the global organization and interplay between components is missing. Here we present our recent advances using solid-state NMR to investigate the molecular organization of fungal and yeast cell surface at atomic resolution: the architecture of Aspergillus fumigatus cell wall during its conidial morphotype transition (1) and the molecular distinction between cell wall and capsular polysaccharides in *Cryptococcus neoformans* (2). (1) Solid-state NMR molecular snapshots of *Aspergillus fumigatus* cell wall architecture during a conidial morphotype transition.

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The small GTPase SEY-1 regulates the morphology and function of the apical endoplasmic reticulum and mitochondria in *Neurospora crassa*

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This study investigates the roles of the dynamin-like GTPase SEY-1 and the DP1 family protein YOP-1 in shaping the smooth-tubular endoplasmic reticulum (ER) and modulating its functional interactions with mitochondria (M) in the filamentous fungus *Neurospora crassa.* Confocal laser scanning microscopy revealed that YOP-1-GFP and SEY-1-GFP localize to the apical, nucleus-free region of the hyphae (12–15 μm), where they co-localize with mitochondria (M) stained with Mitofluor Red 589 (PCC: 0.81 ± 0.01, n=15). Using ER-tracker Blue-White DPX and Mitofluor Red 589 overlapping distributions of tubular ER and M were observed at the hyphal apex in wild-type strains, including regions near the SPK. Mean-shift super resolution imaging and spinning disk confocal microscopy revealed that SEY-1 and YOP-1 form multiple, discrete ER-M contact sites. These contact sites were further corroborated by 3D electron microscopy reconstructions (~120 slices at 30 nm thickness) using focused ion beam-scanning electron microscopy (FIB-SEM) of cryofixed germ tubes (>150 μm). Mutant strains Δ*sey-1* and Δ*sey-1*/Δ*yop-1* displayed reduced ER-M contact sites and fewer apical ER tubules, along with altered mitochondrial morphology compared to wild-type and Δ*yop-1* strains. Calcium imaging with chlortetracycline and Fluo-4 revealed significantly reduced apical calcium levels in Δ*sey-1* and Δ*sey-1*/Δ*yop-1* strains. These mutants also exhibited hypersensitivity to the calcium chelators EGTA and BAPTA. Furthermore, mitochondrial respiration and oxidative stress assays revealed that the absence of SEY-1 conferred resistance to potassium cyanide (KCN), salicylhydroxamic acid (SHAM), H_2O_2, menadione, and plumbagin, consistent with lower levels of reactive oxygen species and polyphosphates. In conclusion, SEY-1 is critical for maintaining the architecture of the apical smooth ER network, regulating calcium homeostasis, and supporting mitochondrial morphology and respiratory functions. These results highlight the significance of ER-M interactions in the cellular physiology of filamentous fungi.

Cytoskeletal dynamics underlying intracellular organization in the entomopathogenic fungus *Metarhizium brunneum*

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The entomopathogenic fungus *Metarhizium brunneum* plays a key role in biological pest control and has also been recognized for its beneficial associations with plant roots. Central to its adaptability and infection success is its capacity for polarized growth and intracellular organization—processes tightly regulated by the cytoskeleton. To dissect the cytoskeletal components involved, this study explored the dynamics of both microtubules and actin filaments in living hyphae of *M. brunneum* using advanced fluorescence microscopy. The actin cytoskeleton was visualized through Lifeact-GFP expression, revealing discrete cortical patches along growing hyphae. Disruption of actin polymerization with Latrunculin B resulted in morphological abnormalities such as apical branching, increased hyphal diameter, impaired endocytosis, and altered cell wall integrity. Organelle tracking showed that actin destabilization significantly reduced the mobility of peroxisomes and lipid bodies, suggesting its direct role in intracellular trafficking and spatial organization. In parallel, the microtubule network was examined through heterologous expression of GFP-tagged β-tubulin. Microtubules formed long longitudinal bundles aligned with the growth axis. Depolymerization by Benomyl disrupted this arrangement, leading to impaired organelle distribution, disorganization of the apical vesicle crescent (AVC), decreased apical wall thickness, and altered mitochondrial positioning. These changes indicate that microtubules are essential for maintaining directionality of growth and organelle delivery. Together, these results provide an integrative view of cytoskeletal function in *M. brunneum*, highlighting the coordinated roles of actin and microtubules in polarized growth, endomembrane trafficking, and morphogenesis. Understanding these mechanisms advances our knowledge of fungal development and supports the rational improvement of fungal biocontrol strategies.

Combining OSMAC and untargeted metabolomics approaches reveal compounds exhibiting potential anti-HIV-1 activities from an endophytic fungus, *Penicillium rubens*, P03MB2

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The persistent burden of HIV-1 in Sub-Saharan Africa highlights the need for innovative treatments, as current antiretroviral therapies cannot eliminate latent proviral reservoirs and face challenges from multidrug-resistant strains. In this study, we explored the potential of *Penicillium rubens* P03MB2, an endophytic fungus from the *Albizia adianthifolia* plant, as a source of novel anti-HIV-1 compounds. The fungus was cultivated in various media (malt extract broth, oats, and rice), with oat media producing crude extracts with significant anti-HIV-1 activity. Active fractions were further analyzed using untargeted metabolomics and molecular networking, revealing clusters of secondary metabolites, including coumarins and other compounds linked to anti-HIV-1 activity. A virtual screening workflow assessed the binding affinities of these metabolites against HIV-1 protease. Additionally, molecular dynamics simulations analyzed ligand-protein complex stability. Binding free energy calculations identified diosgenin as a promising candidate, with a binding free energy of -34.59 kcal/mol, surpassing the co-crystallized ligand ORV. This research demonstrates the potential of secondary metabolites from *Penicillium rubens* as novel anti-HIV-1 agents, laying a foundation for further development of effective antiviral therapies.

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Cell dynamics investigation of the Mucoromycetes fungus, *Phycomyces blakesleeanus*, via nonlinear laser scanning microscopy

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Multiphoton nonlinear laser microscopy (NLM) is a powerful, non-invasive optical technique. Its unique advantages make it a valuable tool for a wide range of applications, from basic research to clinical diagnostics. These advantages include in vivo and label-free imaging, high-contrast images, high-resolution 3D imaging and reduced sample damage and photo-bleaching due to the use of infrared ultrafast pulsed lasers (1). Different NLM modalities provide the structural and functional imaging as well as selective manipulation of cells and intracellular structures. NLM have rarely been used to study filamentous fungi. Here we present a comprehensive application of NLM modalities to investigate the lipid droplets (LDs) and mitochondria dynamics under various environmental conditions. Exposure to 1 mM selenite and temperature shift of 3 degrees outside the optimal range had a clear effect on organelle dynamics, significantly increasing LD number and inducing the appearance of smaller LDs and predominantly tubular mitochondrial morphology. Nitrogen starvation also significantly increased the LD number after 4.5 hours, but induced the appearance of a new morphological type with semicircular tubules. Due to refractive index mismatch, between LDs and cytoplasm, third harmonic generation microscopy enables the visualization and quantified changes of quite small fungal LDs (2). In synergy with two-photon excitation fluorescence, NLM provides fundamental insights into organelle dynamics and physiological adaptation of hyphal metabolism. We also present femtosecond (fs) laser nanosurgery of the cell wall of filamentous fungi, which enables patch-clamp measurements on viable protoplasts released from different regions of hyphae (3). The high precision of fs laser ablation allowed us to cut a small portion of the cell wall without damaging the cell membrane (only a few micrometers away), while the ability to select an area for surgery allowed us to obtain information on the distribution of different ion channels along the hyphae.

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Accessory gene cluster confers high copper tolerance in the cross-kingdom pathogen *Fusarium oxysporum*

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Fungi colonize different niches, from soil and plants to humans, using homeostatic regulatory mechanisms to rapidly respond to changing conditions. Copper is an essential micronutrient which is extremely toxic at high concentrations. Since it is utilized as an agronomic fungicide and employed by macrophages as an antimicrobial weapon through its accumulation in the phagosome, fungi face selective pressure to increase copper tolerance both in agricultural and clinical settings. In fungi, copper excess is sensed by the conserved copper-binding transcription factor Ace1, which activates a battery of genes for copper detoxification [1]. Loss of Ace1 leads to severe copper sensitivity and attenuation of virulence in human pathogens [2]. Here, we surveyed copper tolerance in different isolates of *Fusarium oxysporum* (Fo), a fungal pathogen that causes vascular wilt disease in many important crops and opportunistic infections in humans [3]. Several Fo isolates were highly copper tolerant compared to other plant or human pathogens such as *Fusarium graminearum* or *Aspergillus fumigatus*. We found that high copper tolerance correlated with the presence of an accessory gene cluster encoding a multicopper oxidase and a copper-binding transcription factor homologous to Ace1. Spontaneous loss of the accessory chromosome carrying the cluster resulted in increased copper sensitivity, while introduction of either both genes or the multicopper oxidase gene alone was sufficient to confer high copper tolerance to strains naturally lacking the cluster. Our results demonstrate a key role of this accessory gene cluster in copper tolerance and reveal a genetic mechanism for rapid adaptation of Fo to high copper environments found in agricultural settings or in the human host.

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The Mycology Bioimaging Initiative

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Fungal pathogens present unique imaging challenges due to their non-adherent growth, dynamic morphologies, and the limited utility of fluorescent proteins in non-model organisms. These challenges are compounded by a critical shortage of fungal cell biologists in regions where fungal diseases are most prevalent. To address this disparity, the Mycology Bioimaging Initiative (MBI) was launched to build local capacity in fungal imaging and analysis, particularly in the Global South. In partnership with the Africa Microscopy Initiative, we delivered an intensive workshop for 15 early-career scientists working on pathogenic fungi. Participants received hands-on training in microscopy fundamentals, grant writing for imaging-based research, and open-source image analysis tools. The workshop emphasized practical skills and community building, enabling participants to generate and interpret high-quality imaging data in their home institutions. We benchmarked the impact of this training through a visiting scientist program, where an emerging pathogen (*Emergomyces africanus*) was captured with mammalian cells at high-resolution at Exeter. This data catalyzed the establishment of local imaging workflows upon their return. Our initiative also surveyed the distribution of fungal cell biologists and imaging technologies across Africa, highlighting the urgent need for regional expertise. Key technical challenges addressed include: adapting fluorescent protein tools for *Candida glabrata*, *Rhizopus microsporus*, and *Emergomyces africanus*; developing devices to track buoyant and filamentous fungal growth in 4D; and creating morphotype-specific image analysis pipelines for long-term time-lapse data. We also aim to initiate benchmarking of probes for use across fungal organisms. The MBI demonstrates that with targeted training and collaborative tool development, we can empower a new generation of fungal cell biologists where they are most needed—accelerating efforts to combat antifungal resistance and neglected fungal diseases globally.

Elucidating the role of de-N-glycosylation activity in filamentous fungal homeostasis

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N-deglycosylation is a critical process in the degradation of misfolded glycoproteins (1). Two key enzymes have been identified as playing a pivotal role in this process: peptide N-glycosidases (PNGases) and endo-β-N-acetylglucosaminidases (ENGases) (2). The present study investigates the role of PNGases and ENGases in the homeostasis of the model species Neurospora crassa, focused on the acidic PNGase (pngA) and cytosolic ENGase (gh18-10), respectively, given the previously demonstrated importance of the cytosolic ENGase in the filamentous ascomycete lifestyle (3, 4), while the role of pngA is unknown. The gh18-10 is an active de-glycosylating enzyme, involved in the ERAD process, in contrast to the pngA one. Phenotypic analysis demonstrated that the Δgh18-10 strain exhibits enhanced growth efficiency under variable stress conditions (ER, oxidative, osmotic and hypoxic), and impaired sexual reproduction. RNAseq analysis demonstrated that the deletion of the gh18-10 gene had a severe impact on N. crassa transcriptome, causing induction of plasma membrane and peroxisome genes and a N-acetyl-beta-D-glucosaminidase (NAGase), under ER stress conditions. Deletion of the NAGase gene increased resistance to tunicamycin, and negatively affects sexual reproduction as well, suggesting that NAGase could play an alternative role in N-deglycosylation activity, when ENGase is impaired. In summary, these results offer novel insights into the biological significance of N-deglycosylation in several aspects of fungal cell biology, and contributes to a more comprehensive understanding of glycoprotein homeostasis.

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Exploring the link between azole resistance and fitness in *Aspergillus fumigatus* by using quantitative trait locus (QTL) mapping

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Aspergillus fumigatus is an environmental fungus that can cause life-threatening or debilitating lung diseases. The number of A. fumigatus human infections resistant to the first-line treatment azole drugs have increased over last years which has been linked to the widespread use of azoles fungicides in agriculture. Azole resistance is primarily caused by variants in the gene coding the azole target Cyp51A, however alternative and complementary non-target variants are increasingly recognized as the cause for antifungal resistance which are potentially coupled with variants associated with increased fitness. Recently, it has been demonstrated that *A. fumigatus* harbors the highest known rate of meiotic crossovers during sexual reproduction generating a highly recombinant progeny which allows for fine mapping of traits of interest. Here, we have developed a high-throughput bulk QTL mapping approach to identify variants causing azole resistance in A. fumigatus. Azole sensitive A. fumigatus strains were crossed with environmental and clinical azole resistant strains and pooled F1 progenies were exposed to azoles. Using a custom QTL bioinformatic pipeline we were able to identify not only the variant conferring azole resistance but also complementary variants contributing to general fitness. This approach offers a great potential for identifying the underlying mechanism of complex polygenic traits such as antifungal resistance and fitness.

RNA-Binding protein SsdA shows dynamic localization and transport during hyphal growth in *Aspergillus nidulans*

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Filamentous fungi grow through highly polarized hyphal extension, requiring precise spatial coordination of vesicle fusion and cell wall material delivery at the growing tip. While mRNA transport along hyphae is documented in some fungal systems, the relationship between RNA regulation and polarized growth remains poorly understood. Using Aspergillus nidulans as a model organism, we characterized SsdA, a putative RNA-binding translational repressor homologous to *Saccharomyces cerevisiae* Ssd1 and *Neurospora crassa* gul-1. These proteins are regulated by the conserved morphogenesis-controlling kinase CotA/Cbk1/cot-1. Bioinformatic analysis using an RNA-binding motif that we characterized in S. cerevisiae shows that putative Ssd1/SsdA RNA targets are enriched for cell wall-related proteins across ascomycete fungi, including *Aspergillus*. Live-cell fluorescence microscopy demonstrates that *A. nidulans* SsdA moves in puncta along microtubules, in association with early endosomes. Movement is dependent on the endosomal hitchhiking-mediators PxdA and DipA. Notably, we observed that SsdA puncta are absent from growing hyphal tips. Mutating conserved phosphorylation sites in SsdA alters its localization, as does CotA inhibition, consistent with phosphorylation by the CotA controlling SsdA's RNA-binding or transport. Mutating conserved RNA-binding residues in SsdA abolishes its transport, suggesting that SsdA is hitchhiking on a larger endosomal-associated mRNA-protein complex. Our findings describe the dynamic localization and regulation of SsdA during hyphal growth. We hypothesize that SsdA might regulate tip-specific translation by repressing target mRNAs during transport and releasing this repression near hyphal tips, to co-ordinate synthesis of cell wall proteins with hyphal growth.

An uncharacterized domain within the N-terminal tail of histone H3 regulates the transcription of FLO1 via Cyc8.

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Yeast flocculation relies on cell surface flocculin proteins encoded by the sub-telomeric gene, FLO1 [1]. The expression of FLO1 is antagonistically regulated by the Tup1-Cyc8 repressor complex and the Swi-Snf co-activator complexes [2, 3]. The role of hyperacetylated N-terminal amino acid residues of histone H3 and H4 is well established in the transcription of FLO1 and other Tup1-Cyc8-regulated genes [4]. However, sub-domains within the tails of histone H3 and H4 are yet to be identified and the mechanism by which they regulate the FLO1 transcription is completely unexplored [5]. Upon screening of different H3 and H4 N-terminal stretch deletion mutants, we have identified a new region within the N-terminal tail of histone H3, H3Δ(17–24) regulating the transcription of FLO1 and FLO5. This N-terminal truncation mutant showed higher FLO1 and FLO5 expression by 68% and 41% respectively compared to wild-type H3. Further examination showed reduced Cyc8 and nucleosome occupancy in the upstream regulatory region of active flo1 in the H3Δ(17–24) mutant than H3 wild type cells. The findings also indicate that Hda1 assists in Cyc8 interaction at the active FLO1 template. Altogether, we demonstrate that Tup1 independent interaction of Cyc8 to the active FLO1 gene acts as a transcription limiting factor and Histone H3 N-terminal 17–24 stretch is essential for this interaction. In the absence of the 17–24 stretch, the Cyc8 restrictive effect is altered, resulting in over-expression of FLO1.

Posters

1. Investigation of spore surface proteins in *Mucor lusitanicus* to establish virulence factors

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Mucormycosis is a life-threatening fungal infection caused by Mucorales, predominantly affecting immunocompromised individuals and characterized by intrinsic resistance to most antifungals [1,2]. Spore coat proteins of the CotH family have been identified as key virulence factors: in *Rhizopus delemar*, CotH2 and CotH3 interact with host GRP78 to facilitate invasion [3,4]. Moreover, in *Mucor lusitanicus*, at least two of 17 cotH-like genes have been implicated in normal spore morphology and virulence in murine and invertebrate models [5]. Here, we applied plasmid-free CRISPR-Cas9 technology to generate five targeted cotH knockout mutants in *M. lusitanicus*: MS12+pyrG ΔcotH13 to ΔcotH17. We conducted in silico analyses, in vitro assays, and in vivo studies using *Drosophila melanogaster*, *Galleria mellonella*, and J774.2 macrophage models to assess the impact of each deletion. The ΔcotH13 mutant exhibited significantly reduced virulence in both insect infection models with altered hyphal branching, indicating CotH13's role in structural integrity and pathogenicity. The ΔcotH14 strain displayed accelerated germination at 4 h and increased susceptibility to macrophage-mediated killing, suggesting CotH14 helps coordinate germination timing with immune evasion. Stress assays revealed differential phenotypes: ΔcotH13 showed enhanced resistance to Calcofluor White and SDS, ΔcotH17 was more sensitive to osmotic stress, while XTT assays demonstrated altered mitochondrial activity in ΔcotH15 and ΔcotH17. Additionally, ΔcotH16 spores displayed reduced survival post–macrophage interaction. These findings delineate specialized functions among CotH paralogs in spore viability, stress resistance, and virulence. This work was supported by projects EKÖP 24 4 – SZTE 666, HUN REN 2001007, and TKP2021 EGA 28.

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2. Functional interaction between nucleosome-binding proteins in *Aspergillus nidulans*

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In addition to core histone proteins, compact chromatin structure and gene expression are also regulated by various chromatin-associated structural proteins. These include linker DNA-binding proteins (linker proteins), H1 histone and B-type High-Mobility Group (HMGB) proteins HmbA, HmbB, and HmbC in Aspergillus nidulans. These proteins bind to linker DNA, particularly at the entry and exit sites of nucleosomes, suggesting potential competition or functional overlap among them. Although not integral components of the nucleosome, linker proteins influence both static and dynamic chromatin organization through reversible post-translational modifications such as acetylation, methylation, and phosphorylation. Depending on chromatin context and interacting partners, they can either activate or repress gene transcription. Generally, architectural HMGB proteins promote gene expression, whereas H1 (encoded by hhoA) is repressive and contributes to heterochromatin formation. Despite extensive research into the function of H1 in A. nidulans, its physiological role has remained unclear. While deletions of hmbA and hmbB result in observable phenotypic changes, hmbCΔ mutants appear phenotypically indistinguishable from the wild type. Based on their shared DNA-binding regions, we hypothesized that one of the HMGB proteins (particularly HmbC) might serve a functionally redundant role with H1. To test this hypothesis, we generated all possible combinations of deletion mutants: single, double, triple, and a novel quadruple mutant (hmbAΔ hmbBΔ hmbCΔ hhoAΔ) lacking all known linker proteins. The hmbAΔ hmbBΔ hmbCΔ triple mutant is viable but exhibits severel developmental defects, supporting a collective role for HMGB proteins. The additional deletion of hhoA in this background provided a unique opportunity to test whether H1 plays a compensatory or non-redundant role. These strains underwent detailed phenotypic and molecular analyses, including assessments of growth, stress tolerance, and secondary metabolite production. This study offers a comprehensive framework to explore linker protein cooperation and may uncover a previously unrecognized biological function of H1 in fungal chromatin regulation.

3. Deciphering the molecular mechanisms involved in the sporulation, the DHN-melanin pathway and the biosynthesis of Naphto-Gamma-Pyrones (NGPs) by *Aspergillus tubingensis* G131

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Consumers increasingly seek eco-friendly products, prompting researchers to explore microorganisms to produce bioactive industrial compounds. Our work focuses on *Aspergillus tubingensis* G131 a black filamentous fungus belonging to the section Nigri (1), able to produce NGPs, secondary metabolites known for their antioxidant capacity (2). NGPs are complex molecules whose biosynthetic pathways have not yet been fully elucidated. A previous study showed that the DHN-melanin and NGPs biosynthetic pathways are linked through a common precursor, YWA1, synthetized by a polyketide synthase encoded by albA (3), (5). NGPs production has been reported to be associated with fungal sporulation following culture in a liquid medium under static conditions (4). To decipher molecular mechanisms involved in sporulation, NGPs production and the DHN-melanin pathway, we generated two mutants of *A. tubingensis* G131: ΔalbA and ΔaygA. The latter two genes encode proteins involved at distinct steps of the melanin biosynthesis pathway. Deletion of these genes didn't affect the radial fungal growth. On a solid medium, after 7 days of culture (28°C), the wild-type strain, ΔalbA and ΔaygA have black, brown and green spores respectively. Sporulation was only slightly impaired in the ΔalbA mutant. However, under static liquid culture conditions for 7 days at 28 °C, no NGPs were detected in the ΔalbA extracts, which also lacked antioxidant activity—unlike extracts from the wild-type strain. These findings confirm the role of albA in the biosynthesis of both melanin and NGPs. In contrast, the ΔaygA mutant was still able to produce NGPs, albeit at reduced levels compared to the wild-type, indicating that aygA contributes to NGPs production. To gain further insight into the molecular basis of these phenotypes, a transcriptomic analysis (RNA-seq) is currently underway, comparing the wild-type strain with the ΔalbA and ΔaygA mutants.

4. In situ solid-state NMR analysis of fungal cell wall carbohydrate biosynthesis using selective isotopic labeling.

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The fungal cell wall is a highly organized and dynamic structure, essential for viability, pathogenicity, and host interactions. Its polysaccharide components—mainly β-glucans and chitin—are synthesized via distinct metabolic pathways that can be modulated by environmental conditions. Understanding the biosynthetic routes and structural integration of these sugars under native conditions remains a major challenge due to the limitations of conventional extraction-based methods. In this study, we developed a metabolic labeling strategy using 13C- and 15N-enriched substrates to selectively probe the biosynthesis of key cell wall polysaccharides in Neurospora crassa and Candida albicans. By applying solid-state NMR spectroscopy directly on intact, labeled cells, we monitored the incorporation of isotopes into specific polysaccharide backbones in situ, avoiding chemical disruption of the cell wall. Different 13C-labeled carbon sources (e.g., glucose) were tested to trace carbon flux through central metabolic pathways into cell wall components. The impact of growth conditions on label incorporation efficiency and distribution was assessed, and isotopic scrambling phenomena were characterized by comparing spectral patterns across labeling strategies and species. Our results reveal differential labeling profiles and metabolic routing between fungal species, offering insights into the influence of metabolism on cell wall composition and organization. This work provides detailed roadmap of fungal cell wall metabolism and highlights biosynthetic targets for antifungal strategy development.

5. Tailoring fungal leucine-rich non-ribosomal peptides by mutasynthesis in a heterologous *Aspergillus* host

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The marine filamentous fungus *Asteromyces cruciatus* 763 (Pleosporaceae, Ascomycota), collected from the coastal waters of La Jolla (San Diego, USA), was identified as the natural producer of the cyclic non-ribosomal pentapeptide lajollamide A, whose structure was previously elucidated by HPLC-MS and NMR (Gulder et al., 2012). Although structurally intriguing, leucine-rich cyclic pentapeptides like lajollamide A remain largely underexplored for their antimicrobial properties. Given its weak but promising antibacterial activity, this study aimed to establish a heterologous production platform for lajollamide A and its derivatization via precursor-directed biosynthesis (PDB) in *Aspergillus*. Genome mining in *A. cruciatus* 763 revealed a putative biosynthetic gene cluster (BGC) encoding a single non-ribosomal peptide synthetase (NRPS), predicted to assemble lajollamide A from L-leucine (3x), N-methyl-L-leucine (1x) and L-valine (1x). In order to achieve efficient lajollamide A production we used the model fungus Aspergillus nidulans and cloned the nrps gene from *A. cruciatus* 763 under the strong constitutive tef1 promoter and introduced it into the auxotrophic GR5 strain using PEG-mediated protoplast transformation (Tilburn et al., 1983; Yelton et al., 1984) This resulted in prototrophic transformants generated by multiple random ectopic integration of the introduced plasmid-DNA. Small-scale chemical screening of 50 transformants, analyzed by HPLC-MS and MS/MS confirmed lajollamide A production in 30% of the prototrophic strains along with the identification of a naturally occurring congener, demethyllajollamide. This successful reconstruction of the lajollamide A biosynthetic pathway in Aspergillus represents the first report of a heterologously expressed BGC from the genus Asteromyces and sets the foundation for further derivatization. Feeding various leucine analogues resulted in the biosynthesis of novel lajollamide derivatives highlighting broad substrate tolerance of all leucine-incorporating A-domains. Simultaneous incorporation of up to four leucine analogues led to the biosynthesis of chemically highly functionalized derivatives with presumably enhanced or altered antimicrobial activities.

6. Subcellular localization of the Cornichon Family Protein ERV-14 in the secretory pathway of *Neurospora crassa*

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Filamentous fungal growth requires continuous synthesis of cell wall and plasma membrane (PM) proteins. This process begins in the endoplasmic reticulum (ER), where newly synthesized proteins are incorporated into vesicles coated by the Coat Protein Complex II (COPII). These vesicles bud off from the ER membrane and follow the secretory pathway to their final destination. COPII vesicle formation involves complex activators, inner and outer coat proteins, and specific cargo adaptors, including transmembrane proteins of the Cornichon family. In Saccharomyces cerevisiae, Erv14p functions as a cargo adaptor for proteins such as Axl2p1, which is required for establishing polarized growth and axial budding. This study identified a putative ortholog of Erv14p in *Neurospora crassa* (NCU06922) and analyzed its subcellular localization in growing hyphae. ERV-14 was tagged with fluorescent proteins (GFP and mChFP), and its distribution was observed by confocal microscopy throughout the hyphae, with a prominent signal found near nuclei. Co-expression of ERV-14-GFP and dsRED-NCA-1, a SERCA-type ATPase localized to the perinuclear ER2, revealed partial co-localization near the nuclear envelope. Similarly, co-expression with CSE-7-mChFP, a cargo adaptor linked to the chitin synthase CHS-4, showed partial co-localization in specific endomembrane compartments. ERV-14-GFP also partially co-localized with mChFP-YPT-1, a Rab GTPase associated with early and late Golgi cisternae and the Spitzenkörper4. Finally, ERV-14 showed partial co-localization with putative COPII markers SEC-24 and LST-1. Altogether, these results suggest that ERV-14 transiently associates with COPII components at the ER, although it is likely trafficking with distinct subpopulations of COPII vesicles.

7. The reticulum endoplasmic under control: The critical role of Sey1 in growth development and chromosomal dynamics in the fungus *Podospora anserina*

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The endoplasmic reticulum (ER) is a dynamic organelle whose structural organization is essential for various cellular processes, including the progression of meiotic development. In this study, we investigated the role of Sey1 (an atlastin) protein in regulating ER morphology during both vegetative growth and sexual development in the model fungus *Podospora anserina*. We generated and characterized the Δsey1 mutant, which exhibited reduced vegetative growth and marked alterations in ER organization, as observed through fluorescence microscopy using SEC61::GFP and RTN1::GFP markers. Ring-like structures were detected instead of the typical branched ER network, and the signal failed to reach the hyphal apex. During sexual development, the Δsey1 mutant showed delayed perithecia formation and a high percentage of asci with an abnormal number of spores (1 to 3 instead of 4). Moreover, we observed defects in spindle positioning during meiosis, and genetic material was abnormally distributed. SEC61 and RTN1 markers revealed altered ER localization patterns during meiosis in the mutant. We also constructed double mutants of Δsey1 combined with deletions in genes encoding other ER-shaping proteins (YOP1, YOP2, and RTN1). The Δsey1Δyop1 double mutant displayed the most severe growth phenotype and further disorganization of the ER, suggesting synergistic effects between these membrane curvature regulators. Altogether, our results indicate that Sey1 plays a central role in ER architecture and its loss negatively impacts fungal growth, ER morphology, and sexual development in *P. anserina*. This study contributes new insights into how the ER regulates nuclear dynamics during meiosis through its structural remodeling.

8. Systematic functional profiling of transcription factor networks in *Candida auris*

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Candida auris, identified in 2009 as an emerging fungal pathogen, has been receiving much attention due to its resistance to multiple drugs, leading to notable fatality rates. Despite the risks posed by *C. auris* infection, the pathogenicity and virulence mechanisms of *C. auris* remain largely unknown. Transcription factors (TFs), pivotal regulators of gene transcription in response to external stimuli, exhibit significant evolutionary divergence among species. Studying TFs will enhance our understanding of the unique characteristics of *C. auris*; hence, we aim to elucidate the transcriptional networks of C. auris. Using the DeepTFactor and DNA-binding domain research, we identified 167 putative TF genes and constructed signature-tagged gene-deletion strains in clade I wild type (B8441) background for approximately 70% of TF genes. We investigated the in vitro phenotypes of gene deletion mutants under various stress conditions, including high temperature, antifungal drug, and cell membrane/wall stresses. Furthermore, we assessed the ability of these genes on the production of virulence factors, such as biofilm formation, secreted aspartyl protease activity, and morphogenetic transition. To enable large-scale virulence profiling, we will utilize a *Caenorhabditis elegans* infection model for high-throughput screening of *C. auris* TF mutants. In parallel, we will elucidate pathogenicity-related TFs with high-throughput signature-tagged mutagenesis (STM) murine virulence assay. In conclusion, this study aims to offer powerful insights into the understanding of the pan-drug resistant fungal pathogen and discover novel antifungal targets of *C. auris* through the analysis of its transcriptional networks.

9. Optimization of fermentation conditions and capsule formulation for enhanced bioactive compound delivery from medicinal mushrooms

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The enduring history of medicinal mushroom use in traditional healing practices, dating back to the Neolithic era, highlights their age-old recognition for nutritional and therapeutic benefits. These fungi exhibit a broad spectrum of potential health-promoting activities, including antimicrobial, antiviral, cytotoxic, immunomodulatory, anti-inflammatory, antioxidant, antiallergic, mood-stabilizing, lipid and glucose regulation, digestive support, and blood pressure modulation. This research focuses on four medicinal mushroom species: *Cordyceps militaris, Ganoderma lucidum, Hericium erinaceus*, and *Lentinula edodes*. The fungi were cultivated on a defined artificial media containing 4% carbon sources (glucose, fructose, galactose, sucrose, or starch) and 0.2% nitrogen sources (peptone, yeast extract, corn steep powder, ammonium nitrate, or potassium nitrate), supplemented with essential minerals and 2% agar. The study investigated the impact of varying carbon and nitrogen sources on mycelial growth and secondary metabolite production through submerged fermentation, with quantification via High-Performance Liquid Chromatography (HPLC). The proximate composition and antioxidant activity of fungal extracts were determined both individually and in combination. Response Surface Methodology (RSM) was utilized to optimize mycelial proliferation in relation to temperature, pH, and carbon-to-nitrogen ratio. Finally, a combined capsule formulation of all fungal extracts was developed, aiming to provide a synergistic health boost by maximizing the benefits of diverse secondary metabolites. Encapsulation offers convenient and precise dosing, masks unpleasant taste, improves absorption, ensures quality, extends shelf life, and enables targeted release, potentially offering advantages over traditional tea, coffee, or powder formulations. Intensive scientific research could pave the way for the mainstream adoption of medicinal mushroom capsules in healthcare.

10. Fungal co-culture from a protease-rich fruit (persimmon) enhances inhibitory activity against papain

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Cysteine proteases (CPs) are key enzymes in multiple biological processes, and their dysregulation is implicated in diseases such as osteoporosis, arthritis and cancer. Their inhibition is thus a promising therapeutic strategy. CPs like papain, bromelain and caricain are abundant in edible fruits, where they serve as chemical defenses against pests and pathogens. Interestingly, some fungi can colonize these protease-rich fruits, possibly by producing inhibitory metabolites. In this study, we applied the OSMAC (One Strain–Many Compounds) approach, using co-culture to induce silent biosynthetic pathways. In this study, we isolated fungi from Diospyros kaki (persimmon), a fruit with high CP content. Two strains were identified as Lasiodiplodia sp. and Diaporthe sp. through ITS and β-tubulin sequencing. The fungi were cultured in rice medium under axenic and co-culture conditions for 21 days. Ethyl acetate extracts were fractionated via VLC to remove triglycerides. CP inhibition was assessed using the fluorogenic substrate Z-FR-MCA. While axenic extracts showed weak or no inhibition at 250 µg/mL, the co-culture extract exhibited strong inhibition (60% at 31.25 µg/mL), suggesting induction of bioactive metabolites. Metabolomic profiling using ^{1}H NMR and LC-MS/MS, combined with GNPS molecular networking, revealed differences in the co-culture chemical profile. Several compounds were isolated, and two—lasiodiplodin and de-O-methyl-lasiodiplodin—were structurally identified. In enzymatic assays, lasiodiplodin showed no inhibition against papain at 100 µM, while de-O-methyl-lasiodiplodin showed limited activity (53.73%). These findings underscore the potential of fungal co-cultivation to unlock silent biosynthetic pathways and highlight protease-rich fruits as valuable sources of fungal strains with potential for discovering protease inhibitors. Ongoing studies aim to identify the compound(s) responsible for the observed protease inhibition, advancing our understanding of fungal chemical ecology and biotechnological applications.

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11. Solid-state NMR investigation of the role of the cystine-rich effector CrpA on *Aspergillus fumigatus* cell wall organization

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Aspergillus fumigatus is a filamentous fungus and a major cause of invasive fungal infections in immunocompromised individuals. The conidial cell wall is the first point of contact with the host and plays a crucial role in protecting the fungus from environmental stress and immune detection. This multilayered structure is composed of an inner rigid scaffold of polysaccharides, including β-1,3-glucan, α-1,3-glucan, and chitin, overlaid by a flexible outer matrix enriched in galactomannan and galactosaminogalactan (GAG). These layers are decorated with surface proteins that contribute to morphogenesis, adherence, and immune modulation. CrpA is a cysteine-rich conidial surface protein recently identified as a modulator of immune signaling and fungal virulence. However, its potential structural role in cell wall organization has not been investigated. Understanding how such proteins contribute to the spatial arrangement of wall components is crucial for elucidating mechanisms of fungal immune evasion. To explore this, we used solid-state nuclear magnetic resonance (NMR) spectroscopy, a method capable of revealing the molecular arrangement of insoluble and heterogeneous biological structures without disrupting their native architecture. We used 13C- and 15N-labeled conidia from both the wild-type strain and a CrpA-deficient mutant (ΔCrpA) to examine the detailed composition and spatial distribution of wall polysaccharides. Comparative NMR analysis revealed changes in the relative abundance and surface accessibility of key polysaccharides, particularly β-1,3-glucan and GAG. These findings show that CrpA influences both the composition and surface organization of the fungal cell wall. Its absence leads to altered exposure of immunogenic components, which could affect recognition by host immune cells and contribute to reduced virulence.

12. Understanding the molecular basis of antimicrobial resistance in dermatophytes

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Human fungal infections present unique challenges due to their eukaryotic nature, sharing cellular similarities with host cells and affecting diverse tissue types. These infections range from mild mucocutaneous conditions to life-threatening diseases. Superficial fungal infections, affecting approximately 25% of the global population, represent the most prevalent form. Dermatophytes are highly specialized, keratinolytic filamentous fungi that cause about 70 % of these cases. These pathogens metabolize keratin, invading the stratum corneum to cause chronic skin, hair, and nail infections (1) known as dermatophytosis or tinea, characterized by redness, itching, scaling, and ring-shaped lesions (2). In spite of the ubiquitous nature of these infections, dermatophytosis remains a neglected disease and molecular basis of virulence and pathogenicity is poorly understood. Treatment options include topical and oral medications, primarily allylamines (targeting squalene epoxidase/ERG1) and azoles (targeting cytochrome P450-dependent enzyme/ERG11), both disrupting ergosterol biosynthesis. However, recent reports of resistance to both agents are emerging, further compounding the problem. India bears over 30% of global terbinafine resistance, highlighting the need to investigate genotype-specific resistance patterns (3,4). We have carried out pan-genomic analysis of available genomes of pathogenic fungi to explore patterns associated with resistance, especially for dermatophyte strains in India. In addition, gene manipulation strategies for exploring drug resistance mechanisms have been developed (5), that can easily be adapted to several filamentous fungi. Details of these works will be presented.

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13. Identification and characterization of putative behavior manipulating fungal effectors

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The entomopathogenic fungus *Ophiocordyceps* is well-known for their ability to manipulate ant behavior, creating so-called "Zombie Ants". While the ecological and evolutionary advantage of host manipulation is well-described, the underlying molecular mechanisms of this extended phenotype are still being explored. Previous transcriptomics analyses have identified 587 upregulated fungal secreted proteins during *Ophiocordyceps*-induced summiting behavior in ants. Many lack functional annotations, limiting our understanding of their potential roles as fungal effectors. In this study, we employed an integrative bioinformatic pipeline combining PSI-BLAST and structural homology modeling (using AlphaFold, DALI and PyMOL) to systematically identify and characterize these candidate effectors. Combined with a prior machine learning-based analysis of fungus-ant protein-protein interactions, this approach enabled us to assign putative functions to over half of the previously uncharacterized proteins. Among these candidates, we identified a lytic polysaccharide monooxygenase (LPMO) involved in chitin degradation, which is additionally predicted to bind G-protein-coupled receptors (GPCRs) and trehalose receptors, suggesting a multifaceted role in modulating host physiology and behavior. To experimentally validate the functional relevance of this putative LPMO, we heterologously expressed the gene in the non-manipulating entomopathogen *Beauveria bassiana* using *Agrobacterium*-mediated transformation, and monitored behavior in infected *Camponotus floridanus* ants. Infected ants were housed in controlled arenas equipped with a nest and feeding platform, and behavior was videorecorded over seven days. Behavioral scoring was done manually by counting the amount of ants foraging or feeding. This revealed that ants infected with the LPMO-expressing strain exhibited significantly increased feeding behavior compared to controls, indicating a role in parasitic behavioral manipulation. These findings shed light on the molecular underpinnings of behavioral manipulation by *Ophiocordyceps* and provide a framework for systematic identification and functional characterization of fungal effectors involved in host-parasite interactions.

14. Divergence in mating type genes reflects life cycle variation in scots pine blister rust

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Reproductive strategies are key to shaping the evolution and adaptability of fungal pathogens. Rust fungi (Pucciniales) are obligate plant pathogens known for their complex life cycles and diverse mating systems. *Cronartium p*ini, the causal agent of Scots pine blister rust, exists in two life cycle forms: a heteroecious form that alternates between pine and herbaceous hosts, and an autoecious form restricted to pine. These forms differ not only in host use but also in reproductive strategies, making *C. pini* a valuable model for studying the relationship between mating system variation and life cycle differentiation. Using comparative genomics, we characterized the structure and diversity of mating-type (MAT) loci across both forms. The heteroecious form displayed a classic tetrapolar system, with unlinked, multiallelic homeodomain (HD) and pheromone/receptor (P/R) loci, consistent with obligate outcrossing. In contrast, the autoecious form revealed more flexible patterns. Some individuals were homozygous at MAT loci—suggesting clonal reproduction—while others carried duplicated MAT genes, potentially enabling self-fertility or alternative mating pathways. Among the three identified HD loci, only one was consistently present and highly diverse, implying functional divergence from the others. Notably, expanded allele diversity at the STE3.2 pheromone receptor gene suggests unexpected plasticity at the P/R locus. Although rare, shared MAT alleles between the two forms point to possible historical gene flow or trans-specific polymorphism. Together, these results reveal striking plasticity in the mating system of *C. pini* and suggest that transitions between life cycle forms may be supported by functionally versatile or redundant MAT gene architectures. This work advances our understanding of how reproductive systems can evolve in response to ecological constraints and highlights the importance of mating type genes in facilitating life cycle shifts in rust fungi.

15. CSA-1 and CSA-2 regulate chitin synthase trafficking and fruiting body development in *Neurospora crassa*

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Fungal cells plasticity and polarity are critical for the maintenance of the integrity of the cell wall. Chitin, a key structural component, provides rigidity and mechanical strength. This structural homo-polysaccharide is synthesized by chitin synthases (CHS), a conserved family of glucosyl-transferase enzymes across the fungal kingdom. While several components of the protein machinery regulating intracellular trafficking of CHS in filamentous fungi have been identified, the mechanisms directing CHS to the hyphal apex and septa remain poorly understood. This study focuses on the chitin synthase regulatory proteins CSA-1 and CSA-2 in *Neurospora crassa*, orthologs of *Saccharomyces cerevisiae* Chs4/Skt5. In yeast, Chs4 acts as a critical adaptor for Chs3 localization and activity at the plasma membrane. Additionally, previous research suggested the interaction between CSA-1 and CHS-4 in *N. crassa*. Here, we show that deletion of csa-1 or csa-2 results in defective growth under osmotic and cell wall stress conditions. Notably, expression of CHS4-GFP and CHS-6-GFP is significantly reduced in a Δ*csa-1*/Δ*csa-2* mutant background, suggesting that both regulatory proteins influence CHS gene expression. Furthermore, Δ*csa-1* and Δ*csa-2* strains containing CHS-1/5-GFP, exhibit sterility and chain-like organization of perithecia, indicating impaired fruiting body development. Moreover, GFP-CSA-1 localizes predominantly to the apical region of hyphae, associating with tubular structures that likely correspond to the apical endoplasmic reticulum (ER). These findings highlight CSA-1 and CSA-2 as key factors in chitin synthesis regulation, and suggest a novel role for the apical ER in the trafficking and regulation of CHS complexes. These results provide new insights into the molecular machinery governing cell wall biosynthesis in filamentous fungi.

16. Radionuclide pollution selects for ectomycorrhizal strains with potassium solubilization potential that are able to protect pine host from cesium toxicity.

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Since the nuclear accident at Chornobyl (1986) and the one at Fukushima (2011), research has focused on the impact of radionuclides on the environment. The forests surrounding both power plants are mainly composed of Coniferous trees, which are known to establish ectomycorrhizas. Ectomycorrhizal fungi protect their host plant from metal toxicity, however, considerable inter and intraspecific variation exists. In general, local adapted metal-tolerant strains show higher bioprotection potential. Here we aim at identifying the mechanisms of local adaptation towards radionuclide pollution in the ectomycorrhizal species *Suillus luteus* and explore its potential to protect pine hosts. Strains of *S. luteus* were isolated from radionuclide contaminated and control sites in Fukushima Prefecture and subsequently phenotyped. The results clearly show the absence of distinct tolerance phenotypes for cesium and γ-radiation, as those appear to be constitutive traits. Differences in the capability to grow in potassium-limited conditions have been observed and are associated with the site of isolation. To investigate potential of this fungal trait in protection of pines from cesium toxicity, an in vitro co-culture experiment was performed. Growth results indicate that the presence of the ectomycorrhizal fungus leads to an altered root architecture and increases root growth in presence of cesium. We could not detect differences in general plant stress responses such as anti-oxidative enzyme activity, indicating a distinct mechanism of bioprotection is provided by the ectomycorrhizal fungus. Potassium and cesium concentrations in pine shoots next to transcriptomes will set light on the mechanisms involved. Altogether, our results indicate local adaptation towards environmental pollution with radionuclides in ectomycorrhizal fungi to be a complex phenomenon and in contrast to metal-pollution to be linked to their symbiotic state rather than direct selection by the contaminating factor.

Yoschenko et al., 2011 Lofgren et al., 2024

17. The role of kinesin-3 motor protein KIN2 and the motor adapter HOOK1 in organelle dynamics of *Podospora anserina*

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The long-distance displacement of organelles along elongated cells, like fungal hyphae, depends on the activity of processive motors moving along microtubules. A conceptual frame has emerged from a limited number of models, where HOOK1 forms a functional motor-adapter pair with either class 3 kinesins or dynein for the motility of EE (1), which is used to co-transport, by hitchhiking, peroxisomes and other organelles (2). However, it is not clear to what extent this is a general paradigm, and how it contributes to global intracellular organization. We are interested in understanding the role of kinesin-3 (KIN2) and HOOK1 in organelle dynamics in the ascomycete model Podospora anserina (3), looking for new insights into longstanding questions. KIN2 and HOK1 are required for proper EE and peroxisome displacements, and their single deletion similarly affected the frequency, speed and length of EE and peroxisome movements. However, the lack of HOOK1 but not of KIN2 caused a subapical clustering of EE, which was not observed with peroxisomes. We also detected a low frequency of peroxisome-EE co-transport events in WT and found that the ablation of EE movement through RAB5B deletion did not affect peroxisome movement. Besides, we found that the elimination of KIN2 or HOOK1 did not prevent the motility of other organelles, such as ER, vacuoles and mitochondria, but altered their apical organization generating enlarged peripheral ER, reduced mitochondrial abundance, and vacuole forefront accumulation. And finally, both proteins are required for optimal mycelium growth rate. Our research revealed a major role for KIN2 and HOOK1 in the regulation of P. anserina organelle dynamics.

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18. OxrA protein as part of the antioxidant response in *Aspergillus nidulans*

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Aerobic organisms generate reactive oxygen species (ROS) as a product of their metabolism, which can have both damaging and signaling roles in various cellular processes, including gene regulation, signal transduction and differentiation (1). In a global phosphoproteomic analysis carried out in our laboratory to identify putative proteins and pathways involved in H2O2 signaling (2) H2O2 induced the dephosphorylation of the protein AN3004 (Oxidant resistant or OxrA), which contains a conserved C-terminal TLDc domain (3). From yeast to animals, the deletion or reduced expression of Oxr1 results in sensitivity to oxidative stress and has been shown to regulate the expression of several antioxidant genes (4). Our results indicate that the lack of oxrA results in oxidative stress sensitivity and that oxrA mutants show a delay in asexual and sexual development. Moreover, our data suggest that OxrA acts in an oxidative stress pathway independent of NapA and AtfA, which are the primary transcriptional regulators of the canonical oxidative stress response in fungi Acknowledgments. This work was supported by PAPIIT-UNAM IN215622 and CONACYT scholarship 815580.

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19. Lipid remodeling supports anaerobic growth of a eukaryotic organism

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Lipids are highly diverse in chemical structure which drives the physiochemical properties of biological membranes, enabling essential cellular processes such as division, trafficking, and signaling to occur (1). Lipid remodeling and synthesis maintain these functions, with certain biosynthetic pathways requiring oxygen, in particular the synthesis of polyunsaturated fatty acyl chains (PUFAs). This raises the question of how some eukaryotic organisms have modified their lipid composition to support growth in the absence of oxygen. The inability of many fungi to synthesize PUFAs has led to a lack of understanding in this area. The fission yeast Schizosaccharomyces japonicus is notable for its ability to grow in both aerobic and anaerobic conditions and can produce PUFAs, making it a good eukaryotic model to investigate lipid adaptation (2)(3). Genetic deletion of a desaturase enzyme responsible for PUFA synthesis was conducted in S. japonicus with fitness assessed in both aerobic and anaerobic environments, indicating PUFAs contribute to cell survival. To explore the impact of PUFAs on membrane structure, advanced microscopy with polarity sensitive probes was used. Desaturase deficient cells showed to have more ordered membranes as PUFAs were no longer present. Additionally, when reactive oxygen species (ROS) were induced, desaturase deficient cells were more sensitive than wild-type cells. Current conceptions suggest PUFAs are more prone to reactive oxygen species (ROS), with these results offering a new perspective on the functions of PUFAs. This finding may suggest that PUFAs have a protective effect on cells.

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20. The role of spore surface proteins in *Mucor lusitanicus*

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The Mucorales order includes highly resilient fungi responsible for mucormycosis, a lethal and emerging infection, and the third most common angio-invasive fungal disease [1]. CotH proteins play a central role not only in pathogenicity but also in regulating spore germination and proper spore structure formation [2]. These spore surface proteins are crucial for adhesion, colonization, and infection. In this study, we analysed *Mucor lusitanicus* cotH-disrupted mutants to assess cell wall alterations. Fluorescent staining and transmission electron microscopy (TEM) were used to monitor structural changes, while spore viability was assessed using the XTT assay, Propidium iodide, and the FUN1-staining. Growth abnormalities and germination defects were investigated under various stress conditions and temperatures. The absence of CotH12 caused abnormal septa formation. CotH mutants showed diverse responses to cell wall stressors, with specific gene deletions leading to modifications in the inner spore coat and affecting fungal growth and sporulation. TEM analysis of the cotH9 mutant revealed morphological markers of programmed cell death, indicating a critical role for CotH9 in spore viability. In vivo virulence testing with Drosophila melanogaster showed reduced pathogenicity in the ΔcotH9 strain, suggesting a host-specific virulence function. Additionally, the ΔcotH6 mutant displayed reduced sporulation and abnormal sporangial morphology. Our results highlight that several CotH proteins are indispensable for infection, spore formation, and structural organization. Understanding how *M. lusitanicus* utilizes CotH proteins provides valuable insights into fungal development and pathogenicity, potentially guiding strategies to mitigate mucormycosis.

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21. Improving lipid accumulation in *Rhodosporidium toruloides* via adaptive evolution strategies

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The depletion of fossil resources and rising pollution levels underscore the urgent need for sustainable alternatives to fuels and industrial oils. Oleaginous microorganisms, particularly those producing value-added lipids, have emerged as promising solutions. Among them, *Rhodosporidium toruloides* stands out for its ability to accumulate up to 70% intracellular lipids and remarkable metabolic flexibility. This study aims to leverage that adaptability to enhance lipid production in the *R. toruloides* NRRL-Y-2701 strain through adaptive laboratory evolution (ALE). A dual-selection strategy was applied: (1) environmental pressure promoting lipid overproduction during consecutive evolutionary rounds, coupled with inoculum selection based on an observed lipid-associated flotation phenotype; and (2) selection of evolved clones exhibiting improved phenotypes. After five evolutionary rounds, a progressive increase in lipid yield was observed, from an initial ~35% lipid content to ~65%, which remained consistent for two additional rounds. However, rounds 8 to 10 showed a slight decline to ~55%, raising the possibility of a reached evolutionary plateau or trade-offs in the evolved phenotype. Epifluorescence microscopy following Nile Red staining confirmed the quantitative analysis, a significant increase in lipid droplet size being observed throughout the rounds. The biomass was further analyzed to quantify protein and polysaccharide content. Despite signs of an evolutionary plateau, several evolved clones isolated throughout the process exhibit improved phenotypes, being capable of mobilizing internal lipid reserves to support growth on carbon-free media, highlighting the efficiency of the dual-selection strategy. These results underscore both the potential and constraints of ALE as a tool for metabolic engineering in *R. toruloides* and emphasize the importance of dynamic selection strategies in long-term adaptive experiments. Further genomic and physiological characterization of evolved clones is required to elucidate the mechanisms behind the observed dynamics.

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22. Development of *in vitro* platform techniques for exploring bacteria-fungi interactions in the gut microbiome

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The human gut microbiome is pivotal in determining health and disease states. Despite its significance, the interaction between fungi and bacteria within the gut microbiota—and their subsequent impact on the host—remains underexplored. In this study, we aimed to establish in vitro methodologies to probe these interactions. We selected *Candida albicans* and Saccharomyces cerevisiae as representative intestinal fungi and *Escherichia coli* and *Bacillus subtilis* as representative intestinal bacteria. For co-culturing, we utilized 0.5x blood-heart-infusion (BHI) medium and incubated the cultures anaerobically at 37°C to simulate the intestinal milieu. We undertook RNA sequencing-based transcriptional profiling to monitor fungal and bacterial gene expression changes during co-incubation. Monocultures of both fungi and bacteria served as controls, while co-cultured samples were harvested after 1, 6, and 24 hours of incubation. DEG and KEGG pathway analyses were employed to elucidate shared and distinct features of various bacterial-fungal interactions. Future research will focus on refining multi-omics methodologies for deeper insights into bacteria-fungi interactions and elucidating multiple facets of these interactions.

23. *Pyricularia oryzae* shows high metabolic dynamics during plant infection

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Pyricularia oryzae, the rice blast fungus, causes a destructive plant disease that poses a serious threat to global rice (*Oryza sativa*) production. Many studies addressed the effectors involved in pathogenesis, but the metabolic changes of P. oryzae during plant infection remain poorly understood. Here we used a set of temporal RNA-seq data, including eight different time points that are key stages for disease development, to identify the dynamics of primary metabolism of P. oryzae. We identified 131 sugar metabolic genes and revealed major temporal changes of these genes during rice infection. In addition, not only sugar metabolic genes, but CAZy genes involved in plant biomass conversion and sugar transporters exhibited similar temporal expression patterns. This revealed the substrates used by the fungus at different stages of infection as well as the degradation pattern of the polysaccharides present in rice. Interestingly, key enzymes of the secondary metabolism showed a similar expression profile. The data was compared to plant infection transcriptome datasets of *Zymoseptoria tritici, Colletotrichum higginsianum* and *Colletotrichum graminicola* to compare their metabolic transcriptome patterns. This is the first comprehensive study of primary metabolism, sugar transport and CAZy genes in plant pathogenic fungi during plant infection and has revealed a new level of understanding into their physiology.

24. Comparative study of molecular mechanisms regulating biofilm formation in *Candida* species

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Fungal biofilms, implicated in 60–80% of microbial infections, are clinically significant due to their persistence, immune evasion, and antifungal resistance. Among fungal pathogens, Candida species are ranked as the number one cause of biofilm-associated infections in clinical settings. Biofilm formation in *Candida* species is a highly regulated, multi-stage process involving distinct molecular mechanisms that govern adhesion, morphogenesis, extracellular matrix production, and resistance. While *Candida albicans* remains the most studied species, non-albicans Candida species, such as *C. glabrata, C. auris, C. tropicalis*, and *C. parapsilosis*, exhibit divergent molecular strategies that reflect their varied biology and clinical behavior. Therefore, this review highlights the molecular pathways and regulatory networks underpinning biofilm formation across these major *Candida* species. In C. albicans, biofilm formation is orchestrated by key transcription factors (BCR1, EFG1, TEC1, NDT80) that regulate adhesin genes and hyphal growth (ALS1-7, HWP1). Signal transduction via the Ras1-cAMP-PKA, MAPK, and calcineurin pathways modulates morphogenesis and ECM-related genes such as ZAP1 and FKS1. Farnesol and tyrosol act as quorum-sensing molecules that regulate biofilm density and filamentation, while extracellular vesicles facilitate matrix delivery. In contrast, NCAC species such as *C. glabrata* lack hyphal growth and depend on EPA adhesins, regulated epigenetically and through stress-responsive factors like YAK1, SKN7, and the HOG pathway. *C. auris*, though non-filamentous, utilizes YAK1, HSP90, and sterol biosynthesis pathways, with extracellular vesicles playing a major role in compact, drug-resistant biofilms. *C. tropicalis* shares some regulatory overlap with *C. albicans* (ALS3, EFG1, and HWP1), biofilm formation however, is supported by species-specific β-glucan-based ECM regulation and quorum sensing molecules. *C. parapsilosis* forms pseudohyphal biofilms via BCR1, secreted lipases, and ECM regulators like RBT5. These interspecies differences in gene regulation, signaling, and matrix control highlight the molecular diversity of *Candida* biofilms and underscore the need for species-targeted therapeutic strategies aimed at mitigating *Candida* biofilm-associated infections.

25. Colonization of plant biomass by *Aspergillus niger* depends on transcriptional regulators controlling polysaccharide degradation

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Saprotrophic fungi produce powerful extracellular enzymes to degrade their substrates, and the production of these is controlled by a set of transcriptional regulators. These regulators respond to chemical cues, which vary from host to environment. In industrial fermentation, fungi must colonize their substrates first to degrade them because biomass particle size is larger than those used in lab-based studies. This inspired our evaluation on the impact of individual transcriptional regulators on the colonization process of the fungus. A previous study in our group demonstrated that an *Aspergillus niger* deletion strain of XlnR and AraR, two main transcriptional activators for plant polysaccharide degradation, showed strongly reduced colonization of wheat bran. The main polysaccharides in the cell walls of wheat bran are cellulose and xylan, implying a direct correlation between regulator function and colonization ability. To determine whether this approach could be used to identify the primary transcriptional regulator in *A. niger* for diverse biomasses, we analyzed colonization of the *A. niger* wild type and deletion mutants for seven transcriptional regulators on five different plant biomass substrates of varying polysaccharide compositions. The tested regulators are involved in xylan (XlnR, ClrB, AraR), cellulose (XlnR, ClrA, ClrB), pectin (GaaR, AraR, RhaR), and starch (AmyR) degradation. We are also including the transcriptional repressor CreA to determine whether carbon catabolite repression plays a role in colonization. Analysis was performed using light, stereo, and scanning electron microscopy and compared to transcriptome data from the wild type and mutants on selected substrates.

26. Investigating genetic and environmental factors driving starship-mediated gene transfer in fungi

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Horizontal gene transfer (HGT) is the exchange of DNA between individuals outside of sexual reproduction and plays a significant role in evolution through the introduction of novel genetic material that can facilitate adaptation to challenging environments. While HGT of transposable elements (TEs) is well documented in prokaryotes, its frequency and mechanisms in eukaryotes remain poorly understood. Starships are a newly discovered group of TEs present in multiple fungal species. These TEs can be hundreds of kilobases in size and are able to mobilize numerous genes, including those conferring resistance to environmental stressors such as heavy metals and antifungals. Previous research has demonstrated that Starships are able to move between individuals belonging to the same species, and recent experiments have shown they can also transpose between different fungal genera. Although we have clear evidence of Starship transposition, we have limited information on what drives their mobilization. We designed a high-throughput method that allows us to cross different strains and screen for transfer events. It remains unclear whether specific environmental cues can promote transfer, but our experimental design enables us to investigate multiple factors that may influence this mechanism. We hypothesize that hyphal fusion is necessary for TE movement, and our setup enables us to investigate whether the presence of genes that inhibit anastomosis between genetically dissimilar individuals also prevents Starship transposition, offering insight into their cryptic dispersal dynamics. I will present the ongoing efforts and recent progress towards understanding the factors impacting the occurrence and frequency of eukaryotic HGT by observing Starships in fungi.

27. Characterizing HsbA proteins involved in *Mucor lusitanicus* pathogenicity

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HsbA proteins are galactomannoproteins covalently linked to the fungal cell wall and known for their antigenic properties [1]. In plant pathogenic fungi, they aid in adhesion, surface penetration, and recruitment of hydrolytic enzymes such as cutinase [2]. A conserved HsbA domain has been identified in the immunogenic Mp1 protein of *Talaromyces marneffei*, which modulates host inflammation by binding arachidonic acid [3]. This study focuses on characterizing hsbA knockout and overexpression mutants (pAV1-3) in *Mucor lusitanicus*, model organism of mucormycosis. Gene expression analysis via qPCR showed coordinated activity among HsbA proteins. Growth assays revealed reduced growth in MS12+pAV1 and MS12+pAV3 at 25 °C, 35 °C, and 20 °C, while MS12+pAV2 was impaired at 35 °C and 20 °C. Deletion mutants MS12-ΔhsbA2+pyrG and MS12-ΔhsbA4+pyrG exhibited growth defects at 20 °C. MS12+pAV3 displayed enhanced sporulation but reduced cell viability in XTT assays. We examined the germination capacity of hsbA mutants, where MS12+pAV1-3 mutants showed significantly reduced germination. Scanning electron microscopy revealed that while the control strain produced granule-like extracellular polymeric substances (EPS), overexpression mutants produced net-like EPS structures. Cell surface hydrophobicity, an important virulence factor [4], was measured in hsbA mutants by MATH assay. The cell surface hydrophobicity of several hsbA mutants were significantly increased. Interestingly, overexpression of HsbA proteins reduced virulence in *Drosophila melanogaster* and *Galleria mellonell*a models, whereas deletion mutants showed increased virulence. These results emphasize the central role of HsbA proteins in regulating fungal surface properties, biofilm formation and pathogenicity in *M. lusitanicus*.

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28. Intra- and inter- species Quorum Sensing in the vascular wilt pathogens *Fusarium oxysporum* and *Verticillium dahliae*

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Fusarium oxysporum and *Verticillium dahliae* are two economically important fungal plant pathogens that cause vascular wilts on a wide range of crops. Since the two fungi share the same ecological niches - soil, plant roots and xylem vessels - understanding their molecular interaction could uncover new mechanisms for improved disease control. Quorum Sensing (QS), the ability to chemically detect population density through extracellular molecules, has been reported to control key processes such as development and virulence in a variety of fungi. In *F. oxysporum*, germination of microconidia is inhibited at high cell densities through a QS mechanism which is, in part, mediated by the small secreted peptide alpha-pheromone (Vitale et al. 2019). Here we studied both the intra- and cross-species inhibition of conidial germination by supernatants from high cell density liquid cultures of *F. oxysporum* and *V. dahliae.* Our findings suggest the presence of conserved intra- and interspecies QS signaling mechanisms. Future efforts are directed towards the identification of the secreted compound(s) and the cellular pathways that mediate the QS response across these two vascular pathogens. Keywords: *Fusarium oxysporum, Verticillium dahliae,* Fungal pathogen, Pathogenesis, Quorum sensing, Spore germination.

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29. Mitochondrial complex III inhibiting fungicide increases resistance to widely used azoles

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Management of clinical fungal infections is increasingly difficult due to the rising frequency of antifungal resistance in human fungal pathogens. Azole-based antifungals are pivotal for treatment of human fungal infections, especially in resource limited regions. However, azoles are also key in agriculture where they are used in combination with electron transport chain (ETC) complex III/cytochrome bc1 inhibitors such as Quinone outside and Quinone inside Inhibitors (QoI, QiI) to combat fungal disease in cereal crops, increasing yields and facilitating food production for an ever-increasing population. We demonstrate that exposure of non-pathogenic fission yeast (*Schizosaccharomyces pombe*) cells to the QiI fenpicoxamide-phenol (FNPQiI) prior to, or simultaneously with, azole-based antifungal compounds, supresses azole-mediated growth inhibition and thus azole effectiveness. FNPQiI treatment of *S.pomb*e cells promotes azole resistance by activating the well-characterised oxidative stress response pathway; Qi site blockage leads to mitochondrial dysfunction, increased intracellular reactive oxygen species (ROS) and upregulation of a subset of core environmental stress response genes (CESR). Importantly, exposure of the pathogenic fungi *Cryptococcus neoformans*, an environmentally prevalent but life-threatening human fungal pathogen, and *Zymoseptoria tritici*, the causative agent of septoria tritici leaf blotch (STB) in wheat, to FNPQiI also enhances their resistance to azole-based antifungals. Thus, the widespread use of ETC inhibiting antifungals in agriculture may be counterproductive with respect to controlling the emergence of resistance to other antifungal agents in both environmental fungi and target crop pathogens. Our findings suggest that a reassessment of the combinations of differently acting antifungal agents, and the spraying regimens, used to prevent fungal disease in crops is critical.

30. Expanding the horizons of antifungal research: a novel synergistic approach with betulinic acid and amphotericin B against fungal pathogens

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Invasive fungal infections are becoming increasingly common, and the escalation of antifungal resistance presents significant challenges for treatment, particularly for pathogens associated with mucormycosis. Amphotericin B (AmB) continues to be a cornerstone in therapy, although its nephrotoxic effects and the emergence of resistant strains pose persistent obstacles. Betulinic acid (BA) has demonstrated notable antimicrobial properties and has a similar core structure with ergosterol (ERG), yet its utility as a supplementary agent in conjunction with established therapies remains inadequately investigated. This study explores the synergistic interactions between AmB and BA against various clinically significant pathogens, including *Candida, Aspergillus, Scedosporium, Fusarium* and *Mucorales* fungi. Our findings indicate a remarkable improvement in antifungal efficacy when AmB is administered alongside BA, achieving substantial fungal growth inhibition at minimal BA concentrations (0.125 μg/mL), which are consistent with clinically relevant serum levels while allowing the dose reduction of AmB. Our in silico molecular docking analyses suggested that BA may facilitate AmB's mechanism via pore formation within fungal membranes. The binding affinity observed between AmB and individual BA was marginally greater than that with ERG, an important component of the fungal membranes. The highest affinity, while simultaneously binding multiple molecules, was observed when both BA and ERG were present. This observation is likely attributable to BA's lipophilic nature and its capacity to permeate cellular membranes, potentially leading to the formation of mixed pores consisting of AmB, BA, and ERG, thereby amplifying the overall antifungal effectiveness [1]. These results imply that the integration of BA with AmB represents an innovative and efficacious approach for addressing invasive fungal infections.

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31. Bio-formulated zinc oxide nanoparticles enhance resistance against powdery mildew in melon by modulating physiological responses, transcriptional regulation, and the microbiome

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The application of bio-formulated nanoparticles in plant disease management have gained significant attention due to their multifunctional properties. In this study, we investigated the effectiveness of bio-formulated zinc oxide (ZnO) nanoparticles in enhancing disease resistance against powdery mildew (*Podosphaera xanthii*) in melon (*Cucumis melo L.*). ZnO nanoparticles were synthesized and integrated into a natural polymer matrix to improve their stability, bioactivity, and plant uptake. Application of these nanoparticles resulted in a significant reduction in disease severity, evidenced by less fungal growth and lower disease incidence compared to untreated controls. Physiological analyses showed that ZnO nanoparticles enhanced the plant antioxidant defense systems, particularly increasing the activity of superoxide dismutase (SOD) and catalase (CAT), which are crucial for mitigating oxidative stress. Additionally, treated plants exhibited increased chlorophyll content and improved overall health, indicating a positive effect on plant growth. Transcriptional profiling revealed that the ZnO nanoparticles upregulated key defense-related genes involved in reactive oxygen species (ROS) production, pathogenesis-related proteins, and defense signaling pathways, suggesting a heightened immune response. Moreover, a microbiome analysis demonstrated significant shifts in the microbial communities of melon plants, with a notable increase in beneficial microorganisms that are known to contribute to plant health and pathogen suppression. These findings underscore the multi-faceted role of bio-formulated ZnO nanoparticles, which not only improve plant physiological responses but also enhance disease resistance through transcriptional modulation and microbiome alteration. This study presents ZnO nanoparticles as a promising and sustainable alternative to chemical fungicides, offering a novel approach for managing fungal diseases in melon crops.

32. Distinct trafficking routes of polarized and non-polarized membrane cargoes in *Aspergillus nidulans*

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Membrane proteins mediate the ability of the cells to communicate with their surroundings and adapt to the environment. The great majority of these proteins are plasma membrane (PM) cargoes, such as transporters, channels or receptors and they are thought to be sorted to their final destination via Golgi-dependent trafficking. However, our studies in the filamentous fungus *Aspergillus nidulans* challenged the essentiality of Golgi in the biogenesis of several membrane cargoes. By following the localization of the polarized R-SNARE SynA versus the non-polarized UapA transporter synchronously co-expressed in wild-type or isogenic genetic backgrounds repressible for conventional cargo secretion, we showed that non-polarized membrane cargoes bypass the Golgi on their way to the PM, while in the same cells the cargoes localized at the tip of the growing hyphae follow the conventional Golgi-dependent trafficking route. In wild-type, the two cargoes dynamically label distinct secretory compartments, highlighted by the finding that, unlike SynA, UapA does not colocalize with the late-Golgi. In line with early partitioning into distinct secretory carriers, the two cargoes collapse in distinct ER-Exit Sites (ERES) in a sec31 thermosensitive background, suggesting the existence of distinct ER-exit mechanisms operated by specific COPII subpopulations. Trafficking via distinct cargo-specific carriers is further supported by showing that repression of proteins essential for conventional cargo secretion does not affect UapA trafficking, while blocks SynA secretion. Overall, this work establishes the existence of distinct, cargo-dependent, trafficking mechanisms, initiating at ERES and being differentially dependent on Golgi and SNARE interactions.

33. A novel effector from a finger millet isolated *Magnaporthe oryza*e strain targets the host nucleus and modulates plant defense responses.

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Rice blast, a disease caused by the fungal pathogen *Magnaporthe oryzae*, is a significant threat to rice production, often resulting in substantial yield losses. In East Africa, this fungus affects various cereal crops, including domestic sorghums and millets. Like other fungal pathogens, *M. oryzae* secretes effector proteins to manipulate host immune responses and cellular processes, enabling successful colonization and infection. In this study, we investigated a novel effector (named Mo2829Fm) from a *M. oryzae* high virulent strain isolated from diseased finger millet in Uganda. Gene expression analysis showed a significant induction of Mo2829Fm in the early stages of infection, followed by a decrease in the later phase. Gene deletion in *M. oryzae* and transient expression in *Nicotiana benthamiana* plants demonstrated its involvement in virulence, possibly through the suppression of genes involved in plant immunity, including Pathogen-Related proteins (PR), WRKY transcription factors, and mitogen-activated protein kinases (MAPKs). In silico analysis of the Mo2829Fm predicted it to be a cytoplasmic effector unique to the M. oryzae species. Confocal microscopy revealed a clear nuclear host localization of this effector. The structure of this effector contains two peptides linked by a serine protein linker. Our results showed that one peptide displayed a non-specific subcellular localization, while the latter is localized only in the nuclei. However, both peptides showed to be involved in virulence. Collectively, these findings underscore the distinctive nature of Mo2829Fm as a unique effector in *M. oryzae*, capable of subverting basal immunity by targeting the host nucleus.

34. Understanding hyphal systems in mycelium leather-like materials for enhanced properties

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Mycelium materials are versatile materials offering a wide range of properties depending on parameters including substrate, growth conditions or strain used. More specifically, in the case of leather like biomaterials made of pure mycelium, finding an optimal strain is critical. Pure mycelium materials are made of one and only component: hyphae. The hyphal system of fungi can be divided into three different types: monomitic, dimitic and trimitic, based on the capability of each strain to produce generative, generative and skeletal, or generative, skeletal and ligative hyphae. It has been proven that skeletal hyphae in fungal fruiting bodies provide the most significant contribution to the mechanical strength (69% of the compression modulus) and that ligative hyphae was second in this contribution (19%). In this preliminary study, we observed the hyphal structure of different polyporales fungi featuring monomitic, dimitic or trimitic hyphal systems and compared the hyphal system in a pure culture with the hyphal system in the fruiting body, using optic and scanning electron microscopy. The mechanical properties of each mycelium mat was measured by assessing tensile strength and compared to the growth rate, allowing us to screen for strains with good potential for material production. By linking classic mycology to mycelium materials science, this study provides insights on how to assess if a fungal strain could potentially have desirable mechanical properties for the growth of leather like materials.

35. Functions of the cell wall polysaccharide schizophyllan during vegetative growth of *Schizophyllum commune*

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The fungal cell wall is essential for the shape of hyphae of filamentous fungi, protects against mechanical stress, and interacts with the (a)biotic environment. The cell wall of the mushroom-forming fungus *Schizophyllum commune* consists of two layers: the inner rigid core, and the mobile outer layer. These contain various polysaccharides, including β-(1-3)-glucan and a β-(1-3)-β-(1-6)-glucan called schizophyllan. This polysaccharide has various commercial applications, but its biological function remains unknown. To elucidate the natural function of schizophyllan during vegetative growth, *S. commune* was grown on minimal medium and medium contain 10-fold more buffer. Addition of extra buffer resulted in a decrease in schizophyllan in both the rigid and mobile layer of the cell wall. This decrease in schizophyllan production was associated with higher elasticity of the mycelium. This may indicate that schizophyllan provides rigidity to the mycelium. Schizophyllan is known to have high thermal stability. We showed that presence of schizophyllan increases the shelf life and survival of both *S. commune* spores and bacterial cells against heat treatment and freeze/thaw cycles. Finally, we assessed the ability of S. commune and bacteria to degrade and metabolize schizophyllan. We found that the bacteria used could not utilize schizophyllan as a carbon, whereas *S. commune* itself could. Together, it can be concluded that schizophyllan may form a selective, protective barrier around the hyphae, providing the mycelium strength, carbon storage, and protection against biotic and abiotic stressors.

36. ER import and Irc22: The structural homolog of TRAPAlpha in *S. cerevisiae*

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In eukaryotes the heterotrimeric Sec61 complex mediates the import of membrane and secretory proteins into the Endoplasmic Reticulum (ER). Depending on the substrate, accessory factors can be involved in the process. One such accessory component is the Translocon Associated Protein (TRAP) complex which supports the ER import of specific secretory precursors in mammalian cells by interacting with the Sec61 complex and the ribosome. TRAP is a heterotetrametric complex comprising TRAPa, TRAPb, TRAPg, and TRAPd. TRAPa is involved in insulin biogenesis and N-glycosylation in mammalian cells. All TRAP subunits are only found in metazoan, except TRAPa, which has a structural homolog in fungi, Irc22. Irc22 is a 225 amino acid protein with an N-terminal signal sequence and a C-terminal transmembrane domain. Irc22 is one of the few proteins whose translocation into the ER is fine-tuned by N-terminal phosphorylation of Sbh1/Sec61b and its synthetic with Ssh1/Sec61, which means its concentration in the ER is critical. This is confirmed by its haplo-insufficiency. I found that Δsbh1/Δirc22 yeast are temperature-sensitive and tunicamycin-sensitivity indicated by increased non-spliced Hac1 mRNA, suggesting general ER homeostasis might be affected. The temperature-sensitivity can be compensated on media containing sorbitol, which indicates that Δsbh1/Δirc22 yeast have a cell wall defect like other ER translocation mutants but not Δirc22. I am now investigating the effects of Δirc22 and Δsbh1/Δirc22 on co- and posttranslational protein import into the ER and on N-glycosylation using pulse experiments, blotting for secretory precursors, and reporter constructs. In addition, we plan on screening a yeast secretome GFP library to identify all ER translocation substrates dependent on Irc22.

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37. Self-organization in biology: hyphal heterogeneity improves starch degradation by *A. niger*

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Self-organization is a striking phenomenon that fascinates many. Especially in homogeneous environments, the question of why- and how -heterogeneous structure emerges is intriguing. We find a novel function of self-organization in biology at the colony margin of the fungus *Aspergillus niger*. Here, the collection of interconnected hyphae splits into two sub- populations with differential expression of amylolytic enzymes. These enzymes are secreted and together break down starch in a multi-step process. We show with a mathematical model that concentrating all enzymes at part of the hyphae results in more efficient substrate degradation, as compared to equal distribution over all hyphae. This model result is corroborated by experimental observations of increased metabolic activity of the wild type that displays hyphal heterogeneity as compared to a mutant that lost this heterogeneity. The intermediate product maltose is known to induce upregulation of amylolytic enzymes. Incorporating this in the model enables the formation of hyphal heterogeneity through self-organization. We generalize our results to a large class of multi-step processes and demonstrate wide applicability. Importantly, this shows that self-organization may not be incidental, but favorable, since it enhances efficiency. Due to its impact on system functioning, its study is not only of academic interest but also of practical value.

38. Biophysical profile of the inward inactivating anion current recorded in the native membrane of the sporangiophore and mycelium of *Phycomyces blakesleeanus*

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Functional characterization of novel ion channels in the native membrane of filamentous fungi is a challenging field of research, mostly due to inaccessibility of the membrane for patch clamp recordings. Cytoplasmic droplets (CD) of *Phycomyces blakesleeanus* sporangiophores are an alternative model system for patch clamp measurements (1), that has yielded characterization of several ion currents so far (1,2). With ambition to exceed our research beyond the CD membrane, we have recently developed an advanced protocol for precise laser microsurgery of the single hyphae of *P. blakesleeanus* mycelium (3). A distinct difference was noticed in the profile of dominant currents from sporangiophore and mycelium cell membrane. Here we present the preliminary biophysical profile of the inward inactivated current from both cell surgery-derived protoplasts and CD, so far shown to have a role in export of chloride and nitrate from the cell. It is active in hyperpolarizing range of membrane potential and has a voltage-dependent inactivating pattern starting from -110 mV, with the time constant of inactivation decreasing with the increase of hyperpolarization. It was present in every recording of the cell- surgery derived protoplast membrane. In CD it was most often recorded in large outside-out patches with 1 mM of Ca2+ present in the recording pipette, but was seldom dominant in the recordings of the entire droplet membrane. Interestingly, this current profile is relatively similar to the recorded activity of bestrophin homologue of *Aspergillus nidulans* that was investigated after heterologous expression in yeast (4), while *P. blakesleeanus* genome holds sequences of bestrophin-like channel domains (5). The further interplay between genetical approach and direct recording of ion channels in their natural surrounding would significantly advance the field of filamentous fungi ion channel studies.

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39. Expression and purification of recombinant human collagen type-1A1 in *Pichia pastoris*

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Collagen is the most abundant structural protein in the human body and is essential for tissue strength, elasticity, and repair. Due to its broad applications in tissue engineering, regenerative medicine, and biomaterials, the development of efficient systems for recombinant collagen production has become increasingly important. In this project, recombinant human collagen type I alpha 1 chain (COL1A1) is produced using the methylotrophic yeast *Pichia pastoris* which is widely recognized for its ability to perform eukaryotic post-translational modifications, for secreting proteins at high levels, and for its uses in scaling up production for industrial and biomedical use. The strategy involves integrating the human COL1A1 gene into the P. pastoris genome and its functional expression in this yeast. Recombinant expression vectors were designed and assembled through gene amplification, and DNA ligation, and they are now being used in yeast transformation. The integrity of the constructs will be verified by DNA sequencing before introducing them into P. pastoris. Transformants expressing the COL1A1 gene will then be selected and cultivated under laboratory-scale fermentation conditions to promote protein expression. Following expression, the recombinant COL1A1 protein will be purified using histidine-tag affinity chromatography with Ni-agarose beads, a method that provides both high yield and specificity. Subsequently, SDS-PAGE analysis and immunoblotting will be employed to evaluate molecular weight, protein integrity, and to confirm expression of COL1A1 with specific epitopes. Moreover, HPLC-based analyses will provide detailed information on purity and biochemical properties. Together, these approaches establish a reliable pipeline for recombinant collagen production in yeast. The resulting biomolecule represents a valuable alternative to animal-derived collagen, with significant potential for biomedical research, biomaterials development, and future therapeutic applications.

40. Investigating how lipidome plasticity facilitates eukaryotic adaptation to oxygen deprivation

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Sterols and unsaturated phospholipids are highly important membrane constituents that are critical for maintaining the functional biophysical properties of eukaryotic membranes. Through lipid-lipid interactions, sterols and unsaturated phospholipid acyl tails play essential roles in modulating lateral lipid organization, phase behavior, and the fluidity of biological membranes (1). However, sterol biosynthesis and fatty acid desaturation can only occur in the presence of molecular oxygen (2). Therefore, this raises the question of how, in the absence of oxygen, can functional eukaryotic membrane properties be maintained? By combining transcriptomic profiling, lipidomic analysis by LC-MS, and advanced microscopy, we demonstrate how the fission yeast *Schizosaccharomyces japonicus* adapts to anoxia by leveraging time-dependent lipidome remodeling to generate an oxygen-independent, functional membrane lipidome. Specifically, we reveal the combination of sterol-like triterpenoids - hopanoids (3,4) - alongside glycerophospholipid acyl-chain remodeling to replace unsaturated species with short (C10-12) saturated equivalents (5) serve as a coordinated, synergistic adaptive response to sustain membrane fluidity in anoxia. Using a combination of antifungal drugs and genetic tools to manipulate membrane lipid compositions in vivo, we uncover key differences in the lipid ordering propensities of ergosterol and diplopterol. Furthermore, we provide evidence of physiochemical incompatibility between ergosterol and phospholipid tail asymmetry which negatively impacts cell physiology. Together, these findings highlight a novel co-evolutionary relationship between lipid metabolic adaptations enabling certain eukaryotes to preserve membrane functionality under complete oxygen deprivation, underscoring the critical role of membrane lipid composition in cellular adaptability and fitness.

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41. Ion channels in plasma membrane of filamentous fungi - methodology and advances in Mucoromycota

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The activity of ion channels, driving exchange across the plasma membrane and electrical signaling, is an essential part of cell physiology. Although far better understood in animals, plants and even bacteria, there are a handful of ion channels or plasma membrane ion currents that have been characterized in filamentous fungi. There is evidence, albeit fragmentary, of their expected role in mycelial growth and regeneration, small organic acid exchange, and cellular and long-range signaling. This, still limited, knowledge is mainly the result of two main directions of fungal research: cloning of ion channels homologous to known types in other kingdoms, followed by characterization by expression in heterologous systems (1), and functional characterization in native membrane in specific experimental preparations, such as cytoplasmic droplets from sporangiophores of *Phycomyces blakesleeanus*, which we use (2). In addition, classical extracellular flux measurements with ion-selective electrodes (3) as well as the still underutilized imaging techniques and the registration of gross potential changes in mycelia also promise progress in this field. Recently, we have developed a femtosecond laser-based nanosurgery method for cell wall removal, which allowed us to obtain protoplasts with plasma membrane suitable for patch-clamp registration using *P. blakesleeanus* mycelia hyphae (4). Here we present our findings from both sporangiophore and hyphal membranes. Most of the identified ionic currents carry various anions, while none is potassium-based. ORIC is an ATP-dependent, outwardly rectifying anionic current from rapidly growing aerial sporangiophores that is osmotically dependent (5). ORAC, another sporangiophore anionic current, is blocked by malate and depolarization-activated for long periods of time. Both currents appear to be characteristic of sporangiophores. In hyphae, several types of ionic currents preferentially conduct glutamate rather than chloride, and only a small fraction can be attributed to calcium channels.

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42. Role of pyrimidines transport in the adaptation to the novel antifungal olorofim in *Aspergillus fumigatus*

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Aspergillus fumigatus is an environmental fungus that can infect humans and cause life-threatening or debilitating lung diseases. Azole drugs are the first-line treatment, but the number of human infections caused by azole-resistant A. fumigatus have increased over last years. The development of new antifungals with novel mechanisms of action has been proposed as the most promising intervention to stop and contain the emergence of antifungal resistance. Olorofim acts by inhibiting the de novo synthesis of pyrimidines in a fungal-specific manner and will reach the clinic in the following years. Therefore, a thorough understanding of processes impacting olorofim's effectiveness is urgently needed. Supplementing exogenous pyrimidines (uracil and uridine) upon olorofim exposure completely restores *A. fumigatus* growth. However, when decoupling them, uracil alone is enough to reverse olorofim's activity in all A. fumigatus strains tested while uridine only restores growth in a subset of them. By using functional genomics and transcriptomics we have identified the amino acid starvation-responsive transcription factor CpcA as a regulator of uridine uptake in A. fumigatus. These findings indicate a pre-existent genomic variability in uridine uptake regulation in natural populations of *A. fumigatus* strains that might impact olorofim's performance upon clinical deployment.

43. Adaptive osmoregulation in successive flushes of *Agaricus bisporus* by free amino acids and mannitol

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Agaricus bisporus, a widely cultivated edible button mushroom, is grown on composted substrates in successive flushes. While the first flush yields the largest and highest-quality mushrooms, later flushes exhibit reduced productivity and quality. These differences are due to physico-chemical changes of the substrate such as nutrient and water availability. Mushroom growth relies on maintaining an osmotic gradient between the substrate and fruiting body to allow resource translocation to the mushrooms. Classically mannitol is known as the most important osmolyte in *A. bisporus* mushrooms. In this study, we investigated osmotic regulation during mushroom development over two flushes. We found that under standard cultivation conditions, the profile of organic osmolytes varies significantly between flushes in response to a changing substrate composition. During the first flush, when carbon is still abundant, mannitol is the dominant organic osmolyte supporting fruiting body expansion. In contrast, the second flush occurs under carbon-limited conditions, prompting a shift in osmotic strategy: fruiting bodies accumulate more nitrogen, with free amino acids contributing substantially to osmotic potential, reaching up to 11% of the dry weight. These findings reveal a dynamic osmotic adaptation mechanism in *A. bisporus*, where different osmolytes are utilized depending on substrate nutrient availability. This metabolic flexibility likely helps sustaining growth under suboptimal conditions but may also relate to quality issues observed in later flushes. Understanding the interplay between substrate composition, osmolyte profiles, and fruiting body physiology offers new avenues for optimizing mushroom yield and quality in commercial systems.

44. Developing functional genetics tools for recalcitrant zombie ant fungi: Transformation of *Ophiocordyceps* using *Agrobacterium* and FACS

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Parasites across taxa can adaptively manipulate host behavior to increase their own transmission and survival. Such extended phenotypes have been relatively well-studied at an ecological and host behavioral level. However, their underlying molecular mechanisms are still poorly understood since functional genetics tools have not been developed for most of the non-model organisms involved in these interactions. We set out to develop such tools for *Ophiocordyceps unilateralis* zombie ant fungi. Fungi of this species complex manipulate ants to climb up and bite down on vegetation in locations that benefit fungal spore development and dispersal. Multi-omics and machine learning approaches have produced candidate fungal genes potentially involved in host manipulation by *Ophiocordyces*. Elucidating their functions will provide deeper insights into the molecular pathways driving manipulation phenotypes and could inspire novel insect pest control strategies. To study putative manipulation genes, we have developed protocols to transform *Ophiocordyceps blastospores* (yeast-like cells). We successfully adopted *Agrobacterium tumefaciens*-mediated transformation to produce hygromycin-resistant, green-fluorescent (GFP) blastospores. While Ophiocordyceps cannot produce spores, nor grow reliably on solid media, we used fluorescence-activated cell sorting (FACS) to select transformants. After culturing for a few weeks, these transformed blastospores could be successfully used for -80C storage, genomic DNA isolation for PCR confirmation, and ant infection. As a next step, we aim to incorporate CRISPR/Cas9 technology in *Ophiocordyceps* to edit genes of interest. Ultimately, we aim to create multiple *Ophiocordyceps* knock-out strains that will be used to infect ant hosts and monitor their behavior to confirm their function and involvement in behavioral manipulation. This work will establish *Ophiocordyceps* as a new model organism to study parasite-host co-evolution and resulting extended phenotypes.

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45. Regulatory mechanisms involved in the activation of the ors gene cluster in *Aspergillus nidulans*

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Fungi possess remarkable potential for producing a diverse array of pharmacologically active compounds. With the rise of drug-resistant infectious diseases, there has been renewed interest in screening microorganisms for new therapeutics. Genome sequencing has shown that many antibiotic-producing fungi contain far more gene clusters for natural products than previously thought. However, some of these genes remain inactive under standard laboratory conditions, representing a largely unexplored reservoir for novel drug discovery. *Aspergillus nidulan*s' orsellinic acid gene cluster remains silent under laboratory conditions. As we have previously shown, co-cultivation with bacterium from the genus *Streptomyces triggers* the production of orsellinic acid in *Aspergillus nidulans* by modifying its epigenetic landscape. The SAGA/ADA complex in *Aspergillus nidulans* acetylates histones in the ors gene cluster, allowing the binding of the transcription factor BasR and the subsequent production of orsellinic acid. Due to the pivotal role that BasR plays in the activation of the ors gene cluster, we further investigated the mechanisms of activation of the ors gene cluster by this transcription factor. By conducting DNA-pulldown assays, we identified a putative binding sequence for BasR in the orsellinic acid gene cluster. Additionally, we found that SirE is the HDAC that represses the expression of the ors gene cluster through expression analysis and LC-MS studies. Our data shed light on the molecular regulation of bacterial–fungal interactions and reveal the complex epigenetic mechanisms underlying these processes.

46. Eavesdropping on underground conversations: Molecular mechanisms underlying soil fungal interspecific interactions and their ecological implications

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Despite their contrasting ecological adaptations, plant symbiotic ectomycorrhizal fungi (EMF) and free-living saprotrophic fungi (STF) have been shown to compete for limiting nutrients in soils, such as nitrogen (N), with important effects on decomposition rates of soil organic matter (SOM) and carbon (C) fluxes. Macroscale observations are inconsistent and show that the decomposition of SOM can be suppressed by the presence of EMF outcompeting STF in organic N acquisition, known as the 'Gadgil effect'. However, in certain ecosystems, an opposite 'priming effect' has been observed, whereby the EMF stimulate saprotrophic activity by introducing labile C into the soil. In order to shed light on these inconsistencies, we investigate the fundamental mechanisms of STF-EMF interactions in laboratory-grown cultures at the mycelial and single-hypha level. We are particularly interested in how metabolic processes involved in nutrient acquisition and the secretion of ecologically active compounds influence the outcome of these interactions. Confrontation assays on plates and special microfluidic chips, which allow the observation of single-hyphae encounters, showed a clear effect of varying C and N availability on interaction dynamics. To gain more insights into these effects we performed untargeted metabolite profiling of culture extracts using high resolution LC-MS. We will complement this with bioactivity assays and transcriptome analyses to further assess the ecological relevance of differentially secreted compounds. Furthermore, we are developing a Raman microspectroscopy-based approach to investigate the metabolic responses at the single-hypha scale and in vivo within the microfluidic chip, focusing on both nutrient assimilation and the secretion of metabolites. A deeper understanding of the metabolic interaction dynamics in different nutrient environments might be crucial to determine directions of overall decomposition rates in the context of a changing climate.

47. Functional profiling and evaluation of essential phosphatases as novel therapeutic targets in *Cryptococcus neoformans*

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Phosphorylation, a reversible post-translational modification, is a key regulatory mechanism in cellular processes, enabling dynamic adaptation to environmental changes. The balance between phosphorylation and dephosphorylation, mediated by kinases and phosphatases, is crucial for cellular homeostasis and pathogenicity of fungal pathogens. In *Cryptococcus neoformans*, a fungal pathogen that causes fatal meningoencephalitis, we previously identified 31 phosphatases required for the infectivity and virulence through knockout analysis. However, the essentiality of phosphatases remains unresolved due to the limitations of gene knockout approaches. To address this, we applied a conditional gene expression system followed by random spore analysis of heterozygous knockout mutants to investigate 25 phosphatases for which knockout mutants were previously unobtainable. Through this approach, we found that 12 phosphatases are required for growth, and 15 are essential for the survival of C. neoformans. Using BLAST analysis, we uncovered that five essential phosphatases (iPGM1, PXP1, MET22, IPC1, and GEP4) are either absent or poorly conserved in humans. This lack of similarity suggests a reduced likelihood of off-target effects, making these unique essential phosphatases promising candidates as antifungal drug targets. To further explore this potential, we are currently performing multiomics analyses and virulence assays to elucidate their mechanisms and evaluate their therapeutic value.