



PROGRAM & BOOK OF ABSTRACTS

Chaves, Portugal | 12-16 September, 2026

Welcome to the 41st SMYTE,

The 41st Small Meeting on Yeast Transport and Energetics brings together researchers from different countries working across membrane transport, trafficking, bioenergetics, metabolism and cellular homeostasis. Over five days in Chaves, the meeting will provide a forum to discuss how cells acquire and transport nutrients, produce and use energy, respond to environmental changes and maintain cellular function and homeostasis.

The scientific program reflects the broad scope of the field, covering transporter structure and function, membrane dynamics, signaling and intracellular trafficking, organelle function, metabolism, stress responses, ageing, disease, evolution, biotechnology and metabolic engineering. By bringing together different experimental systems and perspectives, SMYTE continues to foster connections between fundamental cell biology and its applications in health and biotechnology.

We are delighted to welcome you to Chaves and wish you an enjoyable and scientifically rewarding meeting.

The 41stSMYTE Chairs,



Catarina Prista
LEAF, ISA/University of Lisbon



Miguel Teixeira
IBB, IST/University of Lisbon



Sandra Paiva
CBMA, ECUM/University of Minho

Scientific Committee

Catarina Prista, LEAF, ISA/University of Lisbon

Chris Macdonald, University of York

George Diallinas, University of Greece

Miguel Teixeira, IBB, IST/University of Lisbon

Milan Höfer, University of Bonn

Patrick Van Dijck, University of Leuven

Per Ljungdahl, University of Stockholm

Roland Wedlich-Soldner, University of Münster

Sandra Paiva, CBMA, ECUM/University of Minho

Scott Moyer Rowley, University of Iowa

PROGRAM

Day 1

Saturday, September 12th
(Aqua Flaviae Hotel)

17:00 – 18:30 Arrival and check-in.

18:30 – 18:45 Welcome and Opening Session

SMYTE Chairs: Sandra Paiva, Catarina Prista, Miguel Teixeira

18:45 – 19:30 Marcelle Grenson Keynote lecture

CHAIR: Per Ljungdahl

Maya Schuldiner - "Illuminating the dark transporter-ome: functional genomics approaches for uncovering transporter activity in yeast"

19:50 WELCOME RECEPTION

Day 2

Sunday, September 13th
(Nadir Afonso Contemporary Art Museum)

08:45 – 10:10 Session I - Transporter structure and function

CHAIRs: Margarida Casal, Bruno André

08:50 – 9:20 **EMBO Keynote Lecture:** George Dhalluin – "CryoEM reveals unprecedented structural and functional aspects of the elevator-type fungal purine transporter UapA"

09:20 – 9:40 Nitesh Kumar Khandelwal, "Structural insights into ABC transporter regulation by Cryo-EM"

09:40 – 10:00 Roland Wedlich-Söldner, "Formation of paracrystalline arrays of the proton pump Pma1 in the yeast plasma membrane"

10:00 – 10:10 Faezeh Ghasemi, "Ato transporter family evolution: how genetic expansion and fusion drive functional diversification"

10:10 – 10:40 Coffee Break & Poster Session

10:40 – 12:30 Session II - Membrane dynamics, signalling and protein trafficking

CHAIRs: Morten C. Kielland-Brandt, Sandra Paiva

10:40 – 11:00 Christopher Burd, "TMEM87A/B link golgi cargo processing to focal adhesion assembly"

11:00 – 11:20 Mara Duncan, “Slo1, a little protein with big impact on membrane traffic”
11:20 – 11:40 Allyson O'Donnell, “Too sweet to resist: alpha-arrestin-mediated endocytosis during metabolic deception”
11:40 – 12:00 Francesc Posas, “Selective endocytosis protects plasma membrane integrity upon stress-induced membrane domain reorganization”
12:00 – 12:20 Karen Romich, “Sbh1/Sec61beta is a specific signal peptide receptor within the Sec61 channel”
12:20 – 12:30 Takashi Kubota, “Role of alpha-arrestins in antifungal drug resistance in *Saccharomyces cerevisiae* and *Candida glabrata*”

12:30 – 14:30 LUNCH (Aqua-Flaviae Hotel)

14:30 – 16:40 Session III - Organelle function and bioenergetics

CHAIRs: Christopher Burd, Sofia Dimou

14:30 – 14:50 Oliver Schmidt, “Homeostatic control of sphingolipid biosynthesis via the Orm proteins”
14:50 – 15:10 Zdena Palkova, “Life in yeast multicellular communities: role of mitochondria in development and differentiation”
15:10 – 15:30 Per Ljungdahl “Proline catabolism fuels intraphagosomal replication of *Candida glabrata* and *Candida albicans*”

SHORT BREAK (10min)

15:40 – 16:00 Pierre Morsomme, “Golgi ion homeostasis and membrane protein quality control”
16:00 – 16:20 Bruno André, “Role of transporters in maintaining vacuolar redox homeostasis in yeast”
16:20 – 16:40 Sabrina Büttner, “Vacuolar contact sites: dynamic interfaces for lipid exchange and metabolic remodeling”

16:40 – 17:10 Coffee Break & Poster Session

17:10 – 18:40 Session IV - Transport and energetics in cellular homeostasis and stress adaptation

CHAIRs: Chris McDonald, Catarina Prista

17:10 – 17:30 Norma Sánchez, “Deciphering the HOG1-mediated halotolerance network in *Debaryomyces hansenii* through transcriptomics”
17:30 – 17:50 Riko Hatakeyama, “Metabolic intermediate homoserine acts as a cell growth signal”
17:50 – 18:10 Paula Ludovico, “Beyond Biotechnology: yeast as a window into cellular ageing and disease”
18:10 – 18:30 Daniel Wüstner, “Role of Niemann-Pick type C proteins in lipid transport, metabolic regulation and organelle homeostasis during starvation in *Saccharomyces cerevisiae*”
18:30 – 18:40 Anahit Shirvanyan, “The influence of carbon source and extracellular pH on the regulation of H⁺ and K⁺ fluxes in *Saccharomyces cerevisiae* mutants with defects in plasma-membrane and vacuolar ion channels”

19:30 – 21:30 DINNER (Aqua-Flaviae Hotel)

Day 3

Monday, September 14th

(Nadir Afonso Contemporary Art Museum)

08:40 – 09:50 Session V - Fungal diversity and evolution in transport and energetics

CHAIRs: James Konopka, Allyson O'Donnell

08:40 – 09:00 Oriol Gallego, "Physical chemistry and evolution of exocytosis in the *Saccharomyces* genus: lessons from advanced microscopy"

09:00 – 09:20 Hernâni Gerós, "A Quarter century with yeast: From plant Molecular Biology to sustainable viticulture"

09:20 – 9:40 Cristina Silva-Pereira, "From hyphae to highways: decoding mycelial cord formation in fungi rewiring"

9:40 – 9:50 Maria Zolotareva, "Hexose transporters and azole resistance in *Candida* species: a pangenomic in-silico perspective"

9:50 – 10:20 Coffee Break & Poster Session

10:20 – 12:00 Session VI - Transporters and lipid dynamics

CHAIRs: Mara Duncan, Roland Wedlich-Söldner

10:20 – 10:40 Joseph Mindell, "Mechanism of phosphoinositide inhibition of the lysosomal pH via inhibition of the chloride transporter ClC-7"

10:40 – 11:00 Dan Zhang, "Plasma membrane lipid homeostasis and Cdc42 regulation"

11:00 – 11:20 Christos Gournas, "Eisosomes: fungal membrane domains involved in the sensing of phospholipid peroxidation and the protection against ferroptosis."

11:20 – 11:40 Derek Prosser, "Manipulating the sphingolipid pathway to improve disease-related phenotypes in a yeast model of ALS8"

11:40 – 12:00 James Konopka, "Plasma membrane eisosome domains promote the virulence of *Candida albicans*"

12:20 LUNCH BOXES (Aqua-Flaviae Hotel)

13:00 – 20:00 Free Afternoon / Social Event

Day 4

Tuesday, September 15th

(Nadir Afonso Contemporary Art Museum)

08:30 – 10:20 Session VII - Cool Tools for Transport and Energetics Research

CHAIRs: Scott Moyer-Rowley, Gustavo Goldman

08:30 – 08:50 Marco Fumasoni, “Experimentally evolving cellular miniaturization”
(15+5min)

08:50 – 09:10 Sofia Dimou, “Imaging ER membrane-localized chaperone assisted folding of amino acid permeases”

09:10 – 09:30 Marcel Vieira-Lara, “Accelerating mitochondrial genome editing by streamlining biolistic bombardment”

09:30 – 09:50 Chris MacDonald, “Using yeast as a reconstitution platform to study transport processes relevant to photosynthesis”

09:50 – 10:10 Alexandros Karyolaimos, “Engineering a Genetic Coupling Module to Fine-Tune Heterologous Protein Expression in Yeast”

10:10 – 10:20 Alexandre Deschamps, “Real-time monitoring of intracellular cysteine dynamics using the Cystector biosensor”

10:20 – 10:40 Coffee Break & POSTER Session

10:40 – 12:10 Session VIII - Transporters in biotechnology and metabolic engineering

CHAIRs: Hana Sychrová, Patrick Van Dijck

10:40 – 10:50 Björn Johansson, "Identification of xylose transporters in *Chaetomium thermophilum* by integrated transcriptomics and functional genomics "

10:50 – 11:10 Maria João Sousa, “Exploring glucose transport to modulate the fermentative performance of *Torulaspora delbrueckii*”

11:10 – 11:30 Markus Bisschops, “The yeast general stress response: an energy-saving mechanism and tool to increase product yields”

11:30 – 11:50 Isabel João, “Carboxylic acid transporter engineering for the optimization of microbial cell factories”

11:50 – 12:10 John Morrissey, “Engineering sugar transporters in *Kluyveromyces marxianus* for applications in biotechnology”

12:10 – 14:00 LUNCH (Aqua-Flaviae Hotel)

14:00 – 16:00 Session IXa - Transport and energetics in fungal pathogenicity and drug resistance

CHAIRs: Miguel Teixeira, Zdena Palkova

14:00 – 14:20 Scott Moyer-Rowley, “Interaction of ergosterol biosynthesis and pleiotropic drug resistance genes”

14:20 – 14:40 Minerva Araiza-Villanueva, “From Antifungal Activity to Metabolic Access: Chitosan as a Permeabilizing Agent in Yeasts”

14:40 – 15:00 Patrick Van Dijck, “Intracellular iron homeostasis is a key factor regulating azole cidality, tolerance and susceptibility in *Candida albicans*”

SHORT BREAK (10min)

15:10 – 15:30 Adnane Sellam, “Hidden complexity: How chromatin remodelling drives antifungal tolerance and heteroresistance”

15:30 – 15:50 Catarina Pimentel, “Better together: A multifunctional triazole–copper complex bypasses intrinsic and acquired fluconazole resistance”

15:50 –16:00 Carolina Mariano, “Iron antagonizes fluconazole activity in *Candida glabrata* by reducing drug accumulation”

16:00 – 16:30 Coffee Break & POSTER Session

16:30 – 17:30 Session IXb - Transport and energetics in fungal pathogenicity and drug resistance

CHAIRs: Christos Gournas, Dan Zhang

16:30 – 16:40 Patrícia Ataíde, “Regulation of nutrient-dependent dynamics of Ato proteins and pH modulation in *Candida albicans*”

16:40 – 16:50 Qingjuan Zhu, “Functional Characterization of Sterol Importers in Polyene Resistance of *Candidozyma auris*”

16:50 – 17:10 Gustavo Goldman, “Combined Genomic and Pharmacological Screening Reveals Brilacidin as a Potent Synergizer of Caspofungin Against *Aspergillus fumigatus*”

17:10 – 17:35 **Austrian Embassy Plenary Talk**, Karl Kuchler, “Multidrug resistance in pathogenic fungi - when complexity never ends”

SHORT BREAK (10min)

17:45 – 18:55 Nicolau van Uden Keynote Lecture:

CHAIR: George Dhallinas

Elizabeth Miller, “Directional membrane protein traffic via Erv14/cornichon”

18:55 Best poster/oral communication awards

John Morrissey, **FEMS Yeast Research**

SMYTE Chairs, **Portuguese Society for Microbiology**

19:00 Closing Session

Mayor of Chaves: Nuno Vaz

41st SMYTE Chairs: Sandra Paiva, Catarina Prista, Miguel Teixeira

20:00 CONFERENCE DINNER (Quinta de Samaiões)

Day 5

Wednesday, September 16th

(Aqua-Flaviae Hotel)

8:00 - 09:30 – Breakfast

9:30 - 11:00 Accommodation check out

BOOK OF ABSTRACTS

Marcelle Grenson Keynote Lecture

ILLUMINATING THE DARK TRANSPORTER-OME: FUNCTIONAL GENOMICS APPROACHES FOR UNCOVERING TRANSPORTER ACTIVITY IN YEAST

Maya Schuldiner¹;

¹Weizmann Institute of Science

Membrane transporters govern the movement of metabolites, ions, and cofactors across cellular membranes, thereby shaping metabolism, signaling, and organelle function. Yet despite their central role in cellular physiology, many transporters remain poorly characterized, and their transported substrates are still unknown. This knowledge gap persists even in one of the best-studied eukaryotic model organisms, the budding yeast *Saccharomyces cerevisiae*.

A major challenge in transporter discovery stems from the highly interconnected nature of cellular networks, where perturbation of transport processes rapidly leads to indirect physiological consequences that can mask primary transporter functions. To overcome these limitations, we have developed functional genomic strategies for systematic identification of transporter activities. In essence our approach couples the creation of yeast collections that enable rapid, on-demand, degradation of any protein, alongside high throughput metabolic profiling. Combined they are uncovering the functions of previously uncharacterized enzymes and transporters at scale.

These discoveries provide new insight into how intracellular logistics shape cellular organization and highlight the potential of unbiased functional genomics approaches to illuminate the dark transporter-ome of eukaryotic cells.

Session I

Transporter structure and function

EMBO Keynote Lecture

CRYOEM REVEALS UNPRECEDENTED STRUCTURAL AND FUNCTIONAL ASPECTS OF THE ELEVATOR-TYPE FUNGAL PURINE TRANSPORTER UAPA

George Dhallinas¹; George Broutzakos^{1,3,4}; Yiannis Pyrris¹; Emmanuel Mikros⁵; Ifigeneia Akrani⁵; Christos Gatsogiannis^{3,4}

¹Department of Biology, National and Kapodistrian University of Athens, Athens, Greece; ²Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Greece; ³Center for Soft Nanoscience (SoN), University of Münster, Münster, Germany; ⁴Institute of Medical Physics and Biophysics, University of Münster, Münster, Germany; ⁵Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Members of the ubiquitous Nucleobase Ascorbate Transporter (NAT) family are elevator-type transporters that are responsible for the uptake of nucleobases and ascorbate. In fungi, NAT members are also responsible for the specific uptake of antifungal nucleobase analogues, such as oxypurinol, allopurinol or 8-azaguanine. Here, we report full-length cryo-EM structures of UapA, the NAT high-affinity purine transporter from the model fungus *Aspergillus nidulans*, in new inward-facing apo- and substrate-loaded conformations at 2.06-3.5 Å in detergent and lipid nanodiscs. The exceptionally high-resolution structures of UapA reveal the role of water molecules and lipids in substrate-binding, specificity, transporter dimerization and activity. Importantly, the cytosolic N-tail of UapA is structured, interacting with both substrate transporting and dimerization domains, and rationalizing a plethora of genetic findings that established its dual role in transport dynamics and proper trafficking to the plasma membrane. Under the light of the long standing exceptional genetic and functional analysis of UapA, the present study provides novel structural and functional insights into fundamental aspects of eukaryotic elevator-type transporters, which might also contribute to the exploitation of fungal NATs as specific gateways for targeted pharmacological antifungal approaches.

STRUCTURAL INSIGHTS INTO ABC TRANSPORTER REGULATION BY CRYO-EM

Nitesh Kumar Khandelwal¹

¹Department of Chemical Physiology and Biochemistry, Oregon Health & Science University, Portland, OR 97239, USA

Yeast Cadmium Factor 1 (Ycf1) sequesters heavy metals and glutathione into vacuoles to protect cells from toxic stress. Ycf1 belongs to the ATP-binding cassette C-subfamily (ABCC) of transporters, many of which are regulated through phosphorylation of intrinsically disordered regulatory (R) domains; however, the molecular basis of this regulation remains poorly understood. Here, we present cryo-electron microscopy (cryo-EM) structures of Ycf1 in multiple functional states, including phosphorylated active conformations at 3.4 Å and 4.0 Å resolution and a dephosphorylated inactive state at 3.1 Å resolution.

In the phosphorylated active state, we captured a previously unobserved ordered conformation of the intrinsically disordered R-domain. Clear phosphorylation-dependent remodeling of the R-domain promotes extensive electrostatic and hydrophobic interactions with Nucleotide Binding Domain 1 (NBD1) and the lasso motif, stabilizing the transporter architecture while permitting coordinated movements of the NBD1/R-domain complex. In contrast, structures of dephosphorylated Ycf1 reveal that the unphosphorylated R-domain adopts a distinct ordered conformation containing an unexpected hairpin topology positioned within the regulatory region of the transporter. Remarkably, this binding mode resembles that of viral peptide inhibitors of ABC transporters and secreted bacterial WXG100 peptide toxins.

We further demonstrate that the absence of phosphorylation within the hairpin region drives a major conformational rearrangement of the R-domain, supporting a model in which phosphorylation activates Ycf1 by relieving R-domain-mediated autoinhibition. Together, these findings establish a structural framework for phosphorylation-dependent regulation of ABCC transporters and provide new mechanistic insights into how intrinsically disordered R-domains control transporter activity.

FORMATION OF PARACRYSTALLINE ARRAYS OF THE PROTON PUMP PMA1 IN THE YEAST PLASMA MEMBRANE

Aniket Bandyopadhyay¹; Iryna Levkovych¹; Dekai Dong¹; **Roland Wedlich-Söldner**¹

¹University of Münster

Yeast cells adapt to nutrient starvation by endocytic internalization of many plasma membrane (PM) nutrient transporters. Interestingly, one of the most abundant and energy-intensive PM transporters, the P-type ATPase Pma1, is regulated by a distinct mechanism. Upon acute glucose withdrawal, Pma1 undergoes lateral reorganization from a continuous network into discrete clusters.

Using freeze-fracture electron microscopy, we show that these clusters correspond to paracrystalline hexagonal arrays of Pma1 hexamers. Formation of these structures requires the positively charged cytosolic tail of the seven-transmembrane protein Mrh1, suggesting a mechanism mediated by electrostatic interactions with negatively charged lipids at the inner leaflet of the PM. Consistently, while Mrh1 co-clusters with Pma1 in wild-type cells, it remains dispersed in cells lacking phosphatidylserine (Δ cho1).

Quantitative analysis of Pma1 clustering reveals non-linear kinetics consistent with a charge-modulated nucleation process. Stochastic simulations of two-dimensional Pma1 crystal formation recapitulate these dynamics and generate kinetic predictions that we validate experimentally using a series of Mrh1 charge variants.

Functionally, Pma1 clustering results in stronger inhibition of ATPase activity and delays responses to rapid glucose fluctuations. Loss of clustering (Δ mrh1) leads to faster reactivation but ultimately accelerates cellular aging, as evidenced by increased senescence following glucose depletion.

In summary, we show that Pma1 undergoes lipid- and charge-dependent crystallization into hexagonal arrays during glucose starvation, a process that modulates its activity and balances rapid metabolic responsiveness with long-term cellular fitness.

ATO TRANSPORTER FAMILY EVOLUTION: HOW GENETIC EXPANSION AND FUSION DRIVE FUNCTIONAL DIVERSIFICATION

Faezeh Ghasemi¹; Patrícia Ataíde^{1,2}; Cláudia Barata-Antunes^{1,2}; Yiannis Pyrris^{3,4}; João Alves^{1,2}; Vitor Fernandes^{1,2}; Rosana Alves^{1,2}; Alexandra Gomes-Gonçalves^{1,2}; Margarida Casal^{1,2}; Wouter Van Genechten⁵; Jana Nysten⁵; Alistair J. P. Brown⁶; Patrick Van Dijck^{5,7}; Alexandros A Pittis³; George Diallinas^{3,4}; Isabel Soares-Silva^{1,2}; Sandra Paiva^{1,2}

¹Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal;

²Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal; ³ Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology, 70013, Heraklion, Greece.;

⁴Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis 15784, Athens, Greece.;

⁵Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, K.U. Leuven, Leuven, Belgium.; ⁶ MRC Centre for Medical Mycology, University of Exeter, Exeter, England, UK.; ⁷ KU Leuven One Health Institute, Leuven, Belgium.

The commensal and opportunistic pathogen *Candida albicans* exhibits metabolic flexibility that enables adaptation to glucose-restricted niches via the utilization of alternative carbon sources, such as carboxylates. In *Saccharomyces cerevisiae*, monocarboxylate uptake is mediated by regulated plasma membrane transporters, including Ato1 (Ady2), a member of the Acetate Uptake Transporter (AceTr) family. In *C. albicans*, the Ato family has expanded significantly to ten members (Ato1–Ato10) whose specific functions remain unknown. Here, we investigated the roles of CaAto proteins in carboxylate utilization using integrated in-silico and functional approaches. In-silico analyses revealed that while several CaAto proteins retain conserved AceTr motifs, they possess distinct pore radii and substrate-binding sites for acetate or lactate. Expression analyses showed that CaAto1, CaAto2, CaAto3, and CaAto6 exhibit distinct plasma membrane localization patterns and expression levels dependent on the specific carboxylate present. Notably, deletion of ATO1 impaired the expression of Ato2 and Ato3, causing endoplasmic reticulum retention of CaAto2, which suggests the central regulatory role for CaAto1. Functional transport assays identified CaAto1 as a major acetate-specific transporter dependent on the proton motive force, driven specifically by the transmembrane pH gradient, showing low affinity for butyrate and lactate. Finally, comparative analyses uncovered a novel Ato-related protein family in vertebrates fused to the tRNA-modifying-enzyme Sua5/YciO/YrdC, highlighting the broader evolutionary diversification of this family. Overall, the Ato protein family in *C. albicans* plays a critical role in carboxylate utilization, its functional expansion probably contributing to rapid adaptation and pathogenicity in different environmental niches.

Acknowledgments: This work was supported by the MetaFungal project PTDC/BIA-MIC/5246/2020 through the 'Fundação para a Ciência e a Tecnologia' (FCT) and by project TransFactory (COMPETE2030-FEDER-00750700) co-financed by ERDF through COMPETE 2030. FG acknowledges FCT for the 2023.03135.BD_PhD_grant (<https://doi.org/10.54499/2023.03135.BD>). PA acknowledges FCT for 2024.03178.BDANA_PhD_grant. YP was supported by a Fondation Santé Sidney Altman Scholarship. AG-G acknowledges FCT for 2021.08564.BD_PhD_grant (<https://doi.org/10.54499/2021.08564.BD>).

Session II

Membrane dynamics, signalling and protein trafficking

TMEM87A/B LINK GOLGI CARGO PROCESSING TO FOCAL ADHESION ASSEMBLY

Priyanka Sharma¹, Sangyoon Lee², Yamuna Krishnan², **Christopher Burd**¹

¹Department of Cell Biology, Yale School of Medicine, New Haven, CT USA; ²Department of Chemistry, University of Chicago, Chicago, IL USA

TMEM87A is a member of the GOST (GOLD domain seven transmembrane helix) protein family localized to the Trans-Golgi Network. GOST proteins contain a luminal GOLD domain, also found in TMED/p24 cargo-sorting adaptors, and a GPCR-like seven-transmembrane fold, suggesting roles in cargo sorting and Golgi-based signaling. TMEM87A has been described as Elkin, a mechanosensitive protein regulating melanoma cell adhesion, and as GolpCat, a voltage-dependent cation channel that controls Golgi pH, a finding that we corroborated using a chemigenetic pH reporter, denoted SeRapHin.

To define the functional consequences of TMEM87A/B loss, we performed lectin-based screening and observed a selective reduction in terminal α -1,2 fucosylation, a modification that occurs in the Trans-Golgi Network. Consistent with this, loss of TMEM87A/B increased TGN pH, which may impair FUT1/FUT2 activity and thereby reduce α -1,2-terminal fucosylation. Because this glycan modification influences integrin clustering and cell adhesion, we next examined β 1-integrin and found reduced terminal fucosylation in TMEM87A/B knock-out cells. These cells also showed increased cell size, elevated surface active β 1-integrin, and enhanced focal adhesion formation on fibronectin. In parallel, PI3K and mTOR signaling were upregulated, consistent with increased integrin-dependent signaling. Collectively, our data reveal a novel role for TMEM87A/B in regulating cell adhesion through control of TGN pH, maintenance of α -1,2 fucosylation, and preservation of integrin glycosylation and signaling.

SLO1 A LITTLE PROTEIN WITH BIG IMPACT ON MEMBRANE TRAFFIC

Mara C Duncan¹

¹University of Michigan Medical School.

The budding yeast *Saccharomyces cerevisiae* was the first eukaryotic organism to be completely genetically mapped and sequenced. This achievement enabled the development of whole genome yeast libraries including deletion and epitope tagged libraries. However, the early versions of the yeast genome omitted many genes particularly ones smaller than 100 amino acids, leaving such genes understudied. Here, we report the small protein Slo1 binds to proteins important for membrane traffic at the trans-Golgi network and is required for the normal retention of enzymes in the TGN.

TOO SWEET TO RESIST: ALPHA-ARRESTIN-MEDIATED ENDOCYTOSIS DURING METABOLIC DECEPTION

Jillian Herr¹; Emma Bocquillon¹; Kai Fluet¹; Ben Eichenwald¹; Annette Chiang¹; Martin Schmidt¹; Stacy Gellhaus¹; **Allyson O'Donnell**¹

¹University of Pittsburgh

Cancer cells depend on rapid nutrient uptake through plasma membrane (PM) transporters. Many undergo the Warburg effect—shifting from aerobic respiration to aerobic glycolysis—making them heavily reliant on glucose due to reduced ATP generation per glucose molecule. This glycolytic dependence creates a therapeutic window where cancer cells are metabolically distinct from healthy cells.

2-Deoxyglucose (2DG), a toxic glucose analog that inhibits glycolysis, exploits this vulnerability. 2DG has orphan drug status for treating glioblastomas, aggressive brain cancers with a median survival of 14.6 months post diagnosis. Despite clinical use, the full scope of 2DG's cytotoxicity remains unclear. Earlier studies from our lab discovered that 2DG triggers rapid endocytosis of glucose transporters, starving cells of glucose. Alpha-arrestins, a dynamic and understudied class of trafficking adaptors, regulate this 2DG-induced endocytosis.

How widespread is 2DG-induced, alpha-arrestin-mediated endocytosis? We conducted a high-content imaging screen of all yeast PM proteins to identify which respond to 2DG and alpha-arrestins. Nearly all amino acid transporters are rapidly removed from the PM and exhibit increased vacuolar trafficking upon 2DG treatment, consistent with elevated endocytosis. This suggested that 2DG-treated cells might also be starved for amino acids—another nutrient critical for rapidly proliferating cancer cells. Our metabolomics analysis demonstrates that nearly all amino acids are significantly decreased after 2DG treatment, providing a mechanistic explanation for similar observations in human cancer cell lines. Our data further indicate that this endocytic remodeling depends largely on alpha-arrestins, demonstrating that a network of alpha-arrestins becomes activated in response to this drug.

Our studies reveal widespread, alpha-arrestin-dependent membrane proteome remodeling in response to 2DG, removing not only glucose transporters but also amino acid transporters. We propose that this coordinated removal starves treated cells of both glucose and amino acids—metabolites critical for rapid cancer cell proliferation—reframing 2DG's mechanism of action and opening new avenues for enhancing its therapeutic efficacy.

SELECTIVE ENDOCYTOSIS PROTECTS PLASMA MEMBRANE INTEGRITY UPON STRESS-INDUCED MEMBRANE DOMAIN REORGANIZATION

Francesc Posas¹; Gerhard Seisenbacher²

¹IRB Barcelona / Universitat Pompeu Fabra (Barcelona); ²IRB Barcelona

Exposure of cells to osmostress activates the Hog1/p38 family of stress-activated protein kinases (SAPKs). The activation of these highly conserved MAP kinases is essential for generating osmoadaptive responses necessary for cell survival. To adapt to osmostress, the plasma membrane (PM) undergoes extensive remodeling. The plasma membrane is organized into dynamic lipid-protein assemblies that laterally segregate into domains that compartmentalize signaling and trafficking. While highly dynamic, disruption of this lateral organization threatens membrane integrity. As the plasma membrane rapidly reorganizes in response to environmental changes, how cells resolve domain disruption and restore membrane organization remains unclear. In yeast, arrestin-related trafficking factors (ARTs) and the Rsp5 ubiquitin ligase are the principal quality control system for integral plasma membrane proteins. Through an unbiased genetic screen, we show that osmostress triggers Hog1-dependent selective endocytosis and degradation of specific transmembrane transporters. Mechanistically, reduced membrane tension accelerates lateral diffusion of mobile transporters, causing aberrant domain mixing that increases membrane permeability. HOG pathway-mediated phosphorylation of specific arrestins triggers selective ubiquitination and degradation of mislocalized membrane proteins, thereby resetting membrane organization. Failure of this selective remodeling reduces cell viability. We have described a multilayered mechanism linking biophysical changes in membrane tension to selective ubiquitin-dependent plasma membrane remodeling, ensuring membrane integrity and cellular fitness.

SBH1/SEC61BETA IS A SPECIFIC SIGNAL PEPTIDE RECEPTOR WITHIN THE SEC61 CHANNEL

Karin Römisch¹; Lalitha Yadhanapudi¹; Mark Lommel¹; Felipe Santiago-Tirado²; Tamara Doering³; Volkhard Helms¹; Guido Barbieri¹

¹Saarland University; ²Notre Dame University; ³Washington University, St. Louis

Biogenesis of virulence factors in fungal pathogens like *Cryptococcus neoformans* relies on the secretory pathway. In eukaryotes, protein secretion begins with protein translocation across the endoplasmic reticulum (ER) membrane through the heterotrimeric Sec61 channel. The Sec61 channel beta-subunit, Sbh1 in yeast, enhances ER import of proteins with specific signal peptides by an unknown mechanism. Sbh1/Sec61beta consists of a cytosolic intrinsically disordered, non-conserved region (IDR) that has never been visualised in active channel structures, followed by structured 15 amino acids and a transmembrane helix, both of which are conserved. We have shown that conserved residues at the Sbh1 cytosolic/transmembrane interface form a hinge that positions the Sbh1 cytosolic domain across the channel vestibule which is critical for Sbh1-dependent protein insertion. We have shown that Sbh1-dependence is a function of the signal peptide. Chemical crosslinking of purified cytosolic domains of Sbh1 and its paralog Sbh2 to synthetic signal peptides derived from their respective client proteins revealed that the cytosolic domains of Sbh1 and Sbh2 contain specific signal peptide binding sites formed by their IDRs. This has been missed for several decades of Sec61 channel research, because standard reporter proteins for ER translocation contain strong, Sbh1-independent signal peptides. In *C. neoformans* Sbh1 is only required for secretory protein production under infection-like conditions, not for vegetative growth. Deletion of SBH1 renders *C. neoformans* completely avirulent and prevents the biogenesis of numerous virulence factors. Due to the lack of conservation of the signal peptide binding site, Sbh1 may serve as a new drug target for fungal infections. We conclude that Sec61beta homologs directly recognize signal peptides of their substrates and control their entry into the secretory pathway.

ROLE OF ALPHA-ARRESTINS IN ANTIFUNGAL DRUG RESISTANCE IN *SACCHAROMYCES CEREVISIAE* AND *CANDIDA GLABRATA*

Takashi Kubota¹

¹Institute of Medical Sciences, University of Aberdeen

The opportunistic fungal pathogen *Candida glabrata* is a major cause of candidiasis, ranging from superficial infections to life-threatening disseminated disease, and is associated with high mortality, particularly in immunocompromised individuals. A key feature of *C. glabrata* is its capacity to rapidly develop antifungal resistance, often mediated by upregulation of multidrug transporter genes.

In *Saccharomyces cerevisiae*, Pdr5, a major drug efflux transporter, has been suggested to undergo endocytic degradation; however, the underlying mechanisms remain poorly understood. In this study, we investigated the role of alpha-arrestins in regulating drug resistance in both *S. cerevisiae* and *C. glabrata*.

We found that overexpression of Aly2, one of 14 alpha-arrestins, in *S. cerevisiae* increases sensitivity to azole antifungals, even in drug-resistant strains carrying PDR1 or PDR3 gain-of-function mutations. Conversely, an *rpd5-ts* mutant exhibited reduced azole sensitivity. Aly2 overexpression led to a decrease in Pdr5 protein levels independent of transcriptional regulation, consistent with a model in which Aly2 promotes Pdr5 degradation.

Unexpectedly, the opposite effect was observed in *C. glabrata*, where Aly2 overexpression reduced azole sensitivity. Given the limited characterization of α -arrestins in *C. glabrata*, we systematically examined the impact of overexpressing 12 alpha-arrestins on susceptibility to three classes of antifungal agents: azoles, caspofungin, and amphotericin B. Our results suggest that three α -arrestins modulate azole sensitivity, four influence caspofungin sensitivity, and two affect amphotericin B sensitivity.

Together, these findings suggest species-specific roles for alpha-arrestins in antifungal drug resistance.

Session III

Organelle function and bioenergetics

HOMEOSTATIC CONTROL OF SPHINGOLIPID BIOSYNTHESIS VIA THE ORM PROTEINS

Niklas Schomisch¹; **Oliver Schmidt**¹

¹Medical University of Innsbruck, Institute of Cell Biology, Innsbruck, Austria

ORMDL family proteins are conserved regulators of sphingolipid biosynthesis, with the main function to bind and inhibit the rate-limiting enzyme serine-palmitoyl transferase (SPT) in the endoplasmic reticulum (ER) [1]. In humans, dysregulation of SPT activity causes a form of amyotrophic lateral sclerosis (ALS), and is genetically linked to several inflammatory diseases [2,3]. In budding yeast the ORMDL family consists of two proteins, Orm1 and Orm2. Both are embedded in a homeostatic system that adjusts sphingolipid levels to cellular demands [1]. In budding yeast, Orm1 and Orm2 are mainly controlled by phosphorylation downstream of plasma membrane stress signals sensed via the target of rapamycin complex 2 (TORC2) and yeast protein kinase 1 (Ypk1). While this phospho-regulation is primarily a fungal-specific process, it also induces proteolytic destruction of Orm2 [4], which seems to be an evolutionarily conserved mechanism to control the activity of ORMDL proteins. Interestingly, phosphorylated Orm1 is not targeted for degradation, mainly because it is not exported from the ER.

Here, I will describe two sequence determinants in the highly homologous yeast ORMDL proteins that are responsible for their distinct sorting and trafficking behavior and discuss how maintaining a balance between the two ORMDL paralogues may control sphingolipid homeostasis.

[1] Mahawar and Wattenberg, 2025, Bioessays

[2] Mohassel et al., 2021, Nat. Med.

[3] Moffatt et al., 2007, Nature

[4] Schmidt et al., 2019, EMBO J.

[5] Körner; Schäfer et al., 2024, Cell Rep.

LIFE IN YEAST MULTICELLULAR COMMUNITIES: ROLE OF MITOCHONDRIA IN DEVELOPMENT AND DIFFERENTIATION

Zdena Palková¹; Jana Marsikova¹; Vitezslav Plocek¹; Libuse Vachova²

¹Charles University, Faculty of Science, BIOCEV; ²Institute of Microbiology of the CAS, BIOCEV

Life within multicellular spatial communities with complex internal architectures represents the predominant mode of existence for yeasts and other microorganisms in natural environments. Yeast multicellular structures arise primarily through the proliferation of cooperating cells that coordinate their behavior and subsequently differentiate into spatially distinct subpopulations with specialized functions, resembling primitive tissues. Examples include colonies and diverse types of biofilms, which differ in their internal organization, responses to environmental stimuli, and mechanisms of protection against stressors such as nutrient limitation and exposure to toxic compounds (PMID: 40158069, PMID: 40726826). Owing to their unique properties, relative structural simplicity, and greater experimental tractability compared with metazoan systems, spatially organized yeast communities provide a powerful model for investigating fundamental principles of multicellularity. These include the molecular mechanisms underlying intercellular communication, coordination, and differentiation, as well as collective cell responses to nutrient deprivation and environmental stress and their consequences for community development.

Saccharomyces cerevisiae colonies grown on respiratory media constitute an excellent model for studying these processes. During colony development, cells differentiate into two major, spatially separated subpopulations with distinct physiological properties and functions: U cells located in the upper colony layers and L cells residing in the colony interior. Our previous studies demonstrated that these cell types differ markedly in mitochondrial characteristics, respiratory capacity, and the activity of retrograde mitochondrial signaling, which is essential, among other functions, for the viability of a specific L cell subpopulation (PMID: 40158069, PMID: 34070491, PMID: 26992228). We will show how external factors influence the development of multicellular yeast communities (colonies and biofilms), examine the role of mitochondria and mitochondria-associated regulatory pathways in mediating these effects, and demonstrate how alterations in cellular differentiation impact the spatially structured populations. Supported by LUAUS24055 and LasApp Z.02.01.01/00/22_008/0004573.

PROLINE CATABOLISM FUELS INTRAPHAGOSOMAL REPLICATION OF *CANDIDA GLABRATA* AND *CANDIDA ALBICANS*

Anastasiya Ivo Karaivanova¹; Nicolas Hansmann¹; **Per O. Ljungdahl¹**; Fitz Gerald S. Silao¹

¹Department of Molecular Biosciences, The Wenner-Gren Institute, SciLifeLab, Stockholm University, 106 91 Stockholm, Sweden

As a consequence of co-evolution with humans, opportunistic pathogenic fungi of the *Candida* species have adapted to and regulate their metabolism based on nutrients available within infected hosts. We have recently found that the amino acid proline is an important alternative energy source and modulator of fungal virulence. Macrophages, phagocytic cells of the innate immune system, are important in the defense against fungal pathogens. Although the microenvironment of the phagosome is thought to be nutrient poor, *Candida glabrata* and *Candida albicans* cells are known to survive. Here, we show that proline within phagosomes fuels growth and replication of phagocytosed fungal cells. Proline is catabolized to glutamate in the mitochondria via the concerted action of two enzymes of the Proline Utilization (PUT) pathway, Put1 and Put2. The reactions catalyzed are coupled to the reduction of electron carriers that are subsequently oxidized in the electron transport chain generating ATP. By combining time-lapse imaging, immunocytochemistry and biochemical approaches we have mapped key temporal aspects of fungal proline catabolism occurring in situ within macrophages. We observed that proline catabolism is activated upon phagocytosis by RAW264.7 cells or primary bone marrow derived murine macrophages, indicating that proline is indeed present in the phagosome at concentrations sufficient to rapidly induce the expression of PUT pathway components. Consistent with proline catabolism being a driver of virulence, inactivation of PUT1 or PUT2 abolishes the growth and intracellular replication of fungal cells in macrophages.

GOLGI ION HOMEOSTASIS AND MEMBRANE PROTEIN QUALITY CONTROL

Pierre Morsomme¹

¹UCLouvain

Proper protein glycosylation in the Golgi depends on tightly regulated luminal concentrations of divalent cations, yet the molecular mechanisms governing Ca^{2+} and Mn^{2+} homeostasis in this compartment remain incompletely understood. Here, we present complementary findings that shed new light on how the Golgi maintains ionic balance and how key transporters are regulated under stress conditions.

Using the first genetically encoded FRET-based sensors targeted to the *S. cerevisiae* Golgi lumen — GoD4cpV for Ca^{2+} and GoMnLaMP2 for Mn^{2+} — we achieved quantitative, real-time monitoring of both ions in living cells. These probes revealed that glycosylation defects in *pmr1* Δ cells correlate with elevated rather than depleted luminal Ca^{2+} , and that extracellular Ca^{2+} supplementation rescues glycosylation by normalizing luminal Ca^{2+} to wild-type levels. We further demonstrate that Pmr1 and Gdt1 constitute the sole Mn^{2+} import systems of the Golgi, and that Mn^{2+} transport activity — not Ca^{2+} transport — is the primary functional determinant of the P-type ATPase Pmr1. Together, these results reframe Golgi ion homeostasis as a stoichiometric rather than threshold-dependent process, with direct mechanistic relevance to Congenital Disorders of Glycosylation.

Alongside these homeostatic mechanisms, we investigated the fate of Gdt1 under elevated extracellular Mn^{2+} conditions. We show that Gdt1 undergoes rapid and selective degradation in response to manganese excess, through a pathway routing Gdt1 to the vacuole via the multivesicular body (MVB) pathway. The specificity of this response identifies Gdt1 as a unique model for stress-induced Golgi membrane protein quality control.

ROLE OF TRANSPORTERS IN MAINTAINING VACUOLAR REDOX HOMEOSTASIS IN YEAST

Bruno André¹

¹Free University of Brussels (ULB)

The mechanisms that maintain lysosomal redox homeostasis (LRH) remain largely unknown. Given the high iron content and acidic pH of lysosomes, these mechanisms are likely essential for preventing oxidative damage to lipids and proteins. In addition, recent studies have reported that lipid peroxidation (LPO) at the lysosomal membrane can trigger ferroptosis, a non-apoptotic form of cell death that can be induced by drugs to eradicate cancer cells. We use the yeast vacuole as a model to investigate how cells preserve LRH. We will present results showing that redox-sensitive probes, including roGFP2, become oxidized when targeted to the vacuole. This oxidation is more pronounced under respiratory than fermentative conditions and during iron starvation. In yeast, the transition from fermentation to respiration following glucose depletion is associated with the mobilization of intravacuolar Fe^{3+} and its reduction to pro-oxidant Fe^{2+} . We found that this transition is accompanied by increased oxidation of redox probes targeted to the vacuole. Further investigation of how cells adapt to this oxidative challenge revealed that the transition from fermentation to respiration is associated, on the cytosolic side, with decreased cysteine (CSH) levels and increased glutathione (GSH) levels and, on the vacuolar side, with increased GSH content. In addition, we detected the hydropersulfides GSSH and CSSH in isolated vacuoles; these species are known to act as efficient radical scavengers. Finally, we identified several transporters that cooperate to import GSH and hydropersulfides into the vacuole. Overall, these findings enable us to propose a first integrated model of vacuolar redox homeostasis.

VACUOLAR CONTACT SITES: DYNAMIC INTERFACES FOR LIPID EXCHANGE AND METABOLIC REMODELING

Sabrina Büttner¹

¹University of Münster

Eukaryotic cells rely on the coordinated communication between organelles to maintain cellular homeostasis and adapt to changing environmental conditions. Beyond classical vesicular trafficking pathways, membrane contact sites (MCSs) have emerged as critical platforms that enable direct exchange of metabolites, lipids, and ions between organelles. The vacuole, the yeast lysosome and main degradative compartment, is now recognized as a highly dynamic organelle that establishes extensive physical and functional interactions with multiple cellular partners. Focusing on the vacuole as hub of organelle communication, I will address how vacuolar MCSs contribute to the spatial organization and metabolic regulation of the cell. In response to changing physiological states, the vacuole dynamically remodels its interactions with organelles including the perinuclear endoplasmic reticulum, mitochondria, and lipid droplets. These contact sites provide specialized interfaces that coordinate lipid transport, organelle remodeling, and metabolic adaptation. Combining imaging approaches with molecular and functional analyses, we investigate the mechanisms that establish, regulate, and adapt vacuolar connectivity in response to cellular demands.

Session IV

Transport and energetics in cellular homeostasis and stress adaptation

DECIPHERING THE HOG1-MEDIATED HALOTOLERANCE NETWORK IN *DEBARYOMYCES HANSENII* THROUGH TRANSCRIPTOMICS

Norma Silvia Sánchez¹; Minerva Araiza-Villanueva¹; Francisco Padilla-Garfias¹; Antonio Peña¹; Roberto Coria¹

¹Instituto de Fisiología Celular, UNAM

Debaryomyces hansenii is a non-conventional yeast recognized for its remarkable halotolerance, making it a primary model for studying osmotic adaptation mechanisms. Although the High Osmolarity Glycerol (HOG) pathway is a conserved signaling cascade in fungi, its specific regulatory role in *D. hansenii* under high salinity remains to be fully elucidated. This study presents a comprehensive transcriptomic analysis of *D. hansenii* wild-type (WT) and a *hog1Δ* mutant strain when subjected to high salt concentrations.

Using RNA sequencing (RNA-Seq) technology, we identified a set of salt-responsive genes in the WT strain, primarily involved in glycerol metabolism, ion homeostasis, and cell wall reinforcement. Comparative analysis revealed that the absence of the HOG1 gene alters the transcriptional landscape, leading to the regulation of a considerable number of genes compared to the WT under the same stress conditions. Notably, our data suggest that HOG1 is crucial for the adaptation of this yeast to high salt concentrations by orchestrating the induction of primary osmotic defenses genes. However, it is important to note that *D. hansenii* employs a combination of HOG1-independent strategies that converge to maintain cellular viability at high salinities.

These findings provide new insights into the complex regulatory network of halotolerance in *D. hansenii* and highlight the specialized role of the HOG pathway in this extremophilic yeast. Further functional characterization of the differentially expressed genes identified in this study will be essential to fully understand the unique physiological resilience of this species.

METABOLIC INTERMEDIATE HOMOSERINE ACTS AS A CELL GROWTH SIGNAL

Riko Hatakeyama¹

¹University of Aberdeen

Cells adjust their growth in response to nutrient availability, an essential strategy for surviving a fluctuating environment. As nutrient molecules are continuously converted through metabolic reactions, it is unclear which molecules cells measure as proxies for nutrient richness. Here we found that yeast cells monitor the intracellular concentration of metabolic intermediate homoserine, a non-proteinogenic amino acid with no previously reported function as a signalling molecule. Homoserine activates the Target of Rapamycin Complex 1 (TORC1) kinase, an evolutionarily conserved master regulator of cell growth, both in vivo and in vitro. TORC1 activation by homoserine is mediated by the sensor protein Pib2. Our findings establish homoserine as a metabolic checkpoint signal, depending on which cells make growth decisions. Given the conservation of the homoserine synthesis pathway and the Pib2-TORC1 cascade across fungi and plants, our checkpoint model may represent a common principle of cellular nutrient sensing.

BEYOND BIOTECHNOLOGY: YEAST AS A WINDOW INTO CELLULAR AGEING AND DISEASE

Paula Ludovico¹

¹School of Medicine, Life and Health Sciences Research Institute (ICVS), University of Minho

Ageing is driven by conserved cellular and molecular mechanisms that can be effectively investigated using simple eukaryotic model systems. *Saccharomyces cerevisiae* provides a powerful platform for studying cellular ageing, longevity, and age-associated dysfunction due to its rapid growth, low cost, genetic tractability, short lifespan, and extensive molecular tools. Importantly, yeast shares key ageing-related pathways with higher eukaryotes, making it particularly useful for analysing chronological and replicative lifespan, mitochondrial dysfunction, oxidative stress, impaired autophagy, genomic instability, deregulated nutrient sensing, and loss of proteostasis.

This presentation will highlight how yeast models contribute to the understanding of conserved ageing mechanisms and their modulation by environmental, metabolic, and genetic factors. Particular attention will be given to caloric restriction, which extends chronological lifespan through mechanisms involving reactive oxygen species, superoxide dismutase activity, mitochondrial function, and hormetic stress responses. The presentation will also address yeast models of neurodegenerative disease, especially α -synuclein toxicity, where premature ageing is associated with defective autophagy and mitophagy, mitochondrial DNA depletion, activation of the DNA damage response, S-phase arrest, and ribonucleotide reductase degradation.

In addition, the role of amino acid homeostasis will be discussed, with emphasis on charged side-chain amino acids as regulators of chronological lifespan and proteotoxic stress. These findings suggest that specific amino acids influence ageing through pathways involving Sch9, Gcn2, mitochondrial activity, intracellular pH regulation, autophagy, and mitophagy. Finally, the impact of non-caloric sweeteners on yeast ageing will be considered, showing that compounds such as saccharin and cyclamate exert distinct effects on lifespan through osmolarity, oxidative stress, nutrient-sensing pathways, and autophagic regulation.

Overall, *S. cerevisiae* emerges as a versatile and informative model for identifying conserved mechanisms of cellular ageing and for testing interventions with potential relevance to human ageing and age-related diseases.

ROLE OF NIEMANN-PICK TYPE C PROTEINS IN LIPID TRANSPORT, METABOLIC REGULATION AND ORGANELLE HOMEOSTASIS DURING STARVATION IN *SACCHAROMYCES CEREVISIAE*

Daniel Wüstner¹; Katja Thaysen¹; Jacob Marcus Egebjerg¹; Maria Szomek¹; Lynette Nel²; Kelly Frain²; Vladimir Gorshkov¹; Jesper K. Havelund¹; Stephan Werner³; Christoph Pratsch³; Gerd Schneider³; Nils J. Færgeman¹; Frank Kjeldsen¹; Bjørn Panyella Pedersen²

¹University of Southern Denmark; ²Aarhus University; ³Helmholtz-Zentrum Berlin

Niemann-Pick type C (NPC) disease is a rare human disease characterized by accumulation of cholesterol and sphingolipids in lysosomes due to defects in the sterol transporters NPC1 or NPC2. The yeast *Saccharomyces cerevisiae*, lacking the NPC homologs NCR1 or NPC2, provides a well-established model to study these defects. Here, we show that both proteins bind sterols but also sphingo- and phospholipids, an important determinant of the vacuole fusion/fission cycle [1, 2]. Nutrient depletion during stationary growth caused ingestion of lipid droplets (LDs) into the vacuole (lipophagy). Yeast lacking NPC proteins had increased lipid accumulation in fragmented vacuoles, as revealed by quantitative fluorescence and soft X-ray microscopy [3]. Acute glucose restriction (AGR) changes sterol trafficking, turnover of LDs and vacuolar architecture in wild-type and NPC-deficient yeast [4]. Live-cell imaging of the ergosterol analog dehydroergosterol (DHE) revealed enhanced sterol uptake from the plasma membrane in NPC-deficient cells upon AGR. Using proteomics and lipidomics this is explained by change in Target of Rapamycin Complex 2 (TORC2) activity along with changes in sphingolipid and ergosterol content. Glucose resumption caused not only altered metabolic oscillations accompanied by changed mitochondrial morphology but also rescued the vacuole fragmentation phenotype observed in NPC-deficient cells [4, 5]. Together, our study revealed a central role of yeast NPC proteins in lipid transport and metabolism linked to organelle inheritance and energy homeostasis.

THE INFLUENCE OF CARBON SOURCE AND EXTRACELLULAR PH ON THE REGULATION OF H⁺ AND K⁺ FLUXES IN *SACCHAROMYCES CEREVISIAE* MUTANTS WITH DEFECTS IN PLASMA-MEMBRANE AND VACUOLAR ION PUMPS

Anahit Shirvanyan¹; Susanna Musheghyan²; Nicoletta Guaragnella³; Karen Trchounian^{1,2}

¹Scientific-Research Institute of Biology, Yerevan State University, Yerevan, Armenia; ²Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan, Armenia; ³Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Bari, Italy

Environmental factors are the main regulators of cell membrane transport and metabolism. Hence, carbon sources and extracellular pH-dependent changes in plasma or vacuolar transport pathways remain underexplored. The present study aimed to investigate the effects of carbon sources (glucose, fructose, and sucrose) and extracellular pH (4.5 and 6.5) on the regulation of H⁺ and K⁺ fluxes in *Saccharomyces cerevisiae* BY4741 (wild type, WT) and its mutants lacking plasma membrane ATPase (Pma) and vacuolar ATPase (Vma). The biomass production and specific growth rate were monitored using cell growth quantifier (CGQ) biosensors, and the ion flux rate was studied using ion-selective electrodes via the potentiometric method. WT cells showed the highest specific growth rate (0.40 h⁻¹) in 0.5% sucrose at pH 6.5, which was approximately 2.0-fold higher than the values obtained at pH 4.5 and in glucose or fructose. The N, N'-dicyclohexylcarbodiimide (DCCD)-sensitive proton flux rate (JH⁺, 4.8 mM H⁺ min⁻¹ CFU⁻¹) was 1.4-fold higher during 2.0% glucose and 3.4-fold higher during fructose and sucrose fermentation at pH 4.5 compared to those observed at pH 6.5. Regardless of the carbon source, the DCCD-sensitive JH⁺/JK⁺ ratio in the WT decreased by 3.0-fold at pH 4.5 compared to pH 6.5. The Δpma1-007 mutant showed up to 7.0-fold reduced DCCD-sensitive JH⁺/JK⁺ ratio compared with the WT, whereas in the Δvma1 mutant, the same parameter increased 3.0-fold, indicating a compensatory mechanism of Pma. These findings provide new insights into ion flux regulation depending on environmental factors, which may contribute to robust strain construction for yeast-based biotechnological applications.

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Session V

Fungal diversity and evolution in transport and energetics

PHYSICAL CHEMISTRY AND EVOLUTION OF EXOCYTOSIS IN THE *SACCHAROMYCES* GENUS: LESSONS FROM ADVANCED MICROSCOPY

Oriol Gallego¹

¹Universitat Pompeu Fabra

Constitutive exocytosis is a vesicle transport pathway essential for all eukaryotes. Using Single Molecule Localization Microscopy we time-resolved the conformational landscape of the Exocyst Higher-Order Structure (ExHOS), the protein machinery that tethers secretory vesicles to the plasma membrane (PM) in *Saccharomyces cerevisiae*. At the optimal temperature, the ExHOS pulls the vesicle with a stepwise mechanism comprising three metastable states at 27, 18 and 5 nm from the PM. However, below 15°C, nearly 40% of the exocytic events terminate before being completed, a functional breakdown that correlates with the poor cold-tolerance of *S. cerevisiae*. The molecular functions limiting the adaptation of the cell's biology at low temperatures remains poorly understood. Using single-particle tracking we measured how the rates of labelled proteins scale with temperature. Surprisingly, proteins of the exocytic machinery display different behaviors, some deviating from Arrhenius. Cryo-Electron Tomography allowed us to interpret these deviations as quantifiable perturbations of the vesicle movement caused by temperature. A comparative analysis across *Saccharomyces* species with different thermal tolerances identified molecular functions that dominate the adaptation of this essential machinery when cells grow in cold environments.

A QUARTER CENTURY WITH YEAST: FROM PLANT MOLECULAR BIOLOGY TO SUSTAINABLE VITICULTURE

Gonçalo Marques¹, Carolina Moreira¹, Mariana Vale¹, Richard Breia¹, Artur Conde¹, Viviana Martins¹, **Hernâni Gerós¹**

¹Centro de Biologia Molecular e Ambiental, Escola de Ciências, Universidade do Minho

Our group has focused on plant–environment interactions, molecular physiology and secondary metabolism in grapevine, given its socioeconomic importance. A broad range of molecular, biochemical and omics approaches has been combined with field-oriented studies and collaboration with producers, with the aim of translating fundamental knowledge into innovative and sustainable approaches to improve plant resilience to biotic and abiotic stresses. Taking advantage of the genetic and biochemical versatility of yeast as a heterologous expression system, we have investigated grapevine transport systems involved in the movement of water, sugars, compatible solutes, minerals and heavy metals, providing insights into the molecular mechanisms underlying plant–environment interactions and plant–pathogen relationships. Examples include the functional characterization of heavy metal transporters and the elucidation of the role of SWEET sugar transporters in grapevine–pathogen interactions. More recently, yeast has once again become central to our research through the study of native yeast communities associated with grape berries. Combining culture-based approaches, metagenomics and metabolomics, we have investigated how microbial diversity and grape-derived and microbial metabolites shape interactions within the berry microbiome, including beneficial interactions and antagonistic relationships with other microorganisms, as well as their implications for grape health, fermentation and wine characteristics. Thus, yeast has remained a connecting thread linking membrane transport, plant molecular biology and microbial ecology, from fundamental research to field-oriented approaches for sustainable viticulture.

DECODING THE TRANSPORTOME OF *CANDIDA AURIS*: FROM DRUG EFFLUX TO TARGETED ANTIFUNGAL ENTRY

Atanu Banerjee¹

¹Amity Institute of Biotechnology, Amity University Haryana

The multidrug-resistant pathogen *Candida (Candidozyma) auris* has emerged as a significant public health threat because of its persistence in healthcare environments and limited therapeutic options. Consequently, it has also been recently included in the WHO Fungal Priority Pathogens List (WHO FPPL). Elucidating the mechanisms by which antifungal agents cross the fungal cell membrane is essential for overcoming resistance and developing effective therapeutic strategies.

In this talk, I will present research from my laboratory that focuses on the transportome of *C. auris* and its role in antifungal resistance and susceptibility. Our investigations examine membrane transporters that either efflux antifungal compounds from the cell, contributing to drug resistance, or facilitate their uptake, thereby creating opportunities for targeted antifungal delivery. Using functional genomics, heterologous expression systems, and transporter-focused screening approaches, we have identified several transporter proteins that influence the fungal response to clinically relevant antifungal agents and xenobiotics. These studies have revealed novel insights into the role of ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters in antifungal resistance and highlighted the broader contribution of transporter networks to fungal adaptation under antifungal stress. In parallel, we have explored peptide permeases as potential gateways for antifungal entry. We have comprehensively analyzed the Peptide Transporter (PTR) family and identified a transporter, Ptr_C, which plays a central role in importing peptide-based antifungals. Exploiting this feature, we have developed a heterologous system capable of efficiently screening peptidomimetic compounds that can piggyback on Ptr_C to gain cellular entry. Together, our findings highlight that rather than viewing transporters solely as mediators of drug resistance, there is a need to recognize them as crucial targets for antifungal intervention.

Our ongoing studies aim to unravel the molecular mechanisms governing substrate specificity of these transporters as well as to design targeted strategies to mitigate the growing threat posed by *C. auris*.

FROM HYPHAE TO HIGHWAYS: DECODING MYCELIAL CORD FORMATION IN FUNGI REWIRING

Carlos J.S. Moreira¹; Tiago Martins¹; Carolina Silva¹; João Jorge¹; **Cristina Silva Pereira**¹

¹ITQB NOVA

Mycelial cords are large-scale multicellular structures formed by certain decomposer fungi in forest soils, particularly beneath the litter layer. These structures consist of aggregated hyphae organised into high-conductivity channels, reinforced by extracellular matrix components and hyphal fusion. Functionally, cords enable long-distance translocation of water and nutrients, supporting efficient foraging across heterogeneous substrates. Despite their ecological importance, many cord-forming fungi fail to produce these structures under in vitro conditions, suggesting that environmental cues play a critical role in their development.

Forest soils provide a complex substrate rich in plant-derived polymers that may act as signals or priming factors for cord formation. Additional variables such as temperature, pH, moisture, and biotic interactions further influence cord morphology and network architecture. However, the specific signals and genetic mechanisms underlying cord differentiation remain largely unresolved.

We tested the hypothesis that cord formation is a genetically regulated developmental process triggered by specific environmental effectors, analogous to other fungal morphological transitions. Using the model cord-forming species *Armillaria gallica*, we established a controlled cultivation system that enables reproducible induction and disruption of cord formation through defined media conditions.

Scanning electron microscopy revealed cords with a compact, fibrous outer layer displaying pronounced longitudinal ridging and an internal hollow structure characterised by parallel hyphal alignment. Transmission electron microscopy further demonstrated that cords represent a differentiated tissue, with hyphae exhibiting distinct sizes, morphologies, and degrees of compaction. RNA-seq analysis uncovered extensive transcriptional reprogramming during cord initiation and growth, including enrichment in genes associated with cell wall biosynthesis and remodelling.

These findings support the view that mycelial cords arise from a regulated developmental switch. Ongoing work seeks to identify the signalling cues and genetic networks governing this transition, with implications for understanding fungal adaptability and ecological function.

HEXOSE TRANSPORTERS AND AZOLE RESISTANCE IN *CANDIDA* SPECIES: A PANGENOMIC IN-SILICO PERSPECTIVE

Maria Zolotareva¹; Maria Lopes^{1;2}; Inês Vieira Costa^{1;2}; Madalena Silva^{1;2}; Veerle van Berkum^{1;2}; Jolien Vreys⁴; Patrick van Dijck⁴; Diogo Caetano³; Martins Mupueleque^{1;2}; Miguel Cacho Teixeira^{1;2}

¹Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; ²iBB - Institute for Bioengineering and Biosciences, Associate Laboratory i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal; ³INESC MN, Lisbon, Portugal; ⁴Laboratory of Molecular Cell Biology, Department of Biology, Institute of Botany and Microbiology, KU Leuven, Leuven, Belgium

Sugar transporters play an important role in fungal pathogenesis by modulating growth across a wide range of environmental and within-host conditions in *Candida* species. In *Nakaseomyces glabratus*, the emergence of mutations in hexose transporter genes during evolution of azole drug-resistance was previously observed and further demonstrated to result in decreased intracellular drug accumulation, establishing a mechanistic link between hexose transport and pathogenesis [Galocha et al, Commun Biol, 5: 1118, 2022]. These findings raise important questions: how widespread is this mechanism, and could similar adaptations occur across other pathogenic *Candida* species?

To evaluate the strength of the link between drug resistance and hexose transporter function in pathogenic yeasts, we examined interspecific and intraspecific variability in hexose transporter sequences across the genomes of 114 globally distributed strains of three *Candida* species known for their heightened virulence, adaptability to diverse environments, and antifungal drug resistance. Deleterious substitutions in glucose binding sites were identified within the transporter sequence repertoire of fluconazole susceptible and fluconazole resistant strains of *N. glabratus*, *C. auris*, and *C. albicans*. Species-specific differences in hexose transporter diversification and conservation were also identified.

Among *N. glabratus* isolates, loss of transporter function was found to be common, occurring predominantly in HXT2, HXT4/6/7 and HXT6/7, and found statistically correlated to azole-resistance. An additional pool of 31 drug susceptible and 43 drug resistant local isolates was sequenced and their HXT integrity was evaluated. Could modulation of hexose transporter function constitute an evolutionarily recurrent strategy associated with antifungal resistance?

Session VI

Transporters and lipid dynamics

MECHANISM OF PHOSPHOINOSITIDE INHIBITION OF THE LYSOSOMAL PH VIA INHIBITION OF THE CHLORIDE TRANSPORTER CLC-7

Joseph Mindell¹

¹National Institute of Neurological Disorders and Stroke, NIH

Lysosomes process cellular waste and coordinate responses to metabolic challenge. Central to lysosomal homeostasis are phosphoinositide lipids, key signaling molecules which establish organelle identity, regulate membrane dynamics and are tightly linked to the pathophysiology and therapy of lysosomal storage disorders, neurodegeneration, and cancer. Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P₂) interacts with multiple lysosomal membrane proteins and plays a critical role in regulating lysosomal pH by directly inhibiting the chloride/proton antiporter CLC-7, though the molecular mechanism of this inhibition remains unclear. Here, using a combination of functional, structural, and computational analysis, we demonstrate that PI(3,5)P₂ binding dramatically remodels the structure of CLC-7 by inducing close association between cytosolic and transmembrane domains. Disease-causing mutations show increased transport activity through loss of PI(3,5)P₂ binding and subsequent inhibition. Conversely, CLC-7 activation is correlated with dissociation and increased disorder of the cytoplasmic domain along with novel transmembrane domain conformations, revealing a mechanistic link between specific lysosomal lipids, transporter regulation, and the enigmatic basis of the CLC-7 slow gate.

PLASMA MEMBRANE LIPID HOMEOSTASIS AND CDC42 REGULATION

Baicong Mu¹; **Zhang Dan**^{1;2}

¹Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604, Singapore;

²Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore.

Cell polarity is a fundamental feature of eukaryotic cells that underlies the spatial organization of cellular architecture and signaling, thereby directing processes such as polarized growth, migration, morphogenesis, and asymmetric cell division. Central to polarity establishment are signaling networks orchestrated by the conserved Rho-family GTPase Cdc42, which functions as a master regulator of spatial cue integration and cytoskeletal organization. In parallel, accumulating evidence has established that distinct plasma membrane (PM) lipid species—including phosphoinositides, sphingolipids, and sterols—play critical roles in generating membrane heterogeneity and organizing signaling platforms that govern the localization and activity of polarity factors, trafficking machinery, and the actin cytoskeleton. Despite these advances, the mechanistic interplay between dynamic PM lipid remodeling and Cdc42-dependent polarity signaling remains incompletely understood. Here, I will present our recent work exploring the relationship between PM lipid organization and Cdc42 regulation, and discuss how membrane lipid homeostasis may contribute to the establishment and maintenance of cell polarity.

EISOSOMES : FUNGAL MEMBRANE DOMAINS INVOLVED IN THE SENSING OF PHOSPHOLIPID PEROXIDATION AND THE PROTECTION AGAINST FERROPTOSIS.

Christos Gournas¹; Giorgia Di Liberto¹; Fabienne Vierendeels¹; Olga Martzoukou^{1,2}; Bruno André²

¹Labiris Institute, Brussels; ²IBMM, Université Libre de Bruxelles (ULB), Belgium

Ferroptosis is a recently identified form of cell death caused by excess iron-dependent peroxidation of phospholipids. As many cell types are particularly susceptible to ferroptosis, inducing this form of cell death represents a promising treatment strategy. Similarly, inhibition of ferroptosis-protective mechanisms emerges as a promising strategy to tackle persistent fungal infections. Recently, we have established the existence of ferroptosis in yeast and uncovered the main mechanisms that prevent its execution. Specifically, eisosomes, specialized plasma membrane microdomains, expand in quiescent cells and inhibit ferroptosis by hosting and stabilizing the FLP enzymes, which are ubiquinone reductases that regenerate ubiquinol, a lipophilic antioxidant that counteracts phospholipid peroxidation. This mechanism acts in parallel with the established Glutathione peroxidases (Gpx1/2/3), that reduce peroxidised phospholipids. We are currently building on this experience and by exploiting powerful yeast cell biology, genetic, lipidomic and proteomic approaches, we aim to address the following question: How is phospholipid peroxidation sensed to trigger protective signaling responses? Despite the fact that previous studies have identified several ferroptosis-protective mechanisms, whether or how these are coordinated remains unknown. Our results support a model where eisosomes are the main phospholipid peroxidation-sensing sites in yeast and possess the capacity to signal to TORC2 and calcineurin for inducing membrane integrity repair mechanisms. Among those, we identify certain phospholipases as enzymes potentially participating in the repair of damaged phospholipids via phospholipid remodeling as a ferroptosis-protective response. Our work in progress expands our understanding of ferroptosis in yeast and fungi. Importantly, it identifies a potentially broadly conserved mechanism for the sensing of phospholipid peroxidation.

MANIPULATING THE SPHINGOLIPID PATHWAY TO IMPROVE DISEASE-RELATED PHENOTYPES IN A YEAST MODEL OF ALS8

Derek C. Prosser¹; AnnaMari Stump¹

¹Virginia Commonwealth University

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor neuron loss, leading to death typically within 3-5 years of diagnosis. ALS type 8 arises from a P56S mutation in VAPB, a gene involved in membrane transport and contact site formation at the endoplasmic reticulum (ER). VAPBP56S causes ER collapse and stress sensitivity, which may contribute to disease pathophysiology. We created a fungal ALS8 model by introducing analogous mutations in the yeast homolog SCS2, which we used to screen for genes that reduced sensitivity to the ER stress-inducing drug, tunicamycin (TM). Here, we identify sphingolipid degradation as a potential modulator of ALS8 pathogenesis. Overexpression of the sphingosine kinases LCB4 and LCB5 corrected TM sensitivity and reduced ER inclusion size, while deletion of the sphingosine-1-phosphate (S1P) lyase DPL1 increased ER inclusion size. Pharmacological inhibition of sphingosine production with myriocin, or ceramide production with fumonisin B1, both reduced ER stress sensitivity and inclusion size. Since elevated ceramide is linked to cell death, we propose that modulation of the sphingolipid biosynthetic pathway may influence ER homeostasis in ALS.

PLASMA MEMBRANE EISOSOME DOMAINS PROMOTE THE VIRULENCE OF *CANDIDA ALBICANS*

James B. Konopka¹; Carla E. Lanze¹; Shamoon Naseem¹; Anshuman Yadav¹; Robert Morabito¹

¹Stony Brook University

The virulence of *Candida albicans* is promoted by its capacity to resist stressful conditions in the host and to undergo invasive hyphal growth into tissues. Central to these processes is the plasma membrane, an essential barrier that is broadly important for pathogenesis because of its roles in secretion of virulence factors, cell wall synthesis, hyphal morphogenesis, nutrient uptake, and sensing the extracellular environment. The plasma membrane is also on the front line of resisting attack by the host immune system. Our analysis of plasma subdomains known as MCC/eisosome subdomains has revealed that they carry out a broad range of functions that promote virulence. We identified Sur7 as a key eisosome protein that regulates invasive growth and cell wall structural integrity. Furthermore, Sur7 is essential for defending against copper-mediated permeabilization of the plasma membrane, a tactic employed by the innate immune system. Genetic studies demonstrated that both of these functions are due to a novel role of Sur7 in regulating the phosphatases that control the levels of phosphatidylinositol 4,5 bisphosphate (PI4,5P2), which plays critical roles in cell signaling and membrane dynamics. Additionally, we identified important roles in virulence for a set of eisosome proteins known as flavodoxin-like proteins (FLPs; Pst1, Pst2, Pst3, and Ycp4). We discovered that the FLP proteins protect the plasma membrane from lipid peroxidation caused during oxidative attack by the immune system. The FLPs reduce oxidized ubiquinone to ubiquinol so that it can act as an antioxidant to protect membrane lipids. To identify novel eisosome proteins that contribute to *C. albicans* virulence we carried out TurboID proximity labeling studies. The roles of these new eisosome proteins in stress resistance and invasive hyphal growth will be described.

Session VII

Cool Tools for Transport and Energetics Research

EXPERIMENTALLY EVOLVING CELLULAR MINIATURIZATION

Marco Fumasoni¹

¹Gulbenkian Institute for Molecular Medicine (GIMM)

Cell size influences nearly every aspect of cellular physiology and varies drastically among eukaryotes, yet how such diversity evolves without compromising cellular function remains unclear. Using experimental evolution in budding yeast, we selected for progressively smaller cells and identified mutations in conserved nutrient-sensing and cell-cycle pathways that enable sustained reductions in cell size with minimal fitness cost. These findings show that gradual tuning of core regulatory networks can decouple strong decreases in cell size from growth defects, providing an experimental framework to study the evolution of fundamental cellular traits.

IMAGING ER MEMBRANE-LOCALIZED CHAPERONE ASSISTED FOLDING OF AMINO ACID PERMEASES

Sofia Dimou¹; Ioanna Myronidi^{1,2}; Maryam Nejati^{1,2}; Per O. Ljungdahl^{1,2}

¹Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; ²Science for Life Laboratory (SciLifeLab), Solna, Sweden

A major unsolved question in cell biology is how proteins with multiple membrane-spanning segments fold to attain native and functional structures during their co-translational insertion into the endoplasmic reticulum (ER). To understand the underlying mechanistic principles, we are exploiting the well-defined specificity of the yeast ER-membrane localized chaperone Shr3, which is specifically required for the folding of amino acid permeases (AAPs), a protein family of eighteen core members, each comprised of 12 membrane spanning segments. Despite extensive evidence supporting a co-translational role of Shr3 in the biogenesis of AAPs, direct experimental proof has remained elusive. Here, we employed a yeast-derived translational arrest sequence to capture ribosome-associated folding intermediates of the general amino acid permease Gap1. A 22-amino acid arrest peptide, derived from the ribosome quality-control substrate Sdd1, was inserted at the C-terminal end of cytoplasmic loop 10 of Gap1 (Gap1-AP) to stall translation and generate stable ribosome-nascent chain complexes. Biochemical analyses revealed that Gap1-AP co-fractionates and co-immunoprecipitates with ribosomes, the Sec61 translocon, and Shr3, providing direct evidence for co-translational engagement of Shr3 with nascent AAPs. To obtain high resolution structures of AAP folding intermediates in an in situ context, we have developed methods to obtain rough microsomes that preserve ribosome-translocon-nascent chain assemblies. These membrane preparations are being analyzed by cryo-electron microscopy. Together, these findings establish a new experimental platform for visualizing the co-translational folding of membrane proteins in native ER membranes and provide mechanistic insights into how membrane-localized chaperones guide the folding and assembly of polytopic membrane proteins.

ACCELERATING MITOCHONDRIAL GENOME EDITING BY STREAMLINING BIOLISTIC BOMBARDMENT

Malte Marquardt¹; Marcel Vieira-Lara¹

¹Delft University of Technology

The mitochondrial genome (mtDNA) encodes several proteins involved in oxidative phosphorylation. As the organelle does not naturally import DNA or RNA, biolistic bombardment is the only available technique for mtDNA editing. Predominantly used in *Saccharomyces cerevisiae*, the method delivers a gene of interest via bombardment of $\rho 0$ cells (cells lacking mtDNA) with plasmid DNA-covered carrier particles. This yields a subset of cells with plasmid DNA in their mitochondria. To identify this subset, all transformants are crossed to a tester strain with defective mtDNA, allowing the mitochondria to fuse and the mtDNA to recombine. Strains with the introduced gene variant can be identified based on their ability to respire. In this work, we removed bottlenecks in the standard workflow of biolistic bombardment and developed an updated, multiplexed version of the method. We developed plasmids that contain a minimal backbone and are compatible with Golden Gate cloning. To facilitate the transfer of recombinant mtDNA to strains with any nuclear background of choice, we developed a one-step mating protocol accommodating donor and recipient strains of either mating type (a or alpha). We also demonstrated multiplexed bombardment with simultaneous construction of up to five strains on a CEN.PK113-7D background with variants in the mtDNA-encoded ATP9 gene (ATP synthase subunit 9), and are exploring other genes as targets. This improved method holds the potential for higher throughput construction of *S. cerevisiae* strains with engineered mtDNA, reducing laborious screening and shortening design-to-strain time by weeks.

USING YEAST AS A RECONSTITUTION PLATFORM TO STUDY TRANSPORT PROCESSES RELEVANT TO PHOTOSYNTHESIS

Chris MacDonald¹

¹Biology Department, University of York

Photosynthetic carbon fixation by Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is fundamentally limited by its low affinity for carbon dioxide (CO₂) and competing oxygenase activity. To overcome this biophysical bottleneck, aquatic photosynthetic organisms deploy complex chloroplast carbon-concentrating mechanisms (CCMs) that actively transport and compartmentalise inorganic carbon.

The multi-step pathway of inorganic carbon uptake, accumulation, and enzymatic conversion across distinct membrane boundaries is incompletely understood. To drive carboxylation in CCM-containing phototrophs, bicarbonate (HCO₃⁻) is transported across the thylakoid membrane into the thylakoid lumen, yet the precise contributions of specific luminal carbonic anhydrases and their dynamic interactions remain unresolved. Additionally, how thylakoid biogenesis and structural maintenance dictate photosynthetic efficiency remains unclear.

To address these knowledge gaps, we developed a reconstitution platform in *Saccharomyces cerevisiae* for heterologous expression of key photosynthetic factors from diverse phototrophic lineages (*Arabidopsis thaliana*, *Chlamydomonas reinhardtii*, *Synechococcus elongatus*, and *Thalassiosira pseudonana*). Initial validation demonstrates that this yeast chassis can be used to quantitatively measure catalytic activity of specific enzymes like carbonic anhydrases, visualise protein-protein associations within the yeast cytoplasm, and assess thylakoid-like membrane dynamics in a simplified environment. By isolating these components from the complex regulatory network of host chloroplasts, this modular platform enables precise functional analysis of targeted photosynthetic factors. In doing so, it reveals fundamental biophysical principles governing organelle membrane homeostasis and carbon processing.

Ultimately, elucidating this structural and enzymatic interplay between membrane transporters, organellar pH gradients, and localised carbonic anhydrase activity provides essential design principles for engineering high-efficiency carbon-capture platforms in both terrestrial crops and synthetic biology systems

ENGINEERING A GENETIC COUPLING MODULE TO FINE-TUNE HETEROLOGOUS PROTEIN EXPRESSION IN YEAST

Alexandros Karyolaimos¹, Raul Pasadas Castilla¹, Izabella Pomykalska², Berta Nanna-Rosa Syhler¹, Marta Reventós Montané¹, Leonie Johanna Jahn¹, Morten Otto Alexander Sommer¹

¹Biological Research Institute for the Green Transition (BRIGHT), Technical University of Denmark, 2800 Kongens Lyngby, Denmark; ²Laboratory of Synthetic and Applied Microbiology, SB ISIC & SV IBI, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Heterologous protein production in yeast is often limited by the challenge of rapidly identifying genetic designs that combine high expression levels with cellular fitness. Conventional screening approaches typically rely on protein-specific analytical methods, making them laborious and difficult to scale across multiple targets. To address this limitation, we developed a modular genetic coupling strategy that links heterologous protein expression to yeast growth, enabling the functional selection of improved expression designs.

The system was designed to facilitate the optimization of regulatory elements controlling protein production, including promoters, Kozak translation-initiation sequences, secretion signal peptides, and terminators. Libraries of genetic variants can be incorporated into expression constructs and screened based on their effects on cellular growth under selective conditions. This establishes a direct connection between expression performance and an easily measurable phenotype, reducing the need for extensive protein-specific screening.

We validated the platform using several heterologous protein targets, with a particular focus on translation-initiation and secretion-related elements. The system differentiated between genetic designs with distinct expression profiles and enabled the identification of variants that improved protein production relative to commonly used control sequences. Its modular architecture also allows the coupling principle to be adapted to different proteins, expression contexts, and selection systems.

This approach provides a scalable framework for optimizing heterologous protein expression in yeast while accounting for the physiological burden associated with recombinant production. By integrating genetic design with growth-based selection, the platform may accelerate the development of improved yeast strains for the production of difficult-to-express proteins.

This work was supported by the Novo Nordisk Foundation through the Challenge Programme Sustainable Cell Factories (SussCell).

REAL-TIME MONITORING OF INTRACELLULAR CYSTEINE DYNAMICS USING THE CYSTECTOR BIOSENSOR

Alexandre Deschamps¹; Charlotte Felten¹; Bruno André¹; Peter Dedecker²; Brandán Pedre²

¹Université Libre de Bruxelles; ²KU Leuven

Traditional methods used to assess membrane transporter activity – primarily based on radiolabeled substrates – generally fail to capture dynamic, real-time changes in the intracellular distribution of compounds. Genetically encoded biosensors overcome this limitation by enabling real-time monitoring of metabolite dynamics in living cells with subcellular resolution.

As part of an ongoing project investigating the role of cysteine import into the vacuole in vacuolar redox homeostasis, we use a genetically encoded fluorescent cysteine biosensor named Cystector (“cysteine detector”), to monitor intracellular and subcellular cysteine dynamics in real time. This ratiometric green fluorescent biosensor was engineered by inserting a circularly permuted GFP into the periplasmic L-cysteine-binding protein Ngo2014 from *Neisseria gonorrhoeae*. Cystector exhibits exceptional selectivity, minimal pH sensitivity within the physiological range, and an outstanding dynamic range of up to 4500% (Pedre et al., bioRxiv, <https://doi.org/10.64898/2026.01.27.69808>).

We will present results demonstrating the performance of Cystector in the cytosol and mitochondria of yeast cells by monitoring intracellular cysteine dynamics across different genetic backgrounds and growth conditions. Overall, our findings highlight the advantages of genetically encoded, real-time biosensors for investigating intracellular metabolite transport dynamics in yeast.

Session VIII

Transporters in biotechnology and metabolic engineering

IDENTIFICATION OF XYLOSE TRANSPORTERS IN *CHAETOMIUM THERMOPHILUM* BY INTEGRATED TRANSCRIPTOMICS AND FUNCTIONAL GENOMICS

Humberto Pereira¹, Tomas Bock^{2,3}, Katharina Zirngbil^{2,4}, Martin Beck^{2,5,6,7}, Kiran R. Patil^{2,8,9*}, **Björn Johansson**¹, Filipa Pereira^{2,10*}

¹Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga 4710-057, Portugal ²European Molecular Biology Laboratory, Heidelberg 69117, Germany ³Present address: Biozentrum, University of Basel, Basel, Switzerland ⁴Medical Research Council Toxicology Unit, University of Cambridge, Cambridge, UK ⁵Department of Molecular Sociology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany ⁶Cluster of Excellence SubCellular Architecture of Life (SCALE), Goethe University, Frankfurt am Main, Germany ⁷Institute of Biochemistry, Goethe University Frankfurt, Frankfurt am Main, Germany ⁸Medical Research Council Toxicology Unit, University of Cambridge, Cambridge, UK ⁹Department of Biochemistry, University of Cambridge, Cambridge, UK ¹⁰Life Sciences Institute, University of Michigan, Michigan 48109, United States of America

Lignocellulosic biomass represents one of the most abundant renewable feedstock for industrial fermentation. However, efficient utilization of the pentose sugar D-xylose remains a challenge for the preferred industrial organism *Saccharomyces cerevisiae* due to the absence of effective D-xylose transport and metabolism. This study investigates the xylose metabolic pathway in *Chaetomium thermophilum*, a thermophilic filamentous fungus known to proliferate in lignocellulose. Through an integrated multiomics approach, we identified key genes involved in xylose metabolism, including CtXR (xylose reductase), CtXDH (xylitol dehydrogenase), and CtXK (xylulose kinase). These genes were successfully expressed in *S. cerevisiae*, enabling xylose utilization. Additionally, we characterized four putative xylose transporters in *C. thermophilum*, identifying S3B3 as a functional D-xylose transporter actively expressed in *S. cerevisiae*. A C-terminal truncated variant of S3B3 alleviated D-glucose-mediated target degradation but did not enhance xylose uptake in co-fermentation conditions. These findings provide insights into xylose transport and metabolism in thermophilic fungi and contribute to the development of engineered yeast strains for efficient lignocellulosic bioconversion.

EXPLORING GLUCOSE TRANSPORT TO MODULATE THE FERMENTATIVE PERFORMANCE OF *TORULASPORA DELBRUECKII*

Bruna Oliveira¹, Susana R. Chaves¹, Paulo Silva¹, Björn Johansson¹, Ricardo Franco-Duarte¹, **Maria João Sousa¹**

¹CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal

Recent trends have identified the use of non-*Saccharomyces* yeasts as alternative starters to promote biodiversity and quality of fermented foods and beverages (e.g., wine, beer and bakery products). In particular, *Torulaspora delbrueckii* has emerged as a promising candidate due to its capacity to enhance product flavor and aroma, lower ethanol and volatile acidity production, as well as high osmotic and freeze stress resistance. However, significant knowledge gaps remain regarding the physiology, ecology and molecular/cellular mechanisms underlying *T. delbrueckii* behavior. Glucose transport has been shown to limit *T. delbrueckii* growth, namely in the presence of ethanol, hampering its fermentative performance. Until now, only two glucose transporters have been functionally characterized in *T. delbrueckii*, Lgt1 and Igt1. Genome analysis revealed that the genes encoding these transporters are present in a cluster together with two other putative transporter genes. By further analyzing all *T. delbrueckii* available genomes (94), we found that this cluster was conserved in all the strains, and that this yeast encodes between 5 and 7 Hxt transporter genes. Expression analysis of the 4 most conserved *T. delbrueckii* Hxt transporters at different fermentation stages, showed significant different regulation indicating potential diverse relevance. Furthermore, to evaluate if manipulating hexose transport might be a suitable strategy to increase *T. delbrueckii* growth rate and improve its fermentation capacity, we heterologously expressed the low affinity high-capacity *S. cerevisiae* transporter Hxt1 in *T. delbrueckii*, which successfully improved its specific growth rate. These results provide insights to enhance fermentative performance of *T. delbrueckii* through transporter manipulation, paving the way for future optimization efforts.

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THE YEAST GENERAL STRESS RESPONSE: AN ENERGY-SAVING MECHANISM AND TOOL TO INCREASE PRODUCT YIELDS”

Markus MM Bisschops¹; Nuran Temelli¹; Willem H Baris¹; Simon van den Akker¹; Ruud A Weusthuis¹

¹Wageningen University

Retentostats can be used to achieve stable, virtually non-growing, but metabolically active yeast cultures. In these nutrient-limited cultures, the majority of energy-substrate (e.g., glucose) is used for the maintenance energy requirement (MER): energy to maintain cellular homeostasis and activity. Despite the absence of other environmental stresses, the general stress response (GSR) is activated under these extremely slow growth conditions. In this work, we set out to find an evolutionary driver behind this GSR activation and explore if it can be used to express and secrete recombinant proteins in *Saccharomyces cerevisiae*.

To investigate an energetic advantage of the GSR, a strain with reduced GSR was constructed by deletion of the main activating transcription factors Msn2 and Msn4. Chemostat and fed-batch cultures showed that a reduction in GSR significantly increased maintenance energy requirements without impact on theoretical maximum biomass yield or maximal growth rate.

To test if the GSR can be used to strongly express heterologous genes in slow to non-growing yeast cells, the HSP12 promoter was selected and compared to the constitutive TEF1 promoter under slow-growing conditions using expression of a fluorescent protein. The GSR-promoter led to high intracellular fluorescent protein levels in slow growing fed-batch cultures with intracellular protein titers increasing 15-fold, whereas PTEF1-driven protein titers remained virtually constant. The specific production (qP) - growth (μ) rate correlation furthermore predicts a higher productivity at near-zero growth rates. However, when the target protein was secreted, opposite results were obtained. The TEF1 promoter outperformed that of HSP12, leading to higher titres and an inverted qP, μ correlation.

We show that an energetic advantage may drive evolutionary GSR activation under slow-growing, energy-limiting conditions in yeast. This conserved GSR can be used to drive heterologous expression, specifically in slow to non-growing yeast cells, thereby bringing efficient, growth-uncoupled production processes a step closer.

CARBOXYLIC ACID TRANSPORTER ENGINEERING FOR THE OPTIMIZATION OF MICROBIAL CELL FACTORIES

Isabel Soares-Silva^{1,2}, Francisca Ferreira^{1,2}, João Alves^{1,2}, Margarida Casal^{1,2}

¹Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal; ²Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal

Carboxylic acids are among the most prevalent organic compounds in nature and act as essential intermediates in cellular metabolism. The efficient transport of these molecules across the cell membrane is therefore crucial for cellular homeostasis.

Carboxylic acid transporters comprise a heterogeneous group of proteins from diverse families, exhibiting distinct structural features, modes of action, and regulatory mechanisms. A detailed understanding of both the metabolism and transport mechanisms of these compounds has become increasingly vital from both fundamental and applied perspectives.

Despite the broad applicability of carboxylic acids in the polymer, food, agricultural, and pharmaceutical sectors, the current portfolio of fermentation-derived carboxylic acids remains limited. A major bottleneck for their efficient and cost-effective bioproduction is their export through the microbial plasma membrane. While transporter engineering has been the focus of several studies, the optimization of cell membrane transporters for industrial carboxylic acid production still lags behind. This delay results from the inherent difficulties associated with the functional and structural characterization of membrane proteins.

The study of structure-function relationships of carboxylate transporters such as importers from the AceTr family^{1,2} and the highly efficient citrate exporter CexA3 from *Aspergillus niger*, a member of the DHA1 family, has expanded our knowledge of carboxylic acid transport mechanisms. By utilizing combined approaches of phylogenetic analysis, 3D-structure prediction, molecular docking studies, steered molecular dynamics, and site-directed mutagenesis, we have uncovered residues involved in substrate specificity, transporter kinetics, and stability.

These structural and functional insights provide a foundation for engineering transporter proteins, aiming at the development of optimized microbial cell factories for the industrial bioproduction of carboxylic acids.

¹ Rendulić, T., et al. Comput Struct Biotechnol J. 2021;19:4412-4425

² Sousa-Silva, M., et al. J Fungi. 2022; 8:51; 3Alves, J., et al. Comput Struct Biotechnol J. 2023;21:2884-2898

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ENGINEERING SUGAR TRANSPORTERS IN *KLUYVEROMYCES MARXIANUS* FOR APPLICATIONS IN BIOTECHNOLOGY

John Morrissey¹; Lorena Donzella^{1,2}; Maria João Sousa²

¹University College Cork; ²University of Minho

Kluyveromyces marxianus has gained increased attention for potential in industrial biotechnology, in part because of its capacity to assimilate a wide range of sugars. These include disaccharides such as lactose and cellobiose, as well as hexose and pentose monosaccharides. We have deployed genetic, molecular and biochemical methods to study sugar transporters in this yeast. In addition to dedicated transporters for particular metabolites, several different expansions within the major facilitator superfamily (MFS) have resulted in a plethora of structurally similar transporters with different characteristics. In some cases, domestication has also played a role. These transporters are important for use of *K. marxianus* in industrial biotechnology because they enable the use of low cost feedstocks, such as whey permeate, food processing side-streams, or lignocellulosic hydrolysates. To study *K. marxianus* transporters, we developed a screening platform by the targeted deletion of twelve MFS transporters and application of a synthetic biology pipeline for rapid testing of candidate transporters. Using this *K. marxianus* Δ PT platform, we identified several *K. marxianus* putative xylose or arabinose transporter proteins. Two of these, KMAR_10531 and KMAR_60179, were of particular interest and we kinetically characterised them using ¹⁴C-labelled substrates, revealing them to be medium- and low-affinity transporters, respectively. KMAR_10531, an Hgt-like proton symporter, also mediates high-affinity glucose uptake, limiting xylose assimilation in mixed-sugar environments. Using rational mutagenesis guided by in silico structural analysis, we generated the N325V variant, which reduced glucose transport while enhancing xylose affinity. Expression of KMAR_10531 N325V in a pentose-transporter-deficient *K. marxianus* strain enabled simultaneous co-consumption of glucose and xylose in bioreactors, overcoming the diauxic growth bottleneck observed with the native transporter. This work provides the first example of engineering an Hgt-like transporter for altered sugar specificity and establishes a robust platform for complete valorisation of mixed sugars in second-generation biorefineries.

Session IX

Transport and energetics in fungal pathogenicity and drug resistance

INTERACTION OF ERGOSTEROL BIOSYNTHESIS AND PLEIOTROPIC DRUG RESISTANCE GENES

W. Scott Moye-Rowley¹

¹University of Iowa Carver College of Medicine

Candida glabrata (Cg) (aka *Nakaseomyces glabratus*) is the second-leading cause of invasive candidiasis and is associated with a ~3-fold higher mortality than the major *Candida* pathogen, *Candida albicans*. A problematic clinical phenotype associated with Cg is the high frequency with which this yeast develops resistance to antifungal drugs which is likely to contribute to the high mortality associated with this organism. Cg resistance to the azole class of drugs, such as fluconazole, is so high that current recommendations call for the use of echinocandin drugs as first line therapy. The most commonly observed genetic changes leading to azole resistance in Cg are mutations within the PDR1 gene that encodes a transcription factor that regulates expression of a number of different membrane transporters that are thought to export azole drugs and limit their accumulation. Azole drugs directly act to inhibit the enzymatic product of the ERG11 gene and lead to a block in the synthesis of the essential fungal sterol ergosterol. Fluconazole exposure causes induction of ERG11 transcription that depends on the regulatory protein Upc2A. Using a biochemical approach, we determined that the Ypk2 protein kinase co-purified with Upc2A and was required for the fluconazole inducible transcription of ERG genes. Loss of YPK2 caused the resulting mutant strains to exhibit increased susceptibility to the three major classes of antifungal drugs, consistent with the kinase being required for the normal response to these different chemotherapeutic compounds. We also characterized a collection of Cg clinical isolates and, while we found the expected hyperactive alleles of PDR1, we also recovered 4 strains with 2 different forms of ERG11. Coupled with our earlier observations that changes in ERG11 expression led to Pdr1 activation, these data suggest a fundamental link between ergosterol levels and activation of the Pdr1-regulated drug resistance genes.

FROM ANTIFUNGAL ACTIVITY TO METABOLIC ACCESS: CHITOSAN AS A PERMEABILIZING AGENT IN YEASTS

Minerva Georgina Araiza Villanueva¹; Norma Silvia Sánchez¹; Francisco Padilla-Garfias¹; Augusto César Poot Hernandez¹; Martha Calahorra¹; Antonio Calvin Peña Díaz¹

¹Instituto de Fisiología Celular, Universidad Nacional Autónoma de México

Polycationic compounds have emerged as a promising class of antifungal agents due to their ability to interact with the negatively charged components of the fungal cell envelope. As a representative model, the biopolymer chitosan exerts its activity by targeting the cell wall and plasma membrane through electrostatic interactions, leading to varying degrees of cellular impairment. This study investigates the spectrum of biological responses elicited by chitosan in three distinct yeast models: *Saccharomyces cerevisiae*, *Candida albicans*, and *Debaryomyces hansenii*. Our findings reveal that susceptibility is highly species-dependent, with *D. hansenii* displaying extreme sensitivity compared to the relative resistance of the other species. Through physiological assays, including zeta potential measurements and ion efflux analysis, we demonstrated that these polycationic interactions induce membrane permeabilization while preserving the overall structural architecture. Transcriptional profiling further elucidated that resistance is governed by a shield response involving structural remodeling and chemical protection, whereas sensitivity is marked by a crisis state characterized by mechanical stress and oxidative damage. Based on this selective permeabilization, we developed a method for in situ enzymatic measurements. This approach uses chitosan as a molecular sieve that allows small cofactors to diffuse into the cell while retaining large cytoplasmic enzymes, such as glucose-6-phosphate dehydrogenase. This makes the polymer a versatile platform for developing antifungal agents and engineering whole-cell biocatalysts.

INTRACELLULAR IRON HOMEOSTASIS IS A KEY FACTOR REGULATING AZOLE CICALITY, TOLERANCE AND SUSCEPTIBILITY IN *CANDIDA ALBICANS*

Wouter Van Genechten¹; Rudy Vergauwen¹; **Patrick Van Dijck**¹

¹KU Leuven

We investigated the molecular mechanism of tolerance against fluconazole (FLC) in the human fungal pathogen *Candida albicans*. Tolerance is defined as slow growth at supra-MIC concentrations and possibly a driver for resistance development. We previously showed that a combination of the antibiotic doxycycline with FLC results in the absence of tolerance in a iron dependent way. A proteomics approach on purified mitochondria from cells treated with FLC alone or in combination with iron resulted in the upregulation of the vacuolar iron exporter Smf3 upon FLC treatment. A ROS assay revealed that a *smf3Δ/Δ* strain treated with FLC accumulated ROS to a similar extent as that displayed by the WT undergoing a FLC+DOX combination treatment. Thus, deletion of SMF3 mimics the addition of doxycycline in wild type cells. ROS accumulation, in part through altered iron availability from the vacuolar storage pool, is thus the molecular mechanism underlying the synergy between doxycycline and FLC.

We further investigated the role of mitochondrial iron in determining FLC susceptibility. Mitochondrial iron import is mediated by Mrs4, after which iron is primarily incorporated into iron sulfur clusters and heme. We investigated the role of mitochondrial iron import via Mrs4 in azole susceptibility phenotypes. We demonstrate that Mrs4 is essential for fluconazole tolerance under iron-chelating conditions, despite minimal effects on MIC or drug synergy. Loss of Mrs4 results in mitochondrial dysfunction, impaired heme biosynthesis, and accumulation of toxic sterol intermediates during FLC treatment. Collectively, our findings reveal that vacuolar iron export and mitochondrial iron homeostasis critically govern azole tolerance and highlight toxic sterol accumulation as a driver of fungicidal outcomes.

HIDDEN COMPLEXITY: HOW CHROMATIN REMODELLING DRIVES ANTIFUNGAL TOLERANCE AND HETERORESISTANCE

Maria Juliana Mantilla¹; Faiza Tebbji²; Antony Vincent³; Andrew Chatr-Aryamontri¹; **Adnane Sellam**^{1;2}

¹Université de Montréal; ²Montreal Heart Institute; ³Université Laval

Antifungal tolerance refers to the ability of a subset of susceptible cells to withstand drug concentrations above the minimum inhibitory concentration, allowing infectious fungi to persist during treatment and increasing the risk of therapeutic failure. Although mechanisms of antifungal resistance are well characterized, the pathways underlying tolerance remain poorly understood. Here, we undertook a genetic screen of a transcription factor mutant library to uncover novel regulators of azole tolerance in the opportunistic yeast *Candida albicans*. Our screen identified Isw2, the catalytic subunit of the ISW2 ATP-dependent chromatin remodeling complex, as a negative modulator of azole tolerance. Integration of isw2 mutant transcriptomic data with Isw2 genome-wide DNA-binding and targeted nucleosome-occupancy revealed that this chromatin remodeler regulates the boundaries of the nucleosome-depleted region (NDR) at the CRZ1 promoter to mediate a closed chromatin state, thereby restricting CRZ1 transcription. Beyond tolerance, our work delineates a role for Isw2 in modulating azole heteroresistance through Crz1. We further show that Isw2 influences amphotericin B sensitivity via Crz1, likely due to misregulation of genes encoding different vacuolar-associated functions. Our screen also identified mac1, a mutant lacking the copper (Cu) homeostasis regulator Mac1, which exhibited increased azole tolerance. Notably, reducing Cu levels in the growth medium markedly enhanced mac1 tolerance, whereas Cu repletion diminished it. Together, these findings reveal a novel role for chromatin remodeling and Cu homeostasis in shaping antifungal tolerance in *C. albicans*, providing the foundation for the mechanistic analyses presented in this work.

THE ABC TRANSPORTER PDR9 MODULATES *C. NEOFORMANS* TOLERANCE OF HOST CO₂, PLASMA MEMBRANE ASYMMETRY AND VIRULENCE

Damian Krysan¹

¹University of Iowa Carver College of Medicine

As environmental fungi, *Cryptococcus* species must adapt to the mammalian host environment to cause disease. *Cryptococcus neoformans* var. *grubii* is the predominant cause of human cryptococcosis and causes >180K deaths per year globally. However, not all strains of *C. neoformans* can cause mammalian infection even if they possess the three canonical virulence traits of tolerance to mammalian body temperature, synthesis of polysaccharide capsule, and capacity to melanize. Thus, there must be additional virulence traits that distinguish pathogenic and non-pathogenic *C. neoformans* strains. Our group has shown that the ability to tolerate the increase the 100-fold increase in CO₂ concentration between the environment and the host represents a virulence-bottleneck. Overall, clinical isolates of *C. neoformans* have increased CO₂ tolerance while environmental strains have reduced CO₂ tolerance. In addition, we have found that expression of an ABC transporter, Pdr9, negatively correlates with CO₂ fitness and virulence. Here, I will present data supporting this correlation as well as experiments designed to probe the mechanism of Pdr9's effect on CO₂ tolerance and plasma membrane phospholipid asymmetry.

IRON ANTAGONIZES FLUCONAZOLE ACTIVITY IN *CANDIDA GLABRATA* BY REDUCING DRUG ACCUMULATION

Carolina Vitorino Mariano¹; Teresa Pissarro¹; Margarida Gonçalves¹; Catarina Amaral¹; Catarina Pimentel¹

¹Instituto de Tecnologia Química e Biológica António Xavier

Fluconazole is the most widely prescribed antifungal agent worldwide. The drug targets Erg11, an enzyme involved in the biosynthesis of ergosterol, a key component of the fungal cell membrane. However, its fungistatic activity and extensive use have driven the emergence of fluconazole-resistant fungal pathogens. Recently, we found that iron loading conditions decrease fluconazole activity against *Candida glabrata*, an opportunistic yeast, that can cause life-threatening infections. The antagonism between the drug and the metal is evident across a wide range of concentrations. Iron upregulates genes crucial for fluconazole detoxification, namely the ABC transporters Cdr1 and Cdr2, and their regulator, the transcription factor Pdr1. Consistent with these findings, intracellular fluconazole levels are lower under co-treatment conditions and ergosterol depletion is less pronounced than under treatment with fluconazole alone.

The reduced activity of fluconazole under iron loading conditions may have important clinical implications, as this phenotype would not be captured by standard antifungal susceptibility assays, highlighting the need to consider the host environment when selecting antifungal treatment strategies.

REGULATION OF NUTRIENT-DEPENDENT DYNAMICS OF ATO PROTEINS AND PH MODULATION IN *CANDIDA ALBICANS*

Patrícia Ataíde¹; Faezeh Ghasemi^{1;2}; Diana Azevedo¹; Tamara Brkic^{3;4}; Mrinalini Parmar^{3;4}; Margarida Casal^{1;2}; Patrick Van Dijck⁵; Toni Gabaldón^{3;4;6;7}; Sandra Paiva^{1;2}

¹CBMA, University of Minho, Braga, Portugal; ²Institute of Science and Innovation for Bio-sustainability, University of Minho, Braga, Portugal; ³Barcelona Supercomputing Centre (BSC-CNS). Plaça Eusebi Güell, 1-3 08034 Barcelona, Spain; ⁴Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac, 10, 08028 Barcelona, Spain; ⁵Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, K.U. Leuven, Leuven, Belgium; ⁶Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain; ⁷CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Candida albicans is a commensal yeast of the human microbiota that can become an opportunistic pathogen, especially in immunocompromised individuals. Its adaptation to diverse host niches depends on metabolic flexibility during glucose limitation, including the use of alternative carbon sources, such as carboxylic acids and amino acids, which may contribute to growth, persistence, and virulence-associated traits. Acetate Transporter Ortholog (Ato) proteins have been implicated in carboxylate transport and environmental pH modulation. In particular, Ato1 has been characterized as a H⁺/acetate symporter. However, the regulation, localization dynamics, and broader physiological roles of Ato proteins remain poorly understood.

In this study, we investigated how three genes linked to amino acid metabolism, cell wall/environmental adaptation, and amino acid-responsive signalling – GDH2, CWT1, and STP2, respectively – affect Ato protein expression and localization under distinct metabolic conditions. Using CRISPR-Cas9, we generated a collection of *C. albicans* deletion mutants in backgrounds expressing chromosomally tagged Ato1-GFP, Ato2-GFP, or Ato3-GFP fusion proteins. Ato1-3 dynamics were analysed in living cells by fluorescence microscopy, and extracellular pH was monitored over time as a readout of metabolic activity and environmental alkalinization. Additionally, the susceptibility of ato mutants and GDH2, CWT1, and STP2 deletion mutants to clinically relevant antifungal agents was assessed.

Our findings show that GDH2, CWT1, and STP2 differentially influence Ato1-3 expression and plasma membrane localization, in a growth condition-dependent manner. These results suggest that the regulation of Ato proteins is integrated into broader adaptative responses to nutrient availability, linking carboxylate transport, amino acid-responsive metabolism, and pH modulation in *C. albicans*.

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Keywords: *Candida albicans*, carboxylic acids, nutrient sensing, amino acid metabolism, pH modulation, antifungal susceptibility

FUNCTIONAL CHARACTERIZATION OF STEROL IMPORTERS IN POLYENE RESISTANCE OF *CANDIDOZYMA AURIS*

Qingjuan Zhu¹; Maria Szomek²; Daniel Wüstner²; Patrick Van Dijck^{1,3}

¹Laboratory of Molecular Cell Biology, Department of Biology, Institute of Botany and Microbiology, KU Leuven, Kasteelpark Arenberg 31, 3000-Leuven, Belgium; ²Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M DK-5230, Denmark; ³Leuven One Health Institute

Candidozyma auris (formerly *Candida auris*) is an emerging multidrug-resistant yeast responsible for invasive infections and healthcare-associated outbreaks, with limited therapeutic options available. Polyene antifungals, particularly amphotericin B, remain clinically important because they target ergosterol in the fungal plasma membrane. However, reduced susceptibility to amphotericin B in *C. auris* underscores the need to understand how this pathogen remodels its membrane sterol composition in response to antifungal stress.

Our preliminary findings suggest that the uptake of exogenous sterols may contribute to polyene tolerance in *C. auris*. Unlike *Candida albicans*, *C. auris* was able to utilize extracellular cholesterol to restore growth under amphotericin B exposure. Fixed-cell imaging with the fluorescent ergosterol analogue dehydroergosterol (DHE) revealed pronounced species- and clade-specific differences in sterol uptake and intracellular trafficking. *C. albicans* isolates showed weak DHE fluorescence. In contrast, *C. auris* isolates displayed strong DHE fluorescence localized to the plasma membrane, vacuolar membrane, and lipid droplets. Notably, distinct DHE distribution patterns were observed among *C. auris* clades, suggesting clade-specific variation in sterol handling.

Comparative genomic analysis identified candidate *Saccharomyces cerevisiae* Aus1/Pdr11-like orthologs in *C. auris*, encoding putative sterol importers. To investigate their functional relevance, we generated single and double deletion mutants in *C. auris* and overexpressed selected *C. auris* transporter orthologs in *C. albicans* to determine whether they are sufficient to confer cholesterol-dependent growth rescue. Future studies will combine DHE-based sterol uptake imaging, cholesterol-rescue assays under amphotericin B stress, and antifungal susceptibility testing to define the role of these transporters in sterol import and polyene tolerance.

Together, this study aims to elucidate the mechanistic basis of sterol-mediated membrane adaptation in *C. auris*. By defining how specific transporter proteins facilitate the utilization of exogenous sterols under antifungal pressure, this work may identify sterol import pathways as potential targets for improving antifungal therapy against this emerging pathogen.

COMBINED GENOMIC AND PHARMACOLOGICAL SCREENING REVEALS BRILACIDIN AS A POTENT SYNERGIZER OF CASPOFUNGIN AGAINST *ASPERGILLUS FUMIGATUS*

Gustavo H. Goldman¹

¹FCFRP, Universidade de São Paulo

Invasive aspergillosis carries high mortality due to limited antifungals and rising resistance. To identify novel adjunctive therapies, we screened 1,402 compounds for their ability to potentiate the activity of caspofungin (CAS) against the fungal pathogen *Aspergillus fumigatus*. This uncovered brilacidin (BRI), a host-defense peptide mimetic that synergizes with CAS (FIC index 0.39) and potentiates voriconazole (VOR). BRI overcomes CAS resistance in *fks1* mutants and extends synergy to *Candida albicans*, *C. auris*, and *Cryptococcus neoformans*. To dissect BRI's mechanism of action, we employed two complementary genetic screening strategies. First, screening a phosphatase-null mutant library and protein kinase inhibitors identified calcineurin and the cell wall integrity (CWI) MAPK MpkA as critical determinants: Δ mpkA and Δ calA mutants showed altered susceptibility to BRI+CAS, and the combination induced CrzA nuclear translocation, confirming involvement of the CWI pathway. Second, a forward genetic screen of 484 transcription factor null mutants for BRI hypersensitivity revealed Δ sptA, Δ sppA, and Δ hapB as hypersensitive, with enhanced BRI-mediated potentiation of CAS, VOR, amphotericin B, and myriocin. RNA-seq of these mutants versus wild-type under BRI exposure showed significant enrichment for differentially expressed genes involved in ergosterol and fatty acid biosynthesis, and biochemical validation confirmed that Δ sptA accumulates twice the ergosterol of wild-type under BRI challenge. Thus, mutant screening directly linked both the CWI pathway (calcineurin/MpkA) and lipid metabolism to BRI's mechanism. In vivo, BRI+CAS reduced lung fungal burden by 95% in a murine model of aspergillosis, and topical BRI alone ablated disease in a model of fungal keratitis, with no toxicity. Our integrated chemical and genetic screening approach establishes BRI as a promising dual adjuvant for CAS and VOR, revealing that BRI acts through coordinated disruption of membrane potential, CWI signaling, and lipid homeostasis.

Funding: FAPESP, Brazil

BETTER TOGETHER: A MULTIFUNCTIONAL TRIAZOLE-COPPER COMPLEX BYPASSES INTRINSIC AND ACQUIRED FLUCONAZOLE RESISTANCE

Catarina Pimentel¹

¹ITQB NOVA

Azoles remain the most widely used class of antifungal drugs for the treatment of life-threatening fungal infections and, in many resource-limited settings, fluconazole remains the only therapeutic option available. However, the increasing prevalence of resistance is compromising treatment efficacy.

We have recently uncovered the molecular mechanisms by which copper, enhances the efficacy of fluconazole. These findings inspired the design of compounds that combine azoles with copper-binding groups. In this talk, I will present our journey toward the development of these compounds, which culminated in the synthesis of a novel triazole-copper complex with remarkable antifungal activity against all WHO critical-priority fungal pathogens. Beyond the expected inhibition of ergosterol biosynthesis mediated by the azole moiety, the complex modulates the activity of key fungal transporters, which together contribute to its ability to overcome common azole resistance mechanisms and reduce the likelihood of resistance development.

This work supports the concept that copper-azole conjugates can restore or enhance azole efficacy by simultaneously targeting multiple fungal pathways.

Austrian Embassy Plenary Talk

MULTIDRUG RESISTANCE IN PATHOGENIC FUNGI - WHEN COMPLEXITY NEVER ENDS

Karl Kuchler¹ & Collaborators

¹Medical University of Vienna

Candida auris is a newly emerging opportunistic human fungal pathogen, causing global hospital outbreaks of fungal infections of high mortalities in immunocompromised individuals. The pronounced skin tropism, (pan-antifungal) multidrug resistance (MDR) traits against all antifungal drugs, and the ease of transmission in clinical settings prompted the WHO to declare *C. auris* as a top priority pathogen for drug discovery. However, the utmost complexity underlying clinical MDR traits remains as a major challenge to antifungal drug discovery. In my short talk, I will discuss currently known MDR mechanisms in fungal pathogens, possible ways to overcome MDR, as well as newly emerging therapeutic strategies. Finally, I shall discuss as yet underappreciated mechanisms that link pathogen MDR traits and fitness in the context of immune evasion.

This presentation receives generous support from the Austrian Embassy in Lisbon.

Nicolau van Uden Plenary Lecture

DIRECTIONAL MEMBRANE PROTEIN TRAFFIC VIA ERV14/CORNICHON

Liz Miller¹

¹University of Dundee

One quarter of the eukaryotic proteome comprises transmembrane proteins that contribute to diverse cellular and organismal functions. These proteins are synthesised in the endoplasmic reticulum (ER) and then trafficked to downstream compartments via membrane-bound intermediates. Many proteins that reside at the plasma membrane require an ER export receptor, Erv14, to efficiently traffic out of the ER. We have dissected the mechanism by which Erv14 and its fly ortholog, cornichon, reversibly engage cargo proteins. We show that the binding interface is buried within the lipid bilayer, and that the local lipid environment influences transmembrane helix dynamics to modulate the interaction. Our results suggest that in the thin bilayer of the ER, Erv14 engagement with clients optimally presents bivalent ER export signals that engage with the COPII cargo adaptor subunit, Sec24, to drive capture into vesicles. Upon delivery to the thicker membrane of the Golgi, conformational dynamics within the membrane helices of Erv14 likely eject the cargo client. Using new phenotypes associated with loss of Erv14/cornichon function in the fly, we show that this mechanism is likely conserved across eukaryotes and serves at least in part to modulate the rate of protein flux to the plasma membrane.

POSTERS

***DEBARYOMYCES HANSENII* COORDINATES XENOBIOTIC DETOXIFICATION AND ANTIOXIDANT RESPONSES DURING BENZO(A)PYRENE DEGRADATION**

Francisco Padilla-Garfias¹; Minerva Araiza-Villanueva¹; Norma Silvia Sánchez¹; Martha Calahorra¹; Antonio Peña¹

¹Instituto de Fisiologia Celular, Universidad Nacional Autónoma de México

Polycyclic aromatic hydrocarbons (PAHs), including benzo(a)pyrene (BaP), are recalcitrant pollutants that accumulate in diverse ecosystems and represent a major environmental concern because of their toxicity and carcinogenic potential. Finding microorganisms that are capable of efficient degradation of these compounds, while maintaining metabolic activity under stressful conditions, is still one of the main challenges in bioremediation research.

In this study, we combined physiological, molecular, transcriptomic, and biochemical approaches to better understand how the marine yeast *Debaryomyces hansenii* responds to BaP exposure and why it represents an interesting candidate for mycoremediation. Comparative analyses with *Candida albicans*, *Rhodotorula mucilaginosa*, and *Saccharomyces cerevisiae* showed that all species were able to metabolize more than 70% of BaP while maintaining viability. However, *D. hansenii* consistently showed the strongest tolerance and degradation capacity.

Chemical inhibition assays demonstrated that cytochrome P450 activity is required for the initial oxidation of BaP, and functional analyses identified DhDIT2 as an important gene involved in this process. Transcriptomic analyses revealed an extensive cellular remodeling associated with xenobiotic metabolism, oxidative stress adaptation, glutathione homeostasis, membrane dynamics, and energy metabolism. Genes related to cytochrome P450 monooxygenases, epoxide hydrolases, glutathione S-transferases, and antioxidant systems were strongly induced during BaP exposure.

Biochemical analyses further showed that BaP exposure increased reactive oxygen species, lipid peroxidation, and protein oxidation. Even under these conditions, *D. hansenii* activated coordinated antioxidant defenses involving superoxide dismutase, catalase, glutathione peroxidase, and glutathione-dependent pathways, allowing the cells to maintain redox balance during BaP biotransformation.

Altogether, our results show that *D. hansenii* combines stress resistance with efficient xenobiotic detoxification, highlighting its potential as a robust eukaryotic platform for future bioremediation strategies.

NRG1-RTG3 CHIMERIC MODULATOR REGULATES EXPRESSION OF GENES ENCODING PROTEINS INVOLVED IN THE ORGANIZATION OF MITOCHONDRIAL NUCLEOIDS IN *SACCHAROMYCES CEREVISIAE*

Andrea de la Vega-Alvarado¹; Carlos Campero-Basaldua¹; Edgar Ramirez¹; Francisco Padilla-Garfías¹; Leonardo Peraza-Reyes¹; Valeria Gómez-Marín¹; Héctor Quezada²; Alicia González¹

¹Instituto de Fisiología Celular, Universidad Nacional Autónoma de México; ²Instituto de Investigación en Inmunología y Proteómica, Hospital Infantil de México Federico Gómez, Ciudad de México

In the yeast *Saccharomyces cerevisiae*, the Nrg1-Rtg3 chimeric regulator plays an essential role in maintaining mitochondrial DNA integrity. Direct measurement of the mitochondrial DNA gene complement showed that *nrg1Δ* or *rtg3Δ* mutants completely lose the mitochondrial DNA, resulting in a petite phenotype and confirming that the absence of Nrg1-Rtg3 leads to irreversible damage; consequently, these strains are unable to carry out respiratory metabolism.

We report results showing that Nrg1-Rtg3 hybrid regulator acts as a transcriptional activator, modulating the expression of *ILV5* and *MGM101*. Null mutants of Rtg3 or Nrg1 exhibit a significant decrease in *ILV5* and *MGM101* expression. The *ilv5Δ* mutants have a diminished pool of mitochondrial DNA, while *mgm101Δ* strains completely lose their mitochondrial DNA. *Ilv5* and *Mgm101* are important proteins that function as nucleoids to protect the organization and stability of mitochondrial DNA. Additionally, we confirm the DNA-binding site of Nrg1-Rtg3 complex in the promoters of *ILV5* and *MGM101*, indicating positive regulation. Our results also show that expression of other genes encoding proteins involved in maintaining mitochondrial DNA stability, such as *RIM1*, *ACO1*, and *ABF2* are independently regulated by Rtg3 or Nrg1, and not by the Nrg1-Rtg3 chimeric regulator. These results highlight the importance of the regulatory role played by the chimeric Nrg1-Rtg3 transcriptional modulator in maintaining the fermento-respiratory metabolism of the yeast *S. cerevisiae*.

ADAPTATION OF YEAST PLASMA MEMBRANE TO PROLONGED GLUCOSE STARVATION VIA CHARGE-DEPENDENT CLUSTERING OF PROTON PUMP PMA1

Aniket Bandyopadhyay¹; Iryna Levkovych¹; Dekai Dong¹; Roland Wedlich-Söldner¹

¹Institute for Cell Dynamics and Imaging, University of Münster

Yeast cells, as saprotrophs, are forced to adapt to rapidly changing nutrient levels. One level of cellular control involves endocytic internalization of nutrient transporters at the plasma membrane (PM) during nutrient starvation. Interestingly, one of the most abundant PM transporters in budding yeast *Saccharomyces cerevisiae*, the P-type ATPase Pma1, remains at constant levels up to 12 h of glucose depletion. However, upon acute glucose removal, Pma1 relocates laterally from continuous networks in exponentially growing cells to isolated clusters corresponding to crystalline arrays of Pma1 hexamers. The process of Pma1 clustering is dependent on the equally abundant 7 transmembrane protein Mrh1 and its positively charged C-terminal tail. This suggests a mechanism involving negatively charged lipids at the inner leaflet of the PM. Indeed, while in wild type cells Mrh1 co-clusters with Pma1, it remains completely dispersed in cells lacking negatively charged phosphatidylserine (Δ cho1). Quantification of Pma1 clustering reveals highly non-linear kinetics consistent with a charge-modulated nucleation mechanism. Stochastic simulations of 2D-Pma1 crystal formation reproduce our experimental observations and provide kinetic predictions that we further validated using a range of Mrh1 charge-variants. Importantly, Pma1 clustering leads to more robust inhibition of its ATPase activity and a slower response to rapid glucose fluctuations. Failure of clustering (Δ mrh1) is associated with faster activation but ultimately with more rapid aging of yeast cells, as indicated by an increased number of senescent cells during gradual glucose depletion.

Key words: P-type ATPase Pma1, glucose starvation, charge-dependent clustering, nucleation, senescence.

MITOCHONDRIAL RETROGRADE SIGNALING SHAPES DEVELOPMENT OF YEAST MULTICELLULAR COMMUNITIES

Libuse Vachova¹; Martina Humpalova¹; Zdena Palkova²

¹Institute of Microbiology of the CAS, BIOCEV, Prague; ²Charles University, Faculty of Science, BIOCEV, Prague

Unicellular microorganisms, including yeast, can form multicellular structures such as colonies and colony biofilms when growing on semisolid or solid surfaces exposed to air, as well as biofilms at the interface with a liquid phase. Within these structures, cells differentiate into precisely localized subpopulations that perform distinct functions, with their localization and characteristics depending on the type of multicellular structure. We previously demonstrated that mitochondria and mitochondrial retrograde (RTG) signaling play important roles in colony differentiation and identified three specific RTG regulatory branches—the ATO, Cit2, and viability branches—that govern the differentiation of U cells and the L1 and L2 subpopulations of L cells, respectively (Oncotarget 7: 15299, 2016).

Using proteomic analyses of U and L cell subpopulations from alkaline-phase colonies, along with their ancestral cells from acidic-phase colonies, of strains carrying deletions in RTG pathway genes, we identified targets of RTG-dependent regulation in both U and L cells. Combined with microscopic and Western blot analyses, these studies revealed that the RTG pathway predominantly regulates processes in U cells, a subpopulation of long-lived cells with unique physiological properties localized in the upper regions of colonies. In U cells, Rtg proteins activate pathways associated with acetyl-CoA metabolism, the tricarboxylic acid (TCA) cycle, and amino acid biosynthesis, including the transport of metabolic intermediates between mitochondria and the cytosol. RTG regulators also play a key role in the suppression of mitochondrial activity observed in U cells by repressing the expression of mitochondrial ribosomal components and consequently reducing mitochondrial translation (IJMS 22: 5597, 2021). A comparison of the expression patterns of selected RTG pathway targets in colonies and colony biofilms revealed both shared and distinct features of RTG-mediated regulation in these two types of multicellular structures.

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FOLLOWING STEROL DISTRIBUTION IN YEAST WITH IMIDAZOLIUM-BASED STEROL ANALOGUES

Kevin Joseph Dilip¹; Nele Van Wyngaerden²; Annette Janning¹; Christian Schuberth¹; Frank Glorius²; Roland Wedlich-Söldner¹

¹Institute for Cell Dynamics and Imaging, University of Münster; ²Organisch-Chemisches Institut, University of Münster

Sterols are essential components of eukaryotic membranes, yet their intracellular trafficking and organization remain incompletely understood. Here, we investigate sterol uptake and distribution in budding yeast using a combination of genetic and chemical tools. Commercial sterol analogues labelled on the hydrophobic tail are efficiently internalized but undergo rapid esterification and accumulate in lipid droplets, limiting their utility for tracking membrane sterols. Using *hem1Δ* cells, which are defective in sterol synthesis but can be rescued by supplementation with the precursor aminolaevulinic acid (ALA), we establish a system to study exogenous sterol incorporation.

We further explore imidazolium-based sterol analogues, including CHIM-L and a flexible derivative (flex-CHIM), which resist esterification but display poor cellular uptake. Conjugating these probes to the hydrophobic fluorophore BODIPY-FL allows for long term imaging revealing that CHIM uptake is rapid and targets endoplasmic reticulum and other intracellular membranes. In contrast, analogues coupled to less hydrophobic dyes remain anchored to the plasma membrane or fail to enter cells altogether.

Finally, we examine yeast mutants lacking phosphatidylserine, which form large protein-free plasma membrane regions (“void zones”) enriched in sterols. Ongoing work investigates whether CHIM-based probes can access these domains, providing robust tools to visualize sterol-rich membrane microdomains *in vivo*.

Keywords: Plasma Membrane, Fluidity, Sterol depletion, Void zones, CHIMs

CATCHFIRE: A FAST AND REVERSIBLE RE-LOCALIZATION SYSTEM FOR *S. CEREVISIAE*

Dekai Dong¹

¹University of Muenster

We have implemented CATCHFIRE, a fast and reversible chemically controlled protein re-localization system, in *Saccharomyces cerevisiae*. The system enables rapid and tunable association between a protein of interest and a defined partner upon addition of a small-molecule fluorogenic dimerizer. Fluorescence output varies depending on the dimerizer used, allowing flexible experimental design. Importantly, association is reversible, with rapid and efficient dissociation following removal of the dimerizer.

We demonstrate that CATCHFIRE is fully compatible with yeast and provide a plasmid toolkit for endogenous protein tagging. The induced interactions are stable over extended periods, ranging from minutes to days, and allow efficient and temporally controlled accumulation of proteins at designated intracellular sites.

The functional utility of CATCHFIRE is illustrated through targeted disruption of cell polarity. Forced relocalization of the essential polarity factor Cdc24 to ectopic, non-polar sites prevents normal polarization and budding. Importantly, these defects are reversible: removal of the dimerizer or the interaction partner restores polarization and normal cell cycle progression.

CATCHFIRE thus provides a powerful and versatile tool for dynamic control of protein localization in vivo. Potential applications include regulated retention of proteins within membrane compartments, manipulation of organelle contact sites, and probing protein conformational states through conditional tag accessibility.

EISOSOMES AND FLP-MEDIATED REWIRING OF FATTY ACID METABOLISM IN QUIESCENCE MAINTENANCE AND PROTECTION AGAINST FERROPTOSIS

Olga Martzoukou¹; Fabienne Vierendeels²; Giorgia Di Liberto²; Bruno André¹; Christos Gournas²

¹Université Libre de Bruxelles (ULB), Brussels, Belgium; ²LABIRIS Institute, Brussels, Belgium

Quiescence, a reversible cellular state of non-proliferation, enables microorganisms to survive prolonged nutrient limitation and is associated with persistence, aging, and resistance to stress. In yeast, the consorted action of several regulatory pathways is required for quiescence maintenance, among which is the stabilization of the eisosome-localized, ubiquinol-regenerating Flavodoxin-like proteins (FLPs). Notably, this stabilization protects quiescent cells from ferroptosis, an iron-dependent cell death caused by the accumulation of peroxidised phospholipids (PLs). Herein, we further report that quiescent cells lacking FLPs show defects in lipid stockage and metabolism. Specifically, *flpΔ* cells display altered localization of enzymes involved in fatty acid activation and mobilization, such as TAG lipases/acyltransferases and fatty acyl-CoA synthetases, as well as a transient downregulation of peroxisomal enzymes, accompanied by altered lipid droplet abundance following glucose exhaustion. In addition, increased sensitivity to polyunsaturated fatty acids was observed for strains lacking phospholipid-remodeling-associated enzymes, suggesting a potential role in protection from peroxidised phospholipids. We also analyze the temporal and spatial relationship between eisosomes and peroxisomes, with either mitochondria, the endoplasmic reticulum, or lipid droplets using multicolor fluorescence microscopy and Split-Venus reporters, revealing a close association between organelles and a rewiring of certain membrane contact sites (MCSs) in cells lacking FLPs or eisosomes. Our results support a model in which eisosomes and/or FLPs regulate lipid metabolism and selected MCSs to ensure survival of quiescent cells, suggesting that fatty acids may be redirected toward phospholipid remodeling as an alternative membrane-repair mechanism under oxidative stress.

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GENOMIC CHARACTERIZATION OF THE SUGAR TRANSPORTER REPERTOIRE ACROSS 94 *TORULASPORA DELBRUECKII* STRAINS

Bruna Oliveira¹; Ricardo Franco-Duarte¹; Maria João Sousa¹

¹Universidade do Minho

Climate change-driven imbalances in wine composition can be mitigated by the use of non-*Saccharomyces* yeasts such as *Torulaspora delbrueckii*. Under high-sugar stress, a consequence of increased sugar accumulation in grapes, *T. delbrueckii* can lower ethanol yields, increase glycerol production, reduce off-flavors and enhance aroma complexity. Nonetheless, its widespread use is limited by its slower fermentation kinetics and prolonged lag phases compared to *Saccharomyces cerevisiae*, rarely completing fermentation on its own. Because efficient alcoholic fermentation depends heavily on effective sugar uptake, and we have previously found that different *T. delbrueckii* strains can present very different fermentation behavior, in the present work we aimed to broaden the understanding of the sugar transporter repertoire in this species. Using all 94 available genomes in NCBI, we followed a bioinformatics pipeline to identify suitable candidate transporters. Tools such as RagTag, Augustus, BLASTp, InterproScan, DeepTMHMM and DeepLoc 2.0 were used. Our results revealed the presence of a diverse array of transporters across several classes (HXT-like, HGT-like, sensors, mitochondrial transporters, etc). Compared to *S. cerevisiae*, fewer HXT-like transporters were identified, with most strains encoding only four to six. With few exceptions, all strains encode a single mitochondrial transporter, similar to *S. cerevisiae* Hxt14. Regarding the sensor-like proteins, at least two sequences were identified for each strain, though a small number of strains encoded one or three. Interestingly, a small number of strains encoded sequences similar to known HGT-like transporters. Several other classes of transporters (including those for maltose, intracellular sterol, glycerol, vacuolar, inositol and fructose transport), were found to be present across most strains. Altogether, this genomic characterization provides critical information and can pave the way for a better understanding of *T. delbrueckii* behavior and for the selection of strains able to overcome the sugar transport limitations that restrict their widespread use in the wine industry.

EXPLORING CI TRANSPORT BOTTLENECKS IN SYNTHETIC AUTOTROPHIC YEAST

Despina Noti¹; Diethard Mattanovich²; Inge Van Bogaert¹

¹Centre for Synthetic Biology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium;

²Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, BOKU University, Vienna, Austria

Engineering yeast to enable autotrophic growth, offers a promising route towards sustainable bioproduction and CO₂ valorization. However, the performance of such engineered strains remains rather limited due to inefficient inorganic carbon (Ci) assimilation. RubisCO -the enzyme responsible for CO₂ fixation- displays low affinity for CO₂ and competing oxygenase activity. Natural autotrophs have evolved carbon concentrating mechanisms (CCMs), in order to overcome this limitation. CCMs include specialized bicarbonate or carbon dioxide transporters that facilitate elevated CO₂ availability near RubisCO. In contrast, the development of synthetic autotrophy has mainly focused on heterologous pathway optimization, neglecting the importance of Ci transport across membranes.

Our work explores whether enhancing Ci uptake can alleviate this bottleneck in a synthetic autotrophic *Komagataella phaffii* strain . A set of bicarbonate and carbon dioxide transporters from cyanobacteria, algae and diatoms was individually expressed in the engineered host. The growth was evaluated under controlled CO₂ levels, where improved uptake was expected to translate into increased growth.

Preliminary results showed that none of the tested transporters led to a measurable improvement in the strain's growth rate, thus revealing questions regarding transporter functionality or downstream metabolic constraints that hide potential gains in substrate flux. Further work is needed to assess whether the neutral observations are attributed to transporter mislocalization or inactivity, before ruling out the importance of CO₂ transport in this system.

Keywords: Synthetic autotrophy, inorganic carbon transporter, *Komagataella phaffii*, carbon uptake

DECIPHERING CITRATE RECOGNITION BY CEXA THROUGH MOLECULAR DYNAMICS SIMULATIONS

Francisca Ferreira¹; João Alves^{1;2}; Margarida Casal^{1;2}; Isabel Soares-Silva^{1;2}

¹Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal; ²Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The filamentous fungus *Aspergillus niger* is currently the main microbial cell factory for citric acid bioproduction, accounting for approximately 80 % of the global industrial production. Its high secretion capacity is attributed to the efficient citrate exporter CexA, whose structural and functional characterisation has identified critical residues involved in citric acid transport. In our lab, we performed Molecular Dynamics simulations of the predicted 3D structure of CexA from AlphaFold embedded in a yeast membrane, which revealed a putative citrate-binding site located in the intracellular side of the protein. The amino acid residues S178, R181, V185, K380, and S450 were targeted for site-directed mutagenesis, resulting in the design of 14 mutant alleles, which were expressed in *Saccharomyces cerevisiae* CEN.PK 113-5D strain. The phenotypic effects of the mutations were assessed by GFP-tagging the proteins and spot assays, evaluating protein localisation and cell growth on media containing different carboxylic acids as sole carbon sources, respectively. One mutation affected the proper localisation of the transporter at the plasma membrane. Eleven mutant alleles promoted improved cell growth on carboxylic acids compared to cells expressing the native transporter, indicating a decreased export activity, whereas the remaining alleles displayed growth profiles similar to the wild-type CexA. Molecular Dynamics simulations revealed key mechanistic insights into citrate export by CexA, providing a foundation for the rational engineering of membrane transporters for industrial carboxylic acid bioproduction.

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ENGINEERING THE DCT-02 TRANSPORTER TO ENHANCE SUCCINATE BIOMANUFACTURING

Francisca Ferreira¹; João Alves^{1;2}; Margarida Casal^{1;2}; Isabel Soares-Silva^{1;2}

¹Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal; ²Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Succinic (SA) and malic (MA) acids have widespread applications across various industries. As their production still largely depends on petrochemical-based processes, there is an increasing need to develop more environmentally sustainable and efficient bio-based production strategies using microbial factories. The Dct-02 transporter from *Aspergillus niger*, of the Tellurite-resistance/Dicarboxylate Transporter (TDT) family, when expressed in *Saccharomyces cerevisiae* cell factories, promotes the secretion of SA and MA. We engineered Dct-02 with the goal of improving the bioproduction of dicarboxylic acids in microbial factories. The BLAST search used Dct-02's sequence as the query to identify conserved amino acid residues across the TDT family. To identify potential substrate-binding positions, AlphaFold's Dct-02 model was used in molecular docking analyses with dicarboxylic acids. Thirty-one mutant alleles were constructed through site-directed mutagenesis and expressed in *S. cerevisiae* CEN.PK 113-5D and W303-1A *jen1Δ ady2Δ* strains. Dct-02 alleles' subcellular localisation was assessed by GFP-tagging, revealing nine alleles not properly localised at the plasma membrane. For the remaining mutants, we analysed cells' ability to grow on media containing different carboxylic acids and to import/export SA and MA by spot and transport assays. Eleven mutant alleles promoted improved cell growth on carboxylic acids, indicating a decrease of Dct-02 transporter activity, whereas three mutant alleles presented poorer growth, suggesting increased export capacity. The quantification of extracellular accumulation of organic acids evidenced one mutant allele with increased SA and MA secretion compared to the wild-type. The Dct-02 alleles had no impact in the import of radiolabelled dicarboxylates. In conclusion, Dct-02 primarily functions as a SA and MA exporter in *S. cerevisiae*. We engineered a Dct-02 mutant allele with improved export capacity, a feature with implications in succinate bioproduction by yeast.

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DECIPHERING WATER AND SOLUTE TRANSPORT THROUGH GRAPEVINE AND CORK OAK AQUAPORINS USING AQY-NULL *SACCHAROMYCES CEREVISIAE*

Farzana Sabir¹; Graça Soveral²; Catarina Prista¹

¹LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal; ²Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003, Lisboa, Portugal

Aquaporins (AQPs) are channel proteins that regulate the membrane water flux in almost all living organisms. They belong to Major Intrinsic Protein (MIPs) family. Plants have a particularly high diversity and number of AQPs, with diverse substrate profiles for physiologically important solutes. However, identifying the specific transport functions of individual AQP isoform remains difficult due to complex genetic network of plants and tissue-specific regulation of AQPs in planta. To address this, we used aqy-null *Saccharomyces cerevisiae* expression system. This strain provides a clean background, in which a single heterologous AQP can be expressed and functionally characterized without interference from the host's own AQP activity. Using this system, we studied the transport properties of plasma membrane (PIPs), tonoplast (TIPs), and Nodulin26-like (NIPs) intrinsic proteins from two economically important Portuguese crops; grapevine (*Vitis vinifera* L.) and cork oak (*Quercus suber* L.). GFP-tagging confirmed membrane localization of these AQP constructs in yeast cells. Permeability assays by stopped-flow fluorescence spectroscopy showed that grapevine (VvPIP2;1, VvTIP2;1, VvTIP2;2) and cork oak (QsPIP2;4 and QsTIP2;1) AQPs transport water, while NIPs from both plants (VvNIP1;1, VvNIP2;1 VvNIP6;1, QsNIP1;2 and QsNIP6;1) facilitate glycerol transport. Yeast growth/sensitivity assays in the presence of metalloids (arsenic and boron) provided a complementary phenotypic screening of solute transport. Mutagenesis of the aromatic/arginine (ar/R) selectivity filter in yeast-expressed grapevine NIP constructs revealed the regulatory significance of this region for channel conductance. In cork oak, we identified an unexpected mercury-induced activation of QsPIP2;4, dependent on a conserved cysteine69 residue.

Together, these findings emphasize the versatility of yeast as a transporter biology tool, combining genetic manipulation with biophysical assays, difficult to achieve in planta.

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CHARACTERIZATION OF THE MECHANISM OF FILAMENTATION INDUCTION BY *SACCHAROMYCES CEREVISIAE* AND *CANDIDA ALBICANS* MEP2 AMMONIUM TRANSCCEPTORS

Florence Angelucci¹; Ana Sofia Brito¹; Raquel Torres¹; Mélanie Boeckstaens¹; Anna Maria Marini¹

¹Université Libre de Bruxelles

Filamentation is a dimorphic transition linked to virulence of pathogenic fungi, such as *Candida albicans*. This transition is triggered by several environmental cues including low ammonium availability. Under these conditions, Mep2-type proteins, belonging to the conserved Mep-Amt Rh family of ammonium permeases, are needed. Mep2 has been proposed to act as a transceptor integrating both transporter and receptor activities. However, the signaling mechanism remains poorly understood. Two models are currently considered: the classical receptor model, in which Mep2 interacts with downstream signaling partners, and the mechanism-driven model, in which the specific mode of ammonium translocation indirectly promotes filamentation. Indeed, the non-signaling Mep1 and Mep3 proteins are proposed to transport NH_3 and H^+ following NH_4^+ deprotonation, leading to intracellular acidification, whereas Mep2 would transport only NH_3 , resulting in intracellular alkalization. Here, we investigate the role in transport and signaling functions of the cytosolic C-terminal domain (CTD) of *Candida albicans* Mep2 expressed in *Saccharomyces cerevisiae* cells lacking endogenous ammonium permeases. As previously observed in *C. albicans*, CaMep2 Δ 423 truncated variant induces impaired filamentation compared to CaMep2 and CaMep2 Δ 440 expressed in *S. cerevisiae*, highlighting the crucial role of the 423-440 region in signaling function. Our data indicate that, as in ScMep2, CaMep2 CTD fine-tunes the transport activity of the hydrophobic core via an autoinhibitory domain downstream of residue 440 and an enhancer domain upstream to this position. While CaMep2 transport activity appears sensitive to the regulation by the TORC1 effector kinase ScNpr1 in *S. cerevisiae*, ScNpr1 does not mediate efficient phosphosilencing of the autoinhibitory domain of CaMep2. Fusing ScMep2 CTD to CaMep2 hydrophobic core relieves the ScNpr1 requirement, resulting in a constitutively and highly active hydrophobic core capable of promoting filamentation. We will further discuss the intricate link observed between transport and signaling functions of yeast Mep2 proteins and the involvement of their CTD in controlling both functions.

CHARACTERIZATION OF FUNCTIONAL SPECIFICITIES OF RH-TYPE PROTEINS BELONGING TO THE MEP- AMT-RH FAMILY OF AMMONIUM PERMEASES

Zara Esen¹; René Wintjens¹; Mélanie Boeckstaens¹; Fadi Abdulsater¹; Anna Maria Marini¹

¹Université Libre de Bruxelles

Ammonium is a nitrogen source and a key player in pH control in animals, but it can also turn toxic at high concentrations. Ammonium is however not a simple toxic waste, it can accumulate within tumor microenvironment, stimulate immune evasion and favor tumor progression. The specific transport of ammonium is carried out by the permeases of the conserved Mep-Amt-Rh family. These proteins are subdivided into two major subfamilies : Mep-Amt members are widespread in bacteria, fungi and plants, they are present in some invertebrates but absent from vertebrates. Rhesus (Rh) proteins are instead found mostly in vertebrates, invertebrates and in a few bacteria. Human possesses up to five RH genes. Three encode the Rh blood group antigens RhCE and RhD (absent/non-functional in Rh- individuals), and their associated glycoprotein RhAG. The remaining two encode non-erythroid RhBG and RhCG proteins. Some organisms like nematode and specific bacteria possess members of both subfamilies indicating functional specificities still to be deciphered.

One axis of this work focuses on the molecular mechanisms enabling the intrinsic regulation of ammonium transport, an aspect remaining poorly described for Rh proteins. The cytosolic C-terminal domain (CTD) of Mep-Amt proteins contains a highly conserved stretch of ~20 amino acids that constitutes a positive regulatory domain required to activate transport through the hydrophobic core. This domain is however not conserved in Rh proteins. Here, multiple sequence alignments are used to highlight potential conserved regulatory domains in Rh cytosolic tails and Rh truncated variants are characterized by heterologous expression in *Saccharomyces cerevisiae* cells lacking endogenous Mep ammonium permeases, as first approach.

DIVERGENT REGULATION AND TRAFFICKING OF ATO TRANSPORTERS IN *SACCHAROMYCES CEREVISIAE* AND *CANDIDA ALBICANS*

Faezeh Ghasemi^{1,2}, Carolina Fontinha da Silva¹, Ana Pereira¹, Patrícia Ataíde^{1,2}, Margarida Casal^{1,2}, Patrick Van Dijck^{3,4}, Sandra Paiva^{1,2}

¹Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal; ²Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal; ³Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, K.U. Leuven, Leuven, Belgium; ⁴KU Leuven One Health Institute, Leuven, Belgium.

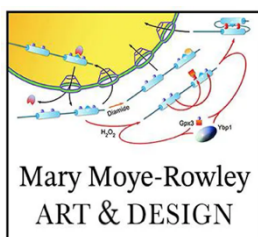
Plasma membrane (PM) transport proteins play fundamental roles in nutrient uptake, signalling, and environmental adaptation in yeast. In *Saccharomyces cerevisiae*, monocarboxylate uptake is mediated by regulated PM transporters, including Ato1 (Ady2), Ato2, and Ato3, members of the Acetate Uptake Transporter (AceTr) family. This family is substantially expanded in the opportunistic pathogen *Candida albicans*, comprising ten Ato proteins (Ato1–Ato10), whose functions remain unknown. High-resolution structures are available only for bacterial orthologues, which assemble into homohexamers. Here, we comparatively characterized Ato proteins in both the non-pathogenic yeast *S. cerevisiae* and the pathogenic yeast *C. albicans*, focusing on sequence conservation, potential cooperative interactions, expression, and permease dynamics.

Sequence homology, 3D structures, and predicted protein–protein interactions of ScAto and CaAto proteins were evaluated using Clustal Omega, AlphaFold, and STRING, respectively. Chromosomal GFP fusions and ATO deletion mutants were generated in prototrophic and auxotrophic strains of *S. cerevisiae* and prototrophic *C. albicans* using homologous recombination and CRISPR-Cas9. Ato protein expression, localization, and inter-paralog cooperative dynamics were analyzed across varying carbon sources, amino acid availabilities, substrate excess, and temperatures using fluorescence microscopy and western blotting.

Our results reveal conserved sequence and structural motifs, but distinct expression and endocytic regulation between species. Ato expression levels were affected by carboxylic acid concentrations and specific amino acids. Deletion of ATO1 and ATO2 influenced the expression of other Ato family members in both species, suggesting potential cross-regulatory or cooperative interactions. Furthermore, glucose did not induce ScAto1 internalization but progressively reduced its levels, whereas in *C. albicans* it triggered rapid CaAto1 internalization and vacuolar degradation.

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