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**31st International Conference on
Yeast Genetics and Molecular Biology**

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Book of Abstracts

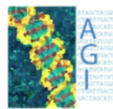
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Plenary sessions

Opening Keynote Lecture

Analysis of cellular networks using systematic yeast genetics and single cell image analysis

Brenda Andrews

University of Toronto, Canada

Speaker: Brenda Andrews

We have developed a combined experimental-computational pipeline for analysis of the effect of genetic perturbations on subcellular compartments and on proteome dynamics in yeast. Our approach uses the Synthetic Genetic Array method to introduce markers of various subcellular compartments, and markers that enable automated image analysis, into yeast arrays. One application of the pipeline involves introducing fluorescent markers of 18 sub-cellular compartments into the yeast deletion array and the essential gene array, which consists of strains expressing temperature sensitive (ts) alleles of essential genes. Single cell image analysis is used to quantify penetrance of genetic perturbations affecting the morphology of sub-cellular compartments.

Another application of our imaging pipeline involves high-throughput fluorescence microscopy of the ORF-GFP fusion collection, and deep learning techniques to analyze protein localization and abundance information for ~75% of the yeast proteome. We have used this pipeline to assess proteome dynamics during the cell cycle, and in response to various genetic perturbations. Recently, we developed PIFiA (Protein Image-based Functional Annotation), a self-supervised approach for protein functional annotation from single-cell imaging data. We used PIFiA to generate protein feature profiles from single-cell images of the ORF-GFP collection. We cluster extracted features into a hierarchy of functional organization, study cell population heterogeneity, and develop techniques to distinguish multi-localizing proteins and to identify functional modules. Finally, we confirm new PIFiA predictions using a colocalization assay, suggesting previously unappreciated biological roles for several proteins.

Microbial Biotechnology Lecture from EFB

Functional amino acids engineering in yeast: From metabolic regulations to biotechnological applications

Hiroshi Takagi

Nara Institute of Science and Technology, Japan

Speaker: Hiroshi Takagi

Amino acids are important not only as protein components of living organisms, but also as nutrients and energy sources. In recent years, many amino acids exist in free form and play important roles in cells, and thus, their physiological functions have been attracting attention. Various foods, beverages, nutritional supplements, and cosmetics, containing these amino acids have been commercialized worldwide. In yeast, amino acid metabolism vary under different growth environments and metabolic modes by regulating anabolic and catabolic processes, including uptake and export. Controlling the amino acid content is expected to contribute to improved productivity and value-added fermented foods and alcoholic beverages. The development of industrial yeast strains that overproduce “functional amino acids” could lead to improvement of fermentation ability, diversity of product taste and flavour, addition of healthy images, or increase of nutritional value. To emphasize these advantages, I named this breeding technology ‘functional amino acids engineering’ (*Biosci. Biotech. Biochem.*, 83, 1449, 2019; *SIMB News*, 71, 8, 2021). The yeast *Saccharomyces cerevisiae*, which is widely used in the fermentation industry, has been certified as ‘Generally Recognized as Safe (GRAS)’ by the US Food and Drug Administration (FDA), demonstrating its high safety, but unlike bacteria, there are few examples of industrialized production of amino acids by fermentation. In this lecture, I will introduce several topics of “functional amino acids engineering” with successful commercialization of alcoholic beverages, focused on the metabolic regulatory mechanisms and physiological functions of amino acids, such as proline, ornithine, leucine, isoleucine, valine, phenylalanine, and lysine, in *S. cerevisiae*.

Plenary Session 1 - Yeast Evolutionary Genomics

Pan-transcriptome reveals a large accessory genome contribution to gene expression variation in yeast

Joseph Schacherer

University of Strasbourg / CNRS, France

Speaker: Joseph Schacherer

Gene expression regulation is an essential step in the translation of genotypes into phenotypes. However, little is known about the transcriptional architecture as well as the genetic effects on gene expression at a population-scale. By taking advantage of a completely sequenced set of more than 1,000 *Saccharomyces cerevisiae* yeast natural isolates, we generated the transcriptome for each isolate and performed an in-depth analysis of the pan-transcriptome, representing 4,977 core and 1,468 accessory genes. These two sets of genes exhibit differential transcriptional behavior, with accessory genes being an underestimated key driver of gene expression variation at the species level. We also found that the global transcriptional landscape corresponds to a two-tier architecture. First, a conserved co-expression network captures major biological functions, reflecting the topological organization of the cell. Second, differentially expressed sets of genes are often specific to different subpopulations and highlight signatures related to adaptations, mostly to domestication processes. Unlike domesticated subpopulations, wild isolates appear to be less differentiated in terms of transcriptional diversity despite their high level of genetic divergence. Finally, we comprehensively characterized genetic associations for gene expression and we found that associated copy number variants (CNV-eQTL) explain a larger fraction of the gene expression variance than associated single nucleotide polymorphisms (SNP-eQTL). Additionally, accessory genes are associated with proportionally more eQTL, which have a significantly higher impact on gene expression variance. Overall, these findings illustrate how the accessory genome largely contribute to genetic effects on gene expression and represent a key component, shaping the transcriptional landscape.

Impact of polyploidisation and inter-specific hybridisation in yeast adaption and evolution.

Daniela Delneri

University of Manchester, United Kingdom

Speaker: Daniela Delneri

Hybrids have higher genetic variation and can combine advantageous traits from both parents. Transcriptional changes, different combinations of protein-protein interactions and type of mitochondria inherited are all factors affecting the phenotype and ability of the hybrids to adapt to new environments. For example, *S. pastorianus* is complex aneuploid hybrid between *S. cerevisiae* and *S. eubayanus* with a strong ability to ferment at low temperature. Expression studies revealed that the *S. eubayanus*-like alleles are significantly over-represented among the genes involved in the cold acclimatization. The presence of functionally redundant parental alleles also impacts on the nature of protein complexes established in the hybrid, where both parental alleles are competing. Interestingly, the majority of the protein complexes established in this hybrid are either exclusively chimeric or uni-specific and redundancy is discouraged, a scenario that fits well with the gene balance hypothesis. One limitation in hybrids evolution is their sterility. By generating allotetraploid (4n) strains between two yeast species we were able to restore fertility and produce viable diploid (2n) hybrid spores. The F1 progeny embedding different combinations of traits showed a large fitness range under different stressors. Using pooled F12 generation segregants of hybrids with extreme phenotype distributions, we identified quantitative trait loci (QTLs) for tolerance to different temperatures, high sugar concentration, acetic acid, and antifungals. We identified QTLs that are species specific, hybrid specific and mitochondria-type-dependent. Such approach opens the door to an unprecedented phenotypic space that can be used to study adaptation trajectories and can be exploited for biotechnological purposes.

Deploying multi-omics to study footprints of horizontal gene transfer events on yeast metabolism

Paula Gonçalves

NOVA FCT, Portugal

Speaker: Paula Gonçalves

Most yeast species belonging to the Wickerhamiella and Starmerella genera (for short, the W/S clade) prefer fructose to glucose as carbon source. Many, if not all, carry in their genomes an unusually high number of genes acquired by Horizontal Gene Transfer (HGT), mainly from bacteria. Over the last few years, we have shown how several of those genes have impacted important metabolic pathways, using genomics and a genetically tractable species, Starmerella bombicola as a model. More recently, we expanded our comparative genomics dataset to include over 60 genomes of W/S clade species. We complemented in silico analyses of these genomes with phenotypic characterization of sugar preference and fermentative profile of 41 species. I will discuss some of our most remarkable findings, such the loss and reinstatement of alcoholic fermentation in the W/S clade through the acquisition of bacterial alcohol dehydrogenases and our most recent hypotheses concerning the evolutionary links between fermentation and fructophily. We also started examining expression of bacterial genes in their new eukaryotic setting, in particular the adaptation of an entire bacterial operon to form a functional thiamin salvage pathway in several Starmerella species. Finally, I will discuss how transcriptomics is helping us to evaluate global expression levels of xenologous genes, from bacteria and filamentous fungi, when compared to native yeast genes in W/S clade species, and to get some insight into which metabolic pathways are significantly impacted by HGT in this group of yeasts.

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Genome Evolution of Agave *Saccharomyces* Populations: A Tale of Recurrent Hybridizations

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Speaker: Lucia Morales

Hybridization between yeast species is common and has crucial consequences in the adaptation of these microorganisms to natural and human-associated environments. Understanding how hybrid genomes evolve and stabilize has far-reaching implications for genetics, evolutionary biology and biotechnology. In this study, we collected thousands of yeasts from traditional agave distilleries and their natural surroundings across Mexico. The genome sequences of over 250 *Saccharomyces* strains revealed that a divergent and highly structured population of *S. cerevisiae* coexists with a *S. paradoxus* population in both settings. Strikingly, genome data also showed that hybrids between these two species are common and widespread across distilleries, while rare in natural reservoirs. The patterns of genetic variation and ploidies of the hybrid isolates strongly suggest recurrent and independent hybridization events. In addition, the genomes of strains from natural areas around the distilleries indicate that hybrids arise from a previously unidentified *S. paradoxus* subpopulation. These analyses also showed that the *S. cerevisiae* population in plants and insects of natural environments is the same as the one in fermentation tanks. Together, our findings depict a natural-history scenario in which a human-associated environment enables hybridization of natural yeast populations. Our research on the agave *Saccharomyces* populations not only contributes to a deeper understanding of hybrid evolution, but also provides essential resources for the genomic profiling and conservation of yeast communities in megadiverse regions of the Global South.

Plenary Session 2 - Systems Level Reconstruction of Cell-Cell Interactions

Yeast-environment interactions: How does the fungus *Cryptococcus neoformans* integrate environmental signals to regulate morphogenesis and pathogenesis?

Xin Zhou^{1,2}, Guillaume Desanti¹, Robin May¹, Ivy Dambuza³, [Elizabeth Ballou](#)^{3,1}

¹University of Birmingham, United Kingdom. ²University of Minnesota, USA. ³MRC Centre for Medical Mycology, University of Exeter, United Kingdom

Speaker: Elizabeth Ballou

In response to changing environmental conditions, the human fungal pathogen *Cryptococcus neoformans* can undergo an inducible change in cell size and ploidy, with implications for pathogenesis. However the molecular mechanisms by which this change is made remain unclear. Here, we report that Reactive Nitrogen Species (RNS) are a major signal driving the frequency and degree of titanization and act by increasing endogenous ROS within the fungus. We show that the accumulation of endogenous ROS is required for the yeast-to-titan transition and is associated with increased genotoxic stress leading to polyploidy. Yet, failure to detoxify this ROS impairs titan cell budding and reduces progeny viability. Therefore, the interface of exogenous RNS and endogenous ROS regulation during host-pathogen interaction represents an Achilles' heel for this major human fungal pathogen.

Mechanisms of cell-cell fusion in the fission yeast

Sophie Martin

University of Geneva, Switzerland

Speaker: Sophie Martin

Sexual reproduction is ubiquitous amongst eukaryotes. This requires alternation of cell-cell (gamete) fusion and genome reduction through meiosis. My lab has been using the yeast sexual reproduction pathway to study how cells polarize to find a mate and mount a fusion reaction. In the fission yeast *Schizosaccharomyces pombe*, sexual reproduction involves the pairing of P- and M-cells, which signal to each other through P- and M-factor pheromones. Formation of cell pairs involves a 'speed-dating' strategy, where each partner secretes pheromones at local mobile polarity sites that stabilize upon signal detection. For cell fusion, the two partner cells organize at the site of cell-cell contact a dedicated actin structure, the fusion focus, which serves to concentrate both signaling and cell wall digestion machineries. I will present our recent work describing the architecture of this fusion structure, and how this leads to cell-cell fusion.

Spatially structured yeast populations: Interactions, differentiation and regulatory networks

Zdena Palkova

Faculty of Science, Charles University, BIOCEV, Czech Republic

Speaker: Zdena Palkova

Yeasts, like other microbes, form organized communities in which cells interact, differentiate, and perform various functions, often to the benefit of the entire community. In spatially structured colonies and various types of biofilms, differentiated cell types are usually specifically arranged within the structure. The coordinated development of communities can be disrupted by the appearance of cells that acquire new properties (e.g., through mutation) and gain a competitive advantage that allows them to disobey community rules and form faster-growing subpopulations. These cells bring variability and opportunities for further population evolution and adaptation, but can disrupt the community from which they emerged. To date, only a few regulatory pathways are known to control the development of spatially structured colonies and biofilms. However, the regulatory components of these pathways are often linked to each other and to cell metabolism in a different way than is the case for single cells in liquid cultures. Here I will discuss two regulatory pathways we have recently identified and highlight their functions and relationships to cell physiology in the context of biofilm and colony formation and development. The first, via the regulator Cyc8p, recognizes a signal from the environment and decides whether a structured biofilm is formed or the cells change their lifestyle and form a less complex structure; the second involves the membrane-bound Whi2p complex and is important for the expansion of cells with new features in the colony structure. This work was supported by COST LTC20036 and GACR 23-06368S.

Plenary Session 3 - Yeast Commensalism and Pathogenesis

How the immune system detects the fungal wall

Neil GOW

University of Exeter, United Kingdom

Speaker: Neil Gow

Immune surveillance and defence against potential fungal pathogens is based on the recognition of this suite of molecules in the fungal cell wall that are recognised by pattern recognition receptors of the innate immune system. We have used a variety of microscopic, forward and reverse genetic and immunological tools to generate a new spatially accurate model of the cell wall and to explore how dynamic changes in the wall influence immune surveillance. We show that immune relevant epitopes can be diffuse or clustered, superficial or buried in the cell wall and they changed during batch culture and between yeast, hypha and other cellular morphologies. We screened libraries of mutants with immune pattern recognition receptors (PRRs) to define the sub set of fungal genes that assemble and regulate immune epitopes. This is revealing novel processes that are important for the assembly of the PRR-ome. These experiments demonstrate that the fungal cell surface is ordered, complex and dynamically changing, making immune recognition a challenging process requiring the concerted action of multiple receptors operating singly and in combination. My presentation will focus on this work that demonstrates that describes recent advances that have generated a scalar model of the cell wall and show it behaves as an ordered and dynamically changing organelle that makes immune recognition a challenging process. Immune recognition requires the concerted action of multiple receptors operating singly and in combination.

Reference: Gow, N.A.R. & Lenardon, M.D. (2022). Architecture the dynamic fungal cell wall. Nature Reviews Microbiology <https://doi.org/10.1038/s41579-022-00796-9>. PMID 36266346

The dual function of the fungal toxin candidalysin

Bernhard Hube

Department Microbial Pathogenicity Mechanism, Hans Knöll Institute, Jena, Germany; Friedrich-Schiller-University, Jena, Germany

Speaker: Bernhard Hube

Candida albicans is both a commensal and an opportunistic pathogen. The adaptation of this fungus to the human host is the result of an ancient, mostly commensal relationship which has led to the development of distinct fungal strategies to survive and proliferate in diverse host niches.

For its transition to a pathogenic phase, *C. albicans* relies on attachment to, invasion into, and damage of epithelial cells. The yeast-to-hypha transition is essential for these pathogenic events. Hyphae production is strongly linked to the expression of hypha-associated genes, a transcriptional pattern which represents a virulence program and anticipatory gene expression mechanisms.

Filaments of *C. albicans* are more adhesive and more invasive than yeast cells. Invasion is accompanied by epithelial damage, however, it does not cause damage *per se*. In fact, most of the damage is due to the polyprotein Ece1, containing the peptide toxin candidalysin. The role of other hypha-associated factors only becomes clear in the context of invasion and host responses to damage. On the other side of this struggle, the host has adapted and evolved mechanisms to prevent invasion, damage, and infection. For example, host cells can initiate repair mechanisms that maintain epithelial integrity and prevent mucosal damage during both *C. albicans* commensal growth and infection. Furthermore, this co-evolution scenario has not only led to the emergence of distinct fungal virulence factors, but also to “avirulence factors” (well known in the plant pathology field) or “immune modulators” (in the field of human immunology), which, once expressed by microbial pathogens, are recognized by the host and trigger microbial clearance via immune responses.

In *C. albicans*, proteins have been found which contribute to both offense and defense and are thus both virulence and avirulence factors. Candidalysin is a prime example of such a dual function factor and a central determinant in *C. albicans* interactions with the host.

From regulated cell death in yeast to anti-aging and anti-fungal treatments

Frank Madeo

Institute for Molecular Biosciences, Austria

Speaker: Frank Madeo

Yeast can undergo programmed cell death displaying diagnostic features of apoptosis. Physiologically, yeast cell death can be triggered by chronological aging. We used chronological aged yeast cells to discover the polyamine spermidine as a natural autophagy inducer. Subsequently, we found Spermidine supplementation to be geroprotective across species. Further, we screened for substances inducing yeast programmed cell death and identified novel natural metabolites that confer broad anti-fungal activity.

Lipid-based immunosuppression fosters the virulence of *Candida albicans* during bloodstream infection

Pauline Basso¹, Eric Dang², Anatoly Urisman¹, Leah Cowen³, Hiten Madhani¹, Suzanne Noble¹

¹UCSF, USA. ²NIH, USA. ³University of Toronto, Canada

Speaker: Suzanne Noble

Candida albicans is the most common cause of fungal infection in humans. IL-17 is critical for defense against superficial fungal infections, but the role of this response in invasive disease is less understood. We show that *C. albicans* secretes a lipase, Lip2, that facilitates invasive disease via lipid-based suppression of the IL-17 response. Lip2 was identified as an essential virulence factor in a forward genetic screen in a mouse model of bloodstream infection. Murine infection with *C. albicans* strains lacking Lip2 display exaggerated IL-17 responses that lead to fungal clearance from solid organs and host survival. Both IL-17 signaling and lipase activity are required for Lip2-mediated suppression. Lip2 inhibits IL-17 production indirectly by suppressing IL-23 production by tissue-resident dendritic cells. The lipase hydrolysis product, palmitic acid, similarly suppresses dendritic cell activation in vitro. Thus, *C. albicans* suppresses antifungal IL-17 defense in solid organs by altering the tissue lipid milieu.

A MAP kinase signaling pathway that regulates penetration peg formation in predator yeasts

Mareike Rij¹, Yeseren Kayacan², Beatrice Bernardi¹, Juergen Wendland¹

¹Hochschule Geisenheim University, Germany. ²University of Ghent, Belgium

Speaker: Mareike Rij

Predator yeasts are either homothallic or heterothallic ascomycetes of the genus *Saccharomycopsis*. These yeasts represent a unique genus of necrotrophic mycoparasites that predate a wide range of yeasts and filamentous fungi. This mycoparasitism can be divided into recognition, adhesion, penetration and killing/nutrient uptake phases. For the penetration of a prey cell a dedicated penetration peg is formed. Penetration pegs grow in a polarized manner into the prey cell and their tips can be brightly stained with dyes recognizing carbohydrate moieties indicating active secretion. Penetration pegs, however, do not contain nuclei, do not grow beyond a prey cell and do not develop into daughter cells. Each penetration peg is thus a one-time investment. MAP kinase signaling has been shown to govern multiple processes: amongst them mating and filamentation in *Saccharomyces cerevisiae* and appressorium formation in *Magnaporthe grisea*. Deletion of the *KSS1/FUS3* map kinase homolog *KIL1* in *Saccharomycopsis schoenii* resulted in avirulent strains as shown by several predation assays. *S. schoenii kil1* cells were unable to form penetration pegs under nutrient-limiting and predation-promoting conditions. Kss1/Fus3 MAP kinases regulate the transcription factor *STE12* in *S. cerevisiae*. *S. schoenii ste12* mutants were non-predacious and similar to *kil1* mutants failed to generate penetration pegs indicating that Ste12 is a major target for Kil1-signaling. Penetration of prey cells may be promoted by multigene families of cell wall degrading enzymes, including chitinases, glucosidases and proteases, which were found to be specifically upregulated during predation in the wild type.

Plenary Session 4- Yeast Biotechnology

A multiplex MoClo toolkit for extensive engineering of *Saccharomyces cerevisiae*

William Shaw¹, [Tom Ellis](#)²

¹Boston University, USA. ²Imperial College London, United Kingdom

Speaker: Tom Ellis

Synthetic biology toolkits are one of the core foundations on which the field has been built and have facilitated our ongoing efforts to reprogram biology for applications across biotechnology. The yeast *Saccharomyces cerevisiae* has benefited from a wide range of toolkits. In particular, the MoClo Yeast toolkit (YTK) allows the rapid construction of multigene plasmids from a library of highly characterised parts for rapidly programming new cellular behaviour in a more predictable manner. While YTK has cultivated a strong parts ecosystem and excels in plasmid construction, it is somewhat limited in the extent and flexibility in which it can create new strains of yeast. Here we describe a new toolkit, the Multiplex Yeast Toolkit (MYT) that extends the capabilities of YTK and addresses strain engineering limitations. MYT provides a set of new integration vectors and selectable markers usable across common lab strains, as well as additional assembly cassettes to increase the number of transcription units in multigene constructs, CRISPR-Cas9 tools for highly efficient multiplexed editing, and three orthogonal and inducible promoter systems for conditional programming of gene expression. With these new tools, we provide yeast synthetic biologists with a platform to take engineering ambitions to exciting new applications.

Metabolic engineering of yeasts for the production of betalain-type natural colors

Irina Borodina

Technical University of Denmark, Denmark

Speaker: Irina Borodina

Betalains are natural red-purple-yellow pigments found in plants of the order Caryophyllales and fungi of the *Amanita* genus. While nearly a hundred natural betalains have been identified, only red pigment betanin is produced commercially, by extraction from red beets. The betanin content in red beets is very low, ~0.2% wet weight, and the downstream process is challenged with impurities, such as geosmin, nitrates, and sugars, co-extracted with the pigment. We developed a yeast-based fermentation process to produce betanin and several other betalain pigments. The work comprised biosynthetic pathway discovery, optimization of pathway expression and precursor supply, and prevention of product degradation. Due to the simple visual detection of betalains, parts of the strain improvement program could be carried out using high-throughput genome engineering and screening. Engineered oleaginous yeast *Yarrowia lipolytica* made ~1.2 g/L betanin in 48-h fed-batch fermentation. According to the life cycle assessment, the fermentation process would have a significantly lower impact on resources and ecosystem quality than the traditional extraction of betanin from beets.

Closing the loop: the power of microbial biotransformations from traditional bioprocesses to biorefineries, and beyond

Paola Branduardi, Immacolata Serra, Stefano Bertacchi, Letizia Maestroni, Riccardo Milanese, Pietro Butti, Vittorio Giorgio Senatore

Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milan, Italy

Speaker: Paola Branduardi

The power of microorganisms in manipulating diverse matrices and in favoring the flux of elements and molecules through biogeochemical cycles developed in natural environments. This also comprises niches created by humans, moving from traditional food and beverage production processes towards polluted sites. Inspired by learning these lessons from nature, we can implement biobased processes at industrial level, for diminishing our dependency on fossil resources and to return molecules to their turnover in a timeframe compatible with our needs and with reduced environmental impact. Here we show some case studies where yeasts, thanks to the innate biodiversity and empowered by engineering their metabolisms, can be exploited for transforming wastes into virgin building blocks or final products. In the logic of biorefineries, these wastes are typically organic biomasses of recent fixation, which constitute residues of traditional and linear production chains, but we will also include an example where the waste derives from fossil sources. These examples of upcycling aim at accomplishing some of the key tasks of the UN 2030 agenda and to create competitive and viable industrial processes that can sustain our willingness to transform our model of growth from linear to circular.

Identification of new genes involved in the regulation of xylose alcoholic fermentation in the thermotolerant yeast *Ogataea polymorpha*

Roksolana Vasylyshyn¹, Justyna Ruchala², Olena Kurylenko³, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

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Speaker: Roksolana Vasylyshyn

Xylose and L-arabinose are important components of renewable feedstocks (lignocellulose and pectin) for biofuel production, being the second and third most abundant sugars in nature. However, since most known yeast strains do not utilize L-arabinose and xylose as carbon sources, a significant amount of carbon in plant residues cannot be used for bioethanol production. Despite significant efforts made by the alcoholic fermentation of basic sugars from lignocellulose hydrolysates and some achievements in this field (especially in xylose fermentation), yeast strains capable of efficient fermentation of L-arabinose have not yet been identified.

Previously, *O. polymorpha* strains were found to be excellent ethanol producers from xylose, but these strains grew poorly on L-arabinose. In contrast, mutants of *O. polymorpha* obtained by UV mutagenesis robustly grew on L-arabinose and still accumulated half of the biomass compared to the parental strain growing on xylose. However, after analyzing the level of ethanol production during high-temperature alcohol fermentation in an environment with 10% xylose or 5% L-arabinose, it was found that the resulting mutants produced 30% more ethanol from xylose compared to the parent strain. Moreover, the ability to ferment L-arabinose in mutants was 6 times higher than in the parent strain during high-temperature alcohol fermentation at 45°C. By sequencing the genomes of the respective strains, two genes have been identified that enable the growth and fermentation of L-arabinose as the sole carbon source. It has been established that damage to the *IRA1* gene positively affects xylose and L-arabinose alcoholic fermentation in *O. polymorpha* yeast.

Biochemical and metabolic engineering of *Saccharomyces cerevisiae* for the production of cannabinoid precursors

Kilan Schäfer^{1,2}, Christina Schmidt¹, Marco Aras¹, Eckhard Boles², Oliver Kayser¹

¹Technical University Dortmund, Germany. ²Goethe University Frankfurt am Main, Germany

Speaker: Kilan Schäfer

Cannabinoids belong to a class of bioactive compounds found in the plant species *Cannabis sativa* and have gained a significant amount of research attention in recent decades. Consequently, the vast pharmacological and therapeutic potentials of the main cannabinoid species, Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD), have been uncovered. This has prompted an exponential increase into both the research and commercialization of these compounds. However, due to the extensive and cost intensive processes involved in directly extracting cannabinoids from plants, approaches to produce these compounds biosynthetically have been the focus of much research in recent years. To date, numerous examples have been presented in which microbial species have been adopted to synthesize Δ^9 -THC and CBD or their precursors, the aromatic polyketide, olivetolic acid (OA), and its prenylated derivative, cannabigerolic acid (CBGA). However, low titers and the requirement of externally supplementing precursor molecules remain as major limitations. Here, we present various approaches to overcome these limitations and implement a range of biochemical strategies using the chassis organism, *Saccharomyces cerevisiae*. By deploying extensive gene editing and rational engineering methods in addition to overexpressing heterologous genes encoding the enzymes responsible for the *de novo* synthesis of OA, we have developed recombinant strains capable of producing up to 82 mg L⁻¹ of OA from glucose in batch cultivations while circumventing the requirement of hexanoate supplementation. We demonstrate our ability to optimize metabolic pathways within *S. cerevisiae* for the increased production of cannabinoid precursors, thus increasing the potential for achieving higher titers of biosynthetically derived cannabinoids.

Hot Topics Session

Novel Warbicin® family of phosphorylation-dependent glucose-uptake inhibitors in yeast and human cells as potential anti-cancer drugs

Ward Vanthienen^{1,2}, Johan Thevelein^{1,2,3}

¹Center for Microbiology, VIB, Belgium. ²Department of Biology, KU Leuven, Belgium. ³NovelYeast bv, Belgium

Speaker: Johan Thevelein

Many cancer cells share with yeast a preference for fermentation over respiration. We isolated Warbicin® A as a compound restoring glucose growth of the yeast *tps1Δ* mutant, which undergoes apoptosis due to hyperactive glucose uptake and catabolism. Warbicin® A and specific structural analogs inhibit glucose uptake by yeast Hxt and mammalian GLUT carriers with compound-specific kinetics. They are the first inhibitors that act on both. Warbicin® compounds inhibit proliferation and trigger cell death in cancer cells in a dose-dependent manner. They also inhibit tumor growth in vivo in mice xenografts. Specific concentrations did not evoke any major toxicity in mice but increase adipose tissue levels, consistent with deviation of glucose into storage organs. Inhibition of yeast sugar uptake depends on sugar phosphorylation, suggesting transport-associated phosphorylation as a target. In vivo and in vitro evidence confirms physical interaction between yeast Hxt7 and hexokinase, while nuclear targeting of NLS-Hxk2 reduces *tps1Δ* glucose sensitivity. We suggest that hexokinase can reversibly interact with glucose carriers and utilize the inhibitory ATP molecule bound in their cytosolic domain, converting it into non-inhibitory ADP and thereby increase the cellular influx of glucose. The yeast *tps1Δ* strain as well as cancer cells are proposed to have defective, permanent interaction resulting in persistent overactive influx of glucose. Based on their chemical structure and hydrophobicity, we suggest that Warbicin® compounds replace the inhibitory ATP in the cytosolic domain of the glucose carriers, preventing its utilization by hexokinase in transport-associated phosphorylation, and thereby reducing the overactive glucose uptake and catabolism.

Engineering phototrophic yeast: physiology, approaches, and applications

Anthony Burnett, Autumn Peterson, William Ratcliff

Georgia Institute of Technology, USA

Speaker: Anthony Burnett

Phototrophic energy production is the base of the vast majority of biological activity on earth, responsible for both nearly all primary production and additional photoheterotrophic metabolism. Microbial rhodopsins are simple light-driven proton pumps, one of two independent origins of phototrophic metabolism that has emerged on Earth and exemplars of horizontal gene transfer across the tree of life. Here, we explore if yeast can utilize rhodopsin-based phototrophic energy production using synthetic biology.

We have successfully transformed *S. cerevisiae* into a facultative photoheterotroph by inserting vacuole-localized rhodopsins from multiple fungal species. This allows light to pump protons into the vacuolar compartment, one of two cellular membranes bearing a rotary ATPase. Modified cells have a fitness advantage under green light, obtain this advantage via increased growth rates, and exhibit altered physiology in the presence of light. These results show the ease with which rhodopsins may be horizontally transferred even in eukaryotes, providing novel biological functions even without extensive evolutionary optimization.

Synthetic phototrophic metabolism in yeast may be used as an experimental tool to probe numerous questions, from the impacts of cellular energy availability to the evolutionary impacts of photoheterotrophy. It may potentially have applications for enhanced bioproduction. We explore the potential uses of this system to research and practical applications, as well as continued extension and optimization of synthetic photoheterotrophy in yeast.

The yeast toxicome: A potential source for new antifungals for food, health, and biotech applications

Sonja Billerbeck

University of Groningen, Netherlands

Speaker: Sonja Billerbeck

Fungal pathogens are an emerging threat to human health and food security. Very few fungicides are available and resistance to these is rising. It is a long-standing challenge to develop new antifungals. As eukaryotic pathogens, fungi offer very few selective drug targets and we urgently need new strategies for antifungal development. Ascomycete yeasts – such as environmental isolates of *Saccharomyces cerevisiae* and related species – have evolved a large set of small protein toxins, so-called yeast killer toxins (or mycocins), to compete against fungi in the environment. Previous research revealed that these toxins exhibit diverse modes of action, thus, indicating that the yeast toxicome might constitute a rich source of functionally diverse but yet-untapped antifungals. We use a combination of synthetic biology, functional genomics, protein engineering, and environmental microbiology to access, understand and engineer the hidden pool of yeast killer toxins toward applications in food, human health, and biotechnology/synthetic biology.

Using AlphaFold to study the signaling complex by which Ssk2 transduces the SDS stress signal between MAPKs pathways in *Saccharomyces cerevisiae*

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Speaker: Beatriz Lavilla García

One of the main Mitogen Activated Protein Kinases (MAPKs) pathways in the yeast *Saccharomyces cerevisiae* is the Cell Wall Integrity (CWI) pathway, which is responsible for the maintenance of the cell wall structure by regulating its synthesis and remodeling in situations that compromise its integrity. We have recently shown that the phosphorylation of Slt2 (the MAPK of the CWI pathway) triggered by stimuli such as the detergent sodium dodecylsulphate (SDS) depends on several components of the High Osmolarity Glycerol response pathway (HOG). These include the MAPK Hog1, the MAPKK Pbs2 and the MAPKKK Ssk2, but not its homologue Ssk22, nor its activator Ssk1 (Jiménez-Gutiérrez *et al.*, 2020).

In order to find potential Ssk2 interactors involved in this signaling crosstalk, we performed a screening by NGS-Y2H (two-hybrid and next generation sequencing) in collaboration with Dr Bernhard Suter from Next Interactions in California. In this screening we found a total of 30 protein interactors of a version of Ssk2 lacking its carboxyl-terminal catalytic end. To further study and validate these results, we first took an *in vivo* approach, looking for interactors whose removal produced a phenotype similar to that caused by the lack of Ssk2 in the presence of SDS. Second, we used the artificial intelligence program for protein folding and interaction prediction AlphaFold to address possible interactions from an *in silico* perspective. Lastly, these putative interactions were evaluated *in vitro* by co-purification techniques.

Plenary Session 5 – Classical Yeast Genetics and Molecular Biology

Sch9S6K -mediated control of DNA repair and DNA damage response during chronological aging

Elisa Ferrari¹, Chiara Lucca¹, Ghadeer Shubassi¹, Marco Foiani^{1,2}

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Speaker: Elisa Ferrari

Survival to UV-induced DNA lesions relies on nucleotide excision repair (NER) and the DNA damage response (DDR). We studied DDR and NER during chronological and replicative aging and found that old cells fail to efficiently repair DNA and activate the DDR. We employed pharmacologic, genetic and mechanistic approaches to rescue DDR and repair efficiency during aging. We found that Torc1, Snf1 and PP2A influence the capability of old cells to repair DNA and to activate the DDR. Moreover, the phosphorylation state of the S6K^{Sch9} kinase can selectively affect NER or DDR efficiency during aging. Altogether our data suggest that DDR and NER are suppressed during aging by metabolic inputs and that metabolic circuits cross-talk with those pathways controlling genome integrity.

Amino acid sensing, vesicle trafficking to the vacuole, cell death and virulence

J. Marie Hardwick

Johns Hopkins University, USA

Speaker: J. Marie Hardwick

Programmed cell death is intrinsic to all life forms, including microorganisms, yet cell death research has focused on animal models. Several suicidal cell death pathways were recently defined in bacteria, revealing ancient origins of animal cell death intertwined with immunity and allaying concerns about the existence of cell death in unicellular organisms. Less is known about fungal cell death and has not yet been explored in most human pathogens. In a genome-wide screen of the *Saccharomyces cerevisiae* deletion collections following a cell death stimulus, we identified death-sensitive and death-resistant strains. By studying cell death-sensitive hit $\Delta whi2$, we uncovered pro-survival functions of Whi2 and demonstrated that Whi2 is required to suppress TORC1 in a novel pathway following depletion of amino acids but not other nutrients, thereby preventing death by gluttonous behaviors. By studying the most cell death-resistant knockouts, we identified pro-death functions of the AP-3 vesicle trafficking complex, which is conserved in the human pathogenic yeast *Cryptococcus neoformans*. The AP-3 vesicle trafficking complex transports newly made membrane-associated proteins to the lysosome/vacuole membrane and results in permeabilization of the vacuole membrane following a death stimulus. Deletion of any AP-3 subunits or some of its cargo proteins, including the Yck3 kinase, results in striking resistance to several cell death stimuli and inhibits permeabilization of the lysosome/vacuole membrane. Further evidence suggests that fungal cell death-resistance is a feature of fungal pathogenesis. This shift in thinking to focus on microorganism cell death raises the possibility of alternative therapeutic strategies for treating fungal infections.

Metabolic Control of TORC1

Claudio De Virgilio

University of Fribourg, Switzerland

Speaker: Claudio De Virgilio

The eukaryotic target of rapamycin complex 1 (TORC1) couples nutrient, energy, and hormonal signals with cell growth, division, and metabolism, and aberrant TORC1 signaling contributes to the progression of human diseases such as cancer and diabetes. Amino acids are important and primeval cues that stimulate TORC1 to promote anabolic processes (such as ribosome biogenesis and protein translation initiation) and inhibit catabolic processes (such as macroautophagy) via the conserved heterodimeric Rag family GTPases. The currently known amino-acid sensitive events upstream of the Rag GTPases, which include vacuolar/lysosomal amino acid transporters and cytosolic leucine, arginine, and S-adenosyl-methionine sensors, all directly or indirectly impinge on GTPase activating (GAP) complexes that control the GTP-loading state of the Rag GTPase heterodimers. Whether intracellular metabolites other than amino acids may be able to control TORC1 through Rag GTPase-independent mechanisms is currently largely unknown. In this context, we recently discovered two metabolites, indole-3-acetic acid and malonyl-CoA, that directly inhibit the TORC1 kinase activity within mammalian and/or yeast cells. Here, we will discuss the underlying molecular mechanisms and implications of these findings for our understanding of how TORC1 adjusts cellular growth in response to fluctuations in nutrient levels.

The natural diversity of the yeast proteome reveals chromosome-wide dosage compensation in aneuploids

Julia Muenzner¹, Pauline Trébulle^{2,3}, Federica Agostini¹, Christoph B. Messner^{2,4}, Henrik Zauber⁵, Martin Steger⁶, Elodie Caudal⁷, Andrea Lehmann¹, Christiane Kilian¹, Anna-Sophia Egger², Fatma Amari¹, Natalie Barthel¹, Kate Lau¹, Matteo De Chiara⁸, Michael Mülleder⁹, Vadim Demichev¹, Gianni Liti⁸, Joseph Schacherer⁷, Toni Gossmann¹⁰, Matthias Selbach⁵, Judith Berman¹¹, Markus Ralser^{1,2,3}

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Speaker: Julia Muenzner

Aneuploidy, a cellular abnormality defined by aberrant chromosome copy numbers, is an important driver of microbial evolution, stress adaptation, drug resistance, and cancer. However, the cellular response to aneuploidy, and especially the extent of dosage compensation occurring at the proteome level, are less well understood. Most studies concerning aneuploidy in yeast have been performed using lab strains, in which aneuploidy is associated with significant fitness costs. Conversely, systematic sequencing and phenotyping of large collections of natural isolates revealed that aneuploidy is frequent and has few – if any – fitness costs in nature. To address these discrepant findings, we developed a platform that yields highly precise proteomic measurements across large numbers of genetically diverse samples, and applied it to natural isolates collected for the 1011 *S. cerevisiae* genomes project, thus covering a wide range of aneuploidies. We integrated these proteomes with transcriptomes and found, as in previous studies, that aneuploid gene dosage is generally not buffered at the transcriptome level. However, we demonstrate that natural yeast isolates attenuate relative protein levels by about 25%, and that dosage compensation occurs chromosome-wide. Furthermore, we observed increased ubiquitin-proteasome levels in natural aneuploid strains, and carried out ubiquitinomics and protein turnover measurements to demonstrate that chromosome-wide dosage compensation correlates with a global increase in protein turnover. Thus, through systematic exploration of the species-wide diversity of the yeast proteome, we shed light on a long-standing debate about the biology of aneuploids, revealing that aneuploidy tolerance can be mediated through chromosome-wide dosage compensation at the proteome level.

The ups and downs of budding yeast's transport of glucose

Luis Montano¹, Marc Sturrock², Iseabail Farquhar³, Kevin Correia⁴, Xiao Wang³, Kim Mailliet³, Yu Huo³, Vahid Shahrezaei⁵, [Peter Swain](#)³

¹Anna Children's Cancer Research Institute, Austria. ²Royal College of Surgeons in Ireland, Ireland. ³University of Edinburgh, United Kingdom. ⁴Unknown, Netherlands. ⁵Imperial College London, United Kingdom

Speaker: Peter Swain

Glucose is many organisms' preferred carbon source, and its transport is complex with budding yeast using at least seven different hexose transporters (HXTs). Here we address why cells might use so many. We show how transcriptional regulation matches levels of transporters by their affinity to current concentrations of glucose and report levels of the HXTs in five other sugars, giving an atlas of their expression. Using mathematical modelling, we argue that one role of the glucose-sensing network is to mitigate a rate-affinity trade-off inherent to transport by facilitated diffusion. By combining time-lapse microscopy with dynamically changing concentrations of glucose, we demonstrate that the HXTs are regulated by a push-pull system of repressors. As glucose rises, repression by one type of repressor, encoded by MTH1 and STD1, is weakened or 'pulled' and repression by the other type, encoded by MIG1 and MIG2, is strengthened or 'pushed'. In falling glucose, cells reverse this push-pull. We show further, via a statistical model comparison with time-lapse data, that the sensitivity of each HXT gene to the repressors matches its expression not only to the range of glucose concentrations coinciding with the transporter's affinity but also - at least for some - to whether glucose is rising or falling. Finally, we indicate how this understanding changes when we consider other hexoses. Together our results underpin how budding yeast excels at importing sugar.

Special Event 1 – Yeast Cell Cycle and modelling Transcriptional regulation

IN MEMORIAM OF STEFAN HOHMANN - **Unicellsys: from osmostress modeling to yeast system biology**

Lilia Alberghina

SYSBIO/Isbe Centre of Systems Biology , University of Milan Bicocca, Milano, Italy.

Speaker: Lilia Alberghina

Stefan Hohmann has been a European leader of the scientific community of yeast genetics, molecular biology and systems biology as it developed in the first years of the XXI century. He started his scientific career at the University of Darmstadt, then moved, as a group leader, at the Catholic University of Leuven, to be called, as professor, by the University of Gothenburg and then, in the same city, by the Chalmers University of Technology. Stefan made of Gothenburg a very relevant site in which to develop basic science and technological applications of yeast systems biology. In 2003 he was the main organiser of the ICYGMB21, which had a great success of attendance. Stefan utilised the meeting to start to structure the small and sparse community of scientists approaching systems biology to better understand the complexity of yeast physiology. He organised the Yeast Systems Biology Network and being the Editor of the series Topics in current genetics, published by Springer Verlag, he asked me and Hans V Westerhoff to plan and to extend invitations for a collective book, which was one of the first to be published to define and present the perspectives of the nascent systems biology. The book was published in 2005 and had a new edition in 2008. In the same years Stefan mobilised the European Systems Biology community to present a proposal for a EU project, Unicellsys, which aimed to lay the foundations of systems biology for eukaryotic cells, from yeast to humans. Stefan was the planner and the coordinator of Unicellsys, which was active from April 2008 to March 2013. Researches from 10 European countries participate and I recall the interesting discussion on how to achieve the targets : “ the overall aim of

Unicellsys is a quantitative understanding of fundamental characteristics of unicellular organisms on how cell growth and proliferation are controlled and coordinated. Quantitative experimentations as well as dynamic mathematical models will be required”. Stefan and collaborators demonstrated in their pioneering work on osmostress that complex functions arise as “ emergent properties”. Of course the aims of Unicellsys were too ambitious to be achieved in just 5 years and Stefan was very disappointed by the non-renewal. Stefan was also aware that the new systems approach was going to require changes in the education, organisation and funding of the new multidisciplinary approach in life sciences. Stefan loved a lot his family and I recall many times when he was proud in relating achievements of one of his children. A long disease, faced with courage by Stefan, ended his life, when he could still give a lot to science and to his family. All of us, who had the fortune to be his friends will keep his memory with affection and may want to complete the lines of work that Stefan envisioned, but was unable to deliver and that now are almost ripe.

SYSTEMS BIOLOGY OF CELL CYCLE CONTROL IN YEAST AND BEYOND

Matteo Barberis

Systems Biology, School of Biosciences, University of Surrey, United Kingdom. Centre for Mathematical and Computational Biology, CMCB, University of Surrey, United Kingdom

Speaker: Matteo Barberis

The eukaryotic cell cycle is driven by waves of cyclin-dependent kinase (cyclin/Cdk) activities that rise and fall with a pattern called “waves of cyclins”. This pattern guarantees coordination and alternation of DNA synthesis with cell division. Cell division dynamics are governed by waves of cyclin/Cdk activity interlocked with transcriptional events. Although details about transcription of cyclins, regulatory subunits of Cdk, are available, the network motifs responsible for the pattern are currently unknown. Here I highlight the power of minimal computer models to capture cell cycle dynamics. Specifically, I show a novel principle of design that ensures cell cycle time keeping through interlocking cyclin/Cdk dynamics with transcription in a budding Yeast Minimal Cell Cycle (YMCC) module. Through computational modeling and analyses of this minimal network, synthetic biology to synthesize the functional minimal module, and quantitative data of mitotic cyclin dynamics, a novel and robust molecular switch is unraveled. This switch highlights the mitotic cyclin/Cdk–transcription factor (TF) axis being pivotal for timely cell cycle dynamics. This minimal cell cycle network can oscillate autonomously, through cyclin/Cdk-mediated positive feedback loops and a TF–mediated cascade among mitotic cyclins. The model predicts, and the experimental testing validates a definite TF activation pattern underlying the design. The YMCC module rationalizes and actuates the quantitative model of Cdk control proposed by the 2001 Nobel Prize recipient Sir Paul Nurse, identifying regulatory motifs that keep a well-timed cell cycle. Altogether, our effort reveals a conserved, functional design principle in cell cycle control.

How transcription factor locate their binding sites in large genomes: a role for intrinsically disordered regions

Naama Barkai

Weizmann Institute of Science, Israel

Speaker: Naama Barkai

Our study address the question of how transcription factors (TFs) detect their binding sites in large genomes. DNA binding domains (DBDs) within TFs bind tightly to short DNA sequence motifs. Those motifs, however, are presents in tens of thousands of genomic locations. Only fractions of those motif occurrences are relevant for TF activity and are bound In-vivo. What distinguish the subset of bound motif sites from the majority of sites that remain unoccupied is a fundamental, but poorly understood aspect of gene regulation. .

Outside their DBDs, TF are enriched in intrinsically disordered regions (IDRs). I will describe our studies implicating those long (>500 aa) IDRs in directing TF binding . I will present results showing that those regions are fundamental for localizing TFs at their target promoters, describe the IDR sequence grammar guiding directing binding preference and provide initial insights of the underlying molecular basis.

Multivariant global control of the major nitrogen-responsive transcription activator, Gln3

Terrance G. Cooper

University of Tennessee Health Science Center, USA

Speaker: Terrance G. Cooper

Gln3 and Gat1 are (NCR-) Nitrogen Catabolite Repression-sensitive transcription activators. In excess nitrogen, TorC1 phosphorylates Gln3 and Tap42, and inhibits Gcn2. TorC1-bound, phosphorylated Tap42-Sit4/PP2A phosphatase complexes are inactive resulting in cytoplasmic Gln3 sequestration as a Gln3-Ure2 complex and minimal NCR-sensitive transcription. Oppositely, in sparse nitrogen, TorC1 activity decreases, Gcn2 (required for nuclear Gln3 localization) is activated, Tap42-Sit4/PP2A complexes dissociate from TorC1 and dephosphorylate Gln3 which becomes nuclear, increasing NCR-sensitive transcription. Dal80/ Gzf3 transcriptional repressors compete with Gln3/Gat1 for promoter binding thereby buffering GATA factor effects during nitrogen availability transitions. Transcription of all GATA-factor genes but *GLN3* is NCR-sensitive, Gln3-dependent, autogenously and cross-regulated. Glutamine levels determine Gln3 binding to its target promoters. In low but not high glutamine, Gln3 must bind to its target promoters before it can exit from the nucleus. Free Sit4 and PP2A dephosphorylate nuclear exiting Gln3 in nitrogen excess, whereas Tap42-Sit4/PP2A complexes do so in low nitrogen. Sit4/PP2A and Ure2 are all required to maintain dephosphorylated cytoplasmic Gln3. Paradoxically, both rapamycin and methionine sulfoximine (Msx), elicit nuclear Gln3 localization but only rapamycin elicits Gln3 dephosphorylation. The explanation – two Gln3-Torc1 interacting sites exist in Gln3. The C-terminal site functions negatively sequestering Gln3 in the cytoplasm in excess nitrogen, whereas rapamycin elicits dephosphorylation of the N-terminal Tor1-Gln3 site which functions positively supporting nuclear Gln3 localization. Recent data show Whi2-Psr1/2 down-regulates TorC1 activity following a shift from high- to low amino acids, but that diminishment has little influence on overall NCR-sensitive protein production. Other proteins, however, change dramatically.

Plenary Session 6 - Yeast as a Model to study mechanism of disease

Yeast as a living test tube to inform on the biology of disease-associated proteins

Tiago Outeiro

University Medical Center Goettingen, Germany

Speaker: Tiago Outeiro

The aging of the human population is resulting in an increase in the number of people afflicted by neurodegenerative disorders such as Parkinson's disease (PD) or amyotrophic lateral sclerosis (ALS), creating tremendous socio-economic challenges. This requires the urgent for the development of effective therapies, and of tools for early diagnosis of the disease. However, our understanding of the molecular mechanisms underlying pathogenesis is still incomplete, hampering progress in those areas. In recent years, the progression made in genetics has considerably contributed to our knowledge, by identifying several novel disease-associated genes. The yeast *Saccharomyces cerevisiae* constitutes an excellent entry-level model system for probing the molecular determinants of protein aggregation. In particular, we are gaining insight into the role of posttranslational modifications (PTMs) on aggregation, and as targets for therapeutic intervention.

Yeast as a model to study mitochondrial disorders

Cristina Dallabona

Department of Chemistry, Life Sciences and Environmental Sustainability; University of Parma, Italy

Speaker: Cristina Dallabona

Mitochondrial disorders (MDs) are a group of clinically heterogeneous inherited diseases due to mutations in either the nuclear or the mitochondrial genomes causing directly or indirectly an energy impairment.

One of the major challenges in the field of mitochondrial medicine is the identification of the genetic basis of pathological conditions with no diagnosis. The detection of novel nuclear variants of unknown significance, often through next-generation sequencing approaches, requires functional validation to confirm pathogenicity and in-depth analysis to assess the molecular mechanisms underlying the disease. Thanks to the fact that most of the nuclear genes involved in MDs are conserved between yeast and human, the yeast *Saccharomyces cerevisiae* has proved significantly useful in contributing to the validation of alleged pathogenic variants and to the comprehension of the molecular basis of numerous mitochondrial diseases.

Furthermore, despite considerable progress in defining the pathogenesis of MDs, effective therapies remain elusive and mostly limited to relieving symptoms. In the attempt to fill this gap, yeast has been extensively used for the discovery of new therapies. High-throughput yeast-based chemical library screenings have been successfully carried out to identify drugs active in different yeast mitochondrial disease models. The possibility of screening collections of thousands of Food and Drug Administration (FDA) approved drugs allows a drug repurposing approach, thus speeding up the drug discovery process by identifying new clinical uses of already available molecules.

The aim of this talk will be to highlight the main advantages offered by the yeast *Saccharomyces cerevisiae* for the study of mitochondrial diseases.

Good and bad about replication-associated recombination

Dana Branzei

IFOM, Italy. IGM-CNR, Italy

Speaker: Dana Branzei

Various findings link replication fork integrity with the ability of cells to engage specialized DNA repair pathways, known as DNA damage tolerance mechanisms, to facilitate replication completion. Cells are equipped with two modes of DNA damage tolerance, one largely error-free, mediated by homologous recombination, and the other error-prone, mediated by mutagenic translesion synthesis polymerases. In parallel with the error-free recombination mode, a genetic pathway known as salvage pathway of recombination operates, although little is known about its mode of action, location and activation. In this talk, I would present evidence from the lab on replisome components modulating DNA damage tolerance pathway choice and on factors that assist Rad51 recombinase in the two modes of recombination, depending on the location of DNA gaps versus the replication fork junction.

Mitochondrial protein import clogging as a mechanism of disease

Liam Coyne¹, Xiaowen Wang¹, Jiyao Song², Thomas Becker², Xin Jie Chen¹

¹State University of New York Upstate Medical University, USA. ²University of Bonn, Germany

Speaker: Xin Jie Chen

Mitochondrial biogenesis requires the import of >1,000 mitochondrial preproteins from the cytosol. Most studies on mitochondrial protein import are focused on the core import machinery. Whether and how the biophysical properties of substrate preproteins affect overall import efficiency is underexplored. Here, we show that protein traffic into mitochondria can be disrupted by amino acid substitutions in a single substrate preprotein. Aac2 is a mitochondrial carrier protein primarily involved in ADP/ATP exchange across the inner membrane in *Saccharomyces cerevisiae*. We found that several missense mutations in Aac2 cause the protein to accumulate along the protein import pathway, thereby obstructing general protein translocation into mitochondria. This impairs mitochondrial respiration, cytosolic proteostasis and cell viability by mitochondrial Precursor Overaccumulation Stress (mPOS). The mutations act synergistically, as double mutant Aac2 causes severe clogging primarily at the Translocase of the Outer Membrane (TOM) complex. This confers extreme toxicity in yeast. We also observed the clogging of the mitochondrial protein translocation channels by mutant Ant1, the mammalian homolog of yeast Aac2, in both cultured human cells and in a mouse model. These mutations in human ANT1 are known to cause autosomal dominant Progressive External Ophthalmoplegia, manifested by muscle weakness and neurological disorders. Our study therefore suggests that the clogging of mitochondrial protein import is a mechanism of disease. More broadly, this work implies the existence of uncharacterized amino acid requirements for mitochondrial carrier proteins to avoid clogging and subsequent disease.

Plenary Session 7 - Yeast cells and organelles as cell factories

Metabolic engineering of non-conventional yeasts *Komagataella phaffii* and *Candida famata* for the production of bacterial antibiotics roseoflavin and aminoriboflavin

Andriy Sibirny^{1,2}, Kostyantyn Dmytruk¹, Justyna Ruchala^{2,1}, Liubov Fayura³, Grzegorz Chrzanowski², Olena Dmytruk³, Andriy Tsyrylnyk³, Yuliia Andreieva³, Daria Fedorovych³, Olena Motyka⁴, Hans Marx⁵, Diethard Mattanovich⁵

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Speaker: Andriy Sibirny

Soil actinomycetes *Streptomyces davaonensis* and *Streptomyces cinnabarinus* synthesize antibiotic roseoflavin (RoF), the natural analog of riboflavin. RoF synthesis starts from flavin mononucleotide and proceeds through immediate precursor aminoriboflavin (AF), which also possesses antibiotic properties. Both flavin antibiotics strongly inhibit growth of many Gram-positive bacteria, including pathogenic ones. RoF accumulation by the natural producers is rather low while AF accumulation is negligible. Yeasts have many advantages as biotechnological producers relative to bacteria, however, no recombinant producers of bacterial antibiotics in yeasts are known. We possess riboflavin and flavin mononucleotide overproducers of the flavinogenic yeast *Candida famata* and riboflavin overproducers of *Komagataella phaffii* (*Pichia pastoris*) which were used as hosts for construction of the producers of flavin antibiotics. Synthetic genes *rosB*, *rosC* and *rosA* with adapted codons for both yeast species, which encode enzymes of RoF synthesis, have been expressed in *C. famata* and *K. phaffii*. Additionally, in riboflavin overproducers, yeast gene *FMN1* coding for riboflavin kinase, was overexpressed. Resulted yeast transformants accumulated AF or RoF. The structure of accumulated antibiotics was confirmed by ultra-HPLC and mass spectrometry (UHPLC-MS). Maximal accumulation of AF in shake flasks reached 5 mg/L (*C. famata*) and that of RoF was near 2 mg/L (*K. phaffii*). Accumulation of RoF in *K. phaffii* in bioreactor reached 130 mg/L which exceeded published data on accumulation of this antibiotic by the recombinant strain of the native producer, *S. davaonensis*. Perspectives of further increase in production of the flavin antibiotics by the recombinant yeasts are discussed.

Sub-organelle engineering to improve the chemical biosynthesis in yeast cell factories.

Yongjin Zhou

Dalian Institute of Chemical Physics, CAS, China

Speaker: Yongjin Zhou

Yeasts are ideal hosts for bioproduction; however, the complex biosynthesis pathway and the tight regulation of cellular metabolism make it challenging in driving the metabolic flux toward product biosynthesis. In particular, the eukaryotic cell metabolism is compartmentalized in sub-organelles, whose membranes are impermeable to various cofactors and metabolites, which thus requires the tailored metabolic engineering strategies to couple the native metabolism and reconstructed biosynthetic pathways. In this talk, we will show that engineering the sub-organelle cofactor supply help to improve the caffeic acid production in *Saccharomyces cerevisiae*. We will report that constructing the cytosolic biosynthesis pathway resulted in compromised fatty alcohol production in the methylotrophic yeast *Ogataea polymorpha*. Alternatively, peroxisomal coupling of fatty alcohol biosynthesis and methanol utilization significantly improved fatty alcohol production by 3.9-fold. Enhancing the supply of precursor fatty acyl-CoA and cofactor NADPH in the peroxisomes by global metabolic rewiring further improved fatty alcohol production by 2.5-fold and produced 3.6 g/L fatty alcohols from methanol under fed-batch fermentation. This talk also will talk the current challenges and feasible strategies in Sub-organelle engineering for improved chemical production in yeasts.

Production of the riboflavin (vitamin B₂) on whey by the flavinogenic yeast *Candida famata*

Justyna Ruchala^{1,2}, Dariya Fedorovych², Alicja Najdecka¹, Kostyantyn Dmytruk², Dominik Wojdyla¹, Andriy Tsyrunyk², Andriy Sibirny^{1,2}

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Speaker: Justyna Ruchala

Many enzymes require FMN and FAD as coenzymes. Riboflavin is the precursor to both of these coenzymes. Riboflavin is important commodity with annual market exceeding 500 million US dollars and is mostly used in agriculture as additive to feed premixes.

Wild-type strains of *Candida famata* were found to be characterized by robust growth on lactose and cheese whey and engineered strains also overproduce riboflavin on whey. The overproduction of riboflavin in whey could be particularly interesting in developing dairy-producing countries such as the United States, the Netherlands, Germany, Ireland, and Poland.

To further enhance riboflavin production on whey, the gene of the transcription activator *SEF1* was expressed under the control of the lactose-induced promoter of the native β -galactosidase gene *LAC4*. These transformants produced elevated amounts of riboflavin on lactose and especially on whey. The strain with additional overexpression of gene *RIB6* involved in the conversion of ribulose-5-phosphate to riboflavin precursor 3,4-dihydroxy-2-butanone-4-phosphate showed the highest titer of accumulated riboflavin in flasks during cultivation on whey. Activation of riboflavin synthesis was also obtained after overexpression of the *GND1* gene that is involved in the synthesis of the riboflavin precursor ribulose-5-phosphate. The best-engineered strains accumulated 2.5 g of riboflavin/L on whey supplemented only with ammonium sulfate during batch cultivation in a bioreactor with high yield.

According to our data, the abundant by-product of milk industry could be used as effective substrate for riboflavin production using riboflavin-overproducing strains *C. famata* capable to lactose utilization.

Unlocking the yeast powerhouse: metabolic engineering of *Yarrowia lipolytica* via manipulation of mitochondrial transporters

Gennaro Agrimi

University of Bari. Department of Biosciences, Biotechnology and Environment, Italy

Speaker: Gennaro Agrimi

The optimization of microbial cell factories through metabolic engineering has predominantly relied on the manipulation of genes that encode soluble enzymes. As eukaryotes yeasts are endowed with membrane-enclosed organelles, metabolic reactions taking place in a cellular compartment are connected to the metabolic network through the action of intracellular transporters whose largest family is that of the mitochondrial carriers (MCs). Several studies have shown that the manipulation of MC expression levels can significantly alter the distribution of metabolic pathway substrates, intermediates, and products within cells. Thus, these proteins can be interesting targets for metabolic engineering approaches aimed at redirecting the metabolic flux toward the production of molecules of interest.

This principle has been applied in recent years to guide the metabolic engineering of *Yarrowia lipolytica*, a non-conventional yeast that is increasingly being used as a microbial cell factory. By manipulating the expression levels of mitochondrial transporters, we have successfully increased the production of organic acids, modified the yeast's fermentation profile obtaining an inversion of the citric/isocitric acid secretion and improved the production of specialized lipids. These findings suggest that manipulating mitochondrial transporters is an effective metabolic engineering strategy for *Yarrowia lipolytica* and other yeasts.

Plenary Session 8 - Synthetic and Systems Biology

Mathematical modeling of yeast: a driver for innovation in biotechnology and human medicine

Feiran Li

Tsinghua University, China. Chalmers University of Technology, Sweden

Speaker: Feiran Li

Metabolism, the interplay of biochemical reactions inside a cell to generate energy and components to maintain life, is an important layer of information for understanding the functioning of organisms. In recent years, constraint-based metabolic models have emerged as a common approach for studying metabolism systematically. However, the rapid growth of 'Big-data' presents an opportunity to use deep learning to uncover relationships between genotype and phenotype. We leveraged these two techniques to gain a better understanding of cell metabolism and investigate large-scale relationships among genotype, phenotype, and environment. Such work can find its broad application ranging from cell factory design in metabolic engineering, mechanism identification in evolutionary biology to hypothesis testing in systems biology.

Mapping Genetic Interaction Networks in Yeast and Human Cells

Charles Boone

University of Toronto, Canada

Speaker: Charles Boone

We've generated a comprehensive genetic network in yeast cells, testing all possible 18 million gene pairs for genetic interactions. Negative interactions connected functionally related genes and coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. We've examined trigenic interaction networks and how different environments modulate the global yeast genetic interaction network. We are now exploring how different genetic backgrounds influence the global yeast genetic network. To test for conservation of the general principles of genetic networks, we are utilizing CRISPR-Cas9 technology to conduct genome-wide screens and map genetic interactions in haploid human cells.

Large scale genome engineering of yeast cell factories

Pascale Daran-Lapujade

Delft University of Technology, Netherlands

Speaker: Pascale Daran-Lapujade

To be successful, the biobased economy requires all powerful microbial cell factories that can perform optimally in harsh industrial environments. The construction of such microbes requires extensive genome engineering, both to add new functionalities and to rewire existing ones. While 'plug-and-play' designer genomes might become a reality in the future, extensive genome engineering remains a challenge, even in the highly tractable and popular yeast *Saccharomyces cerevisiae*.

Chromosome engineering, meant to enable large scale, modular remodelling of pathways and functions in *S. cerevisiae* is an attractive approach for the construction of cell factories. We recently demonstrated how synthetic chromosomes, assembled in vivo from a large number of DNA parts, can be used as orthogonal expression platforms to rewire native cellular processes and implement new functionalities. Using supernumerary 'test chromosomes' mostly composed of DNA non-coding in yeast, we addressed fundamental questions regarding chromosome design, assembly efficiency and fidelity as well as stability and functionality. The potential of modular, specialized chromosomes was then demonstrated by the assembly in vivo, in two transformation steps, of neochromosomes carrying 20 native and 21 heterologous genes, designed for the de novo production of anthocyanins, native to plants, from glucose. This work paves the way for future microbial, synthetic cell factories with modular genomes in which core metabolic networks, localized on satellite, specialized NeoChrs can be swapped for alternative configurations and serve as landing pads for addition of functionalities.

Prediction of representative yeast phenotypes using multi-attribute subset selection

Konrad Herbst, Taiyao Wang, Elena Forchielli, Meghan Thommes, Ioannis Paschalidis, [Daniel Segrè](#)

Boston University, USA

Speaker: Daniel Segrè

The phenotypic characterization of multiple microbial strains under many environmental conditions is increasingly used to complement genomic studies. Large phenotypic data can help understand and engineer microbial systems, but are costly and challenging to generate and interpret. Given an array of phenotypes (e.g., yield across many species and growth media), we thus ask how to best choose subsets of conditions that are informative about the whole dataset, enabling efficient system identification and providing a basis vector in phenotype space. To that end, we devised a mixed integer linear programming approach to choose predictor and response attributes for a phenotypic matrix. The algorithm will find which (predictor) phenotypes are most informative about all other (response) phenotypes, such that each response phenotype can be expressed as a linear combination of predictor phenotypes. We applied the algorithm to microbial phenotypic datasets, including a set of fitness measurements for 462 yeast strains under 38 carbon sources. The algorithm identifies environments that can be used as features to predict growth under other conditions, providing biologically interpretable metabolic axes for strain discrimination. Our approach could be used to reduce the number of experiments needed to identify a strain or to map its metabolic capabilities. The generality of the algorithm makes it appropriate for addressing attribute selection problems in other areas of systems biology.

Closing Ceremony

***Saccharomyces* variation across the world**

Gianni Liti

IRCAN, France

Speaker: Gianni Liti

An understanding of natural variation is crucial to efforts in current biology and to decipher the dynamics of genome evolution. The budding yeast, *Saccharomyces cerevisiae*, has emerged as a leading system for population genomics studies due to its small, well-characterized genome and experimental tractability. In the past decade, we assembled a large collection of natural isolates of *S. cerevisiae* and its closest relative *S. paradoxus* strains and characterized them at the genomic and phenotypic levels. We applied different sequencing and computational approaches to investigate origin, evolution, secondary contacts, and domestication of the species. These data provide a comprehensive view of genomic diversity in budding yeast and expose pronounced population-level differences.

Yeast Synthetic Biology: Past Present and Future

Presenter: Jeff Boeke

Satellite workshop 1 - Yeast Ecology and Fermentations: the intimate relationship of *Saccharomyces cerevisiae* and fermented foods

Yeast-insect associations: from ecology and evolution to winemaking

Irene Stefanini

Laboratory of Microbiology and Virology, Department of Life Sciences and Systems Biology, University of Turin, Italy

Speaker: Irene Stefanini

It is now acknowledged that yeasts and insects can interact in several ways. The association can be ephemeral, allowing insects to localize food thanks to yeast volatile metabolites, or persistent, resulting in positive effects on the microorganism and the host. Whereas a yeast-based diet is necessary for the proper development of insects, yeasts benefit from a persistent association with insects in multiple ways. Ecological studies on *Saccharomyces cerevisiae*-social wasp associations have shown that the budding yeast finds in the insects' gut an environment where they can reside all year long, be vectored in various natural sites, and even mate. Hence, the wasp gut not only preserves the local *Saccharomyces cerevisiae* genetic and phenotypic diversity but, by providing an environment suitable for mating, can also promote the increase of yeast diversity. Besides the impact on *Saccharomyces cerevisiae* ecology and evolution, the association with social wasps can play a relevant role for other non-airborne fungi. Indeed, like *S. cerevisiae*, other yeast species not found on pristine grapes are present on ripened grapes and are known to influence the fermentation of grape musts. Social wasps can vector those yeast species to the vineyard, and the composition of their gut mycobiota is influenced by the characteristics of the environmental matrix surrounding the vineyard. The disclosure of yeast-insect associations is providing fundamental insights on yeast ecology and evolution, as well as on links between natural environments and resources and human activities.

From vineyard to winery: *S. cerevisiae* microbial diversity driving wine fermentation

Jean-Luc Legras

Univ Montpellier, France. INRAE, France. Institut Agro Montpellier, France

Speaker: Jean-Luc Legras

What drives the diversity of *S. cerevisiae* yeast strains that accomplish wine fermentation?

Recent genetic and genomic studies have shown that wine strains clustered in a specific population adapted to the wine environment, but we need to better know the genetic bases of a better fitness in the wine environment.

In order to understand how yeast adapted to the wine environment we built 3 x 4 recombinant populations containing either wine strains, Mediterranean oak strains, and strains from both origins, that were grown for 24 serial fermentations in a typical Sauvignon grape must. This experimental evolution led to an improvement of the fitness of the recombinant Mediterranean oak/wine populations. Several changes in the frequencies of allelic variations observed in specific regions, such as for SSU1 - ECM34, and MET10 involved in MET10, as well as other regions, shows that adaptation to the wine environment involves multiple regions. This adaptation may also have unexpected consequences. Indeed H₂S production varies according to the origin of the yeast population. Interestingly, we found that the amplification of the CUP1 genes impacts the activity of the sulfur assimilation pathway, and provides an interesting example.

Nevertheless, the wine ecosystem contains at least two compartments : cellars and vineyards. Looking for the evolution of wine yeasts raise the questions of the connection between these two. The measures of gene flow between these two compartments, indicate that they are connected, but the identification of the main vectors, especially insects, remain a central question to be better explored.

The yeast consortia involved in tree sap fermentations performed by indigenous Australian peoples

Vladimir Jiranek^{1,2}, Cristian Varela³

¹University of Southampton, United Kingdom. ²The University of Adelaide, Australia. ³Australian Wine Research Institute, Australia

Speaker: Vladimir Jiranek

Even in Australia, few know of the fact that Aboriginal Peoples used a variety of sugar-rich plant materials to produce fermented beverages. Examples include *way-a-linah* and *tuba*, made from the sap of the cider gum (*Eucalyptus gunnii*) and exudate from the lopped inflorescences of the coconut palm (*Cocos nucifera*), respectively. Our pioneering work has begun to define the microbiome associated with these processes, with a view to increasing awareness of these ancient practices, defining the compositional and sensory properties of the products and describing new strains if not species of yeast. To date we have identified over 1,000 isolates from the distinct population profiles found according to the source material. Whilst highlighted through metagenomic analysis, novel yeast species have yet to be successfully cultured. Comprehensive growth and phenotypic profiling of the culturable isolates is revealing properties with potential applications in more common beverage fermentation processes and beyond.

Generating custom yeast strains for traditional and precision fermentation

Quinten Deparis

VIB – KU Leuven Center for Microbiology, Belgium. CMPG Laboratory of Genetics and Genomics, KU Leuven, Belgium. Leuven Institute for Beer Research (LIBR), Belgium

Speaker: Quinten Deparis

Our research focuses on characterizing, comparing and understanding different industrial yeasts from across the world, and using these to improve various fermentation processes, from beer and wine to biofuels, lipids, proteins and chemical compounds. Over the past years, we collected thousands of yeast strains from various niches, including some medieval breweries, distilleries and spontaneous fermentations. Fermentation assays revealed enormous differences in aroma production, fermentation efficiency and stress tolerance. In addition, DNA analysis revealed the history and domestication of today's yeasts, and also opened the doors to understanding and improving aroma formation. Using these resources, our team is producing several new, superior yeast variants with specific properties and aroma profiles for applications in the production of fermented beverages as well as various precision fermentation applications. While most of our research has focused on *Saccharomyces* species, recent advances in yeast genome engineering tools and characterization have led us to explore beyond *Saccharomyces* strains for beverage applications and renewable production of food ingredients and biochemicals.

Evolutionary potential of novel interspecific lager yeast hybrids to brewing fermentative environments

Jennifer Molinet¹, Juan Navarrete¹, Pablo Villarreal¹, Roberto Nespolo², [Francisco A. Cubillos](#)¹

¹Universidad de Santiago de Chile, Chile. ²Universidad Austral de Chile, Chile

Speaker: Francisco A. Cubillos

Saccharomyces pastorianus is widely used to produce lager-pilsner beer at low temperatures. Given that the exact *S. cerevisiae* and *S. eubayanus* parental genomes of *S. pastorianus* are not available, the complex molecular origin and how genome plasticity prompted a greater fitness in lager hybrids is unknown. Here, we determined the genome plasticity and evolvability of *S. cerevisiae* x *S. eubayanus* laboratory hybrids under different environmental conditions. For this, we generated a large set of novel interspecific lager yeast hybrids using a genetically rich collection of wild Chilean strains for beer wort fermentation. We generated 31 interspecific hybrids at two temperatures (12 °C and 25 °C). These hybrids showed similar fermentation capacities to their parental strains, with no evidence of positive heterosis. To ameliorate the hybrid's fermentative performance, four F1s were selected for genetic improvement through a process of adaptive evolution. The different evolved lines showed higher fitness in the same evolution environment than the ancestral hybrids, demonstrating that hybrids greatly improve their fitness. Hybrids generated at low temperature, and which retained the mitochondria of *S. eubayanus*, showed the greatest improvements in fermentation capacity, similar to the commercial Lager strain. OMICs analysis demonstrated signatures of selection that explained the greater fitness. Most genetic changes occurred in the *S. cerevisiae* genome portion, while to a lower extent in *S. eubayanus*. Changes in *S. eubayanus* were in mitochondria-related genes, suggesting a mito-nuclear interaction. This study provides novel lager hybrids demonstrating the greater genomic plasticity of these strains to fermentative environments.

Isolation and phenotypic tests of cocoa fungal communities during fermentation

Rebecca Ghisolfi¹, Gabriele Bellotti¹, Francesca Bandini^{1,2}, Cristian Bortolini³, Vania Patrone¹, Edoardo Puglisi^{1,2}, Lorenzo Morelli¹

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²National Biodiversity Future Centre (NBFC), Italy. ³Soremartec srl (Ferrero group), Italy

Speaker: Rebecca Ghisolfi

To fulfill the market needs in terms of implementation and diversification of end products, a great deal of research has been conducted on cocoa bean fermentation in order to gather information on microbial communities and the dynamics of fermentation. Due to its complexity and heterogeneous factors that impact cocoa bean fermentation, the resulting final quality is variable. The final purpose of the study is to assess candidate yeast starters to obtain a standardized fermented mass, increase production reducing waste and unexpected results that are typical of a spontaneous process, conducted in not always well-controlled conditions.

A polyphasic analysis was carried out on fresh fermenting cocoa beans using both culture-dependant and culture-independent methods for a clearer comprehension of the fungal evolution that occurs during fermentation. The comparison of the two analyses employs both culturable and non-culturable microorganisms, allowing a broader overview of cocobiota. Moreover, phenotypic tests, designed to simulate the fermentation conditions, were performed to screen the temperature, ethanol and acid tolerance. Giving targeted scores to the growth capacity at different stresses, it was possible to study the fungal strains that could be able to survive better in the most extreme conditions that occur during cocoa bean fermentation.

Therefore, the present study allowed us to thoroughly explore the microbiota of fermented cocoa beans and to analyze culturable microorganisms from the perspective of stress resistance.

The core fungal microbiota of grapevines

Kate Howell¹, Di Liu²

¹University of Melbourne, Australia. ²China Agriculture University, China

Speaker: Kate Howell

Microbial ecology and activity in wine production influences grapevine health and productivity, conversion of sugar to ethanol during fermentation, wine aroma, wine quality and distinctiveness. We characterized the spatial and temporal dynamics of fungal communities associated with the grapevine (grapes, flowers, leaves, and roots) and soils over an annual growth cycle in two vineyards to investigate the influences of grape habitat, plant developmental stage (flowering, fruit set, veraison, and harvest), vineyards, and climatic conditions. Fungi were influenced by both the grapevine habitat and plant development stage. The core microbiome was prioritized over space and time, and the identified core members drove seasonal community succession. The developmental stage of veraison, where the grapes undergo a dramatic change in metabolism and start accumulating sugar, coincided with a distinct shift in fungal communities. Co-occurrence networks showed strong correlations between the plant microbiome, the soil microbiome, and weather indices. Our study describes the complex ecological dynamics that occur in microbial assemblages over a growing season and highlight succession of the core community in vineyards; likely contributors to the flavour and aroma of wines.

Satellite Workshop 2 - Yeast probiotics, trained immunity, and the fungal microbiome

Candida albicans interaction with gut microbiota: who does what and how

Marilena Pariano¹, Monica Borghi¹, matteo Puccetti², Giorgia Renga³, Luigina Romani¹

¹Dept. Medicine and Surgery, University of Perugia, Italy. ²Dept. Pharmaceutical Science, University of Perugia, Italy. ³Dept Medicine and Surgery, University of Perugia, Italy

Speaker: Luigina Romani

Candida species are commensal colonizers of the human body but also the most prevalent opportunistic human fungal pathogens especially in the ever-growing population of vulnerable patients in the hospital setting. Evidence suggests that the gastrointestinal tract is the main source of fatal disseminated *C. albicans* infections. Major risk factors for disseminated candidiasis include damage to the mucosal intestinal barrier, immune dysfunction, and dysbiosis of the resident microbiota. Thus, the prevention of disseminated infection requires a better understanding of *C. albicans*' interaction with the local immune system and microbiota. IL-22 and the indoleamine 2,3-dioxygenase (IDO)1 enzyme are key players of host-Candida interaction in the gut. Because IL-18 regulates both IL-22 and IDO1, this anticipates an intriguing role for IL-18 in host-fungus symbiosis. We found that deficiency of IL-18 in mouse intestinal epithelial cells promotes *Candida albicans* pathogenesis by decreasing IL-22 bioavailability, promoting a Th17/Treg cell imbalance and altering the fecal microbiota composition through the expansion of Prevotellaceae and TM7. Antibiotic treatment and fecal transplantation studies indicated a pathogenic role of Prevotellaceae in mucosal candidiasis via the succinate/HIF- α pathway. Thus, perturbations of the inflammasome pathways leading to IL-18 deficiency may contribute to mucosal candidiasis via modulation of host immunity and the microbiota.

Intrahost diversity of *Candida* species as invasive or commensal samples from single isolates or metagenomes

Hannah Snell¹, Terrance Shea¹, Daniel Floyd², Johnny Atallah², Michael Mansour², [Christina Cuomo](#)¹

¹Broad Institute, USA. ²Massachusetts General Hospital, USA

Speaker: Christina Cuomo

Candida species are members of the human microbiome, however people who are immunocompromised are at higher risk for developing candidiasis and invasive candidemia. We have little understanding of the evolution of infective *Candida* strains when at-risk patients present with these diseases in the clinic. We sought to characterize the diversity of *Candida* present in patients with candidemia, comparing bloodstream isolates to those found at other body sites. The patient cohort included adult subjects with candidemia categorized among

three different groups: individuals with leukemia/hematopoietic stem cell transplantation

(HSCT), individuals with indwelling catheters, and individuals with other reasons for candidemia. We carried out whole genome sequencing (WGS), whole metagenomic sequencing (WMS), and internal transcribed spacer (ITS) sequencing for cultured patient blood isolates, stool samples, and skin swabs, respectively. Most commonly *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis* were detected, with additional cases of five other fungal species. We found that blood isolates (WGS) from the same patients are highly identical to each other and to *Candida* from stool (WMS), however in-depth comparisons of WGS blood and WMS stool samples revealed genetic variation unique to each sample or present at different frequencies in WMS data. In many cases, the primary species detected by ITS analysis from skin swabs also matched the WGS and WMS data. Our results support that a single *Candida* species often colonizes multiple body sites, with a connection between a single clonal strain in the gut and the blood, highlighting a short evolutionary path during the progression of infection.

Role of yeasts on vaccine development and manufacturing

Rino Rappuoli

Fondazione Biotechnopolo di Siena, Italy

Speaker: Rino Rappuoli

The vaccine world made a quantum jump in 1979 when Pablo Valenzuela ,and Bill Rutter published in *Nature* the “synthesis and assembly of hepatitis B virus surface antigen particles in yeast.” This paper described a recombinant yeast expressing the surface antigen of HBV which was self-assembling into virus-like particles (VLP) or nanoparticles of 22nm, identical to those found in the plasma of chronically infected people. The work described the first “recombinant DNA vaccine,” the first “nanoparticle based vaccine” and the first “vaccine made in yeast.” The HBV vaccine was licensed in 1986 and is now used to vaccinate virtually every new-born in the planet. The technology was used a decade later to produce the Human papillomavirus which is now used to prevent cervical cancer in adolescents. More recently, the HBV nanoparticles have been engineered to express the surface antigen of *Plasmodium falciparum* and to make a vaccine against malaria which has been recommended for use in Africa by WHO.

***Candida auris* undergoes cellular aggregation via adhesin-dependent and -independent pathways**

Chloe Pelletier^{1,2}, Alistair J. P. Brown², [Alexander Lorenz](#)¹

¹Institute of Medical Sciences (IMS), University of Aberdeen, United Kingdom. ²MRC Centre for Medical Mycology, University of Exeter, United Kingdom

Speaker: Alexander Lorenz

Candida auris is a human fungal pathogen that causes severe nosocomial infections with high mortality rates. Due to its resistance to antifungal drugs and environmental persistence, these infections are difficult to treat and eliminate from healthcare settings. Therefore, understanding the genetics and life cycle of this fungus is critical for developing novel therapies. Studies have identified five geographical clades that exhibit phenotypic and genomic differences. One interesting phenotype primarily of clade III strains is cell aggregation, which has been linked to reduced virulence in mouse and *Galleria mellonella* infection models. However, it is challenging to distinguish morphology-dependent causes from clade-specific genetic factors when comparing strains with different clade affiliations. In this study, we identified two types of aggregation: one induced by antifungal treatment and the other controlled by growth conditions and only present in strains with the ability to aggregate. The latter type depends on an ALS-type adhesin, which is differentially expressed during aggregation. Furthermore, we found that macrophages cannot clear aggregates, suggesting that aggregation might provide a benefit during systemic infection and could facilitate long-term persistence in the host. Overall, this study provides new insights into the relationship between *C. auris* aggregation and virulence, which can inform the development of better treatment strategies.

Discovering pathways of pediatric inflammatory bowel disease through yeast and human genetics

Sabrina Chau¹, Neil Warner², Jing Li¹, Michael Costanzo³, Charlie Boone³, Aleixo Muise², Marc Meneghini¹

¹Department of Molecular Genetics, University of Toronto, Canada. ²SickKids Inflammatory Bowel Disease Center and Cell Biology Program, Canada. ³Donnelly Centre, University of Toronto, Canada

Speaker: Marc Meneghini

Translating mRNAs in eukaryotes are surveilled by the ribosome-associated Ski2/3/8-complex, which targets aberrant transcripts for degradation. The SKI-complex was originally discovered in yeast due to its role in repression of the L-A dsRNA mycovirus. Although a direct antiviral role for the SKI-complex is uninvestigated in humans, it is known to repress antiviral signaling and the interferon response. Homozygous mutation of the human homologs of *SKI2* or *SKI3* cause Trichohepatoenteric syndrome, a severe form of infant-onset inflammatory bowel disease (IBD). Non-syndromic forms of IBD are highly prevalent and have emerged as a model genetic disease with many associated mutations. The incipient paradigm of IBD is that these genetic systems influence mucosal tissue interaction with microbes and viruses that populate the gastrointestinal tract. We investigated a potential broad role of the SKI-complex during IBD and discovered 81 patients with rare and damaging mutations in one copy of hSKI2 or hSKI3 using analysis of whole exome sequencing (WES) data from 1762 pediatric IBD cases. Using humanized yeast, we are characterizing the functional consequences of these IBD-associated mutations with a quantitative flow cytometry assay. To identify new candidate IBD genes, we screened for antiviral systems that act with the yeast SKI-complex and found *Vms1*, a conserved tRNA hydrolase that also functions during translational surveillance. Inspection of the pediatric IBD WES dataset uncovered 30 patients with rare and damaging mutations in the human *VMS1* homolog, *ANKZF1*, suggesting that it acts with the SKI-complex to protect against IBD. Our ongoing work advances this hypothesis.

Metabolic and immunomodulatory potential of yeasts and lactobacilli for the development of probiotic-based therapies

Damariz Rivero^{1,2}, Stefano Nenciarini², Alessia Ciccione^{1,2}, Benedetta Cerasuolo², Marco Pallecchi³, Luisa Ponticelli², Gianluca Bartolucci³, Duccio Cavalieri²

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Speaker: Damariz Rivero

Gut microbiota is essential for humans, helping the assimilation of food components as well as the prevention of pathogen invasions through host immune system modulation and production of beneficial metabolites such as short-chain fatty acids (SCFAs). Several factors, including changes in diet habits due to the progressive Westernization of the lifestyle, are linked to the onset of dysbiosis statuses that impair the correct balance of the gut environment. It is therefore crucial to deepen the interactions between commensal and diet-derived microorganisms that can influence our health, in order to develop targeted therapeutic approaches. Investigating these interactions through co-cultures between human- and fermented food-derived lactobacilli and yeasts led us to understand how the strains' fitness and their metabolic products rely on nature and concentration of the species involved, producing either cooperative or competitive dynamics. Moreover, single cultures of yeasts and lactobacilli proved to be ideal candidates for developing immune-enhancing products, given their ability to induce trained immunity in blood-derived human monocytes in vitro. Conversely, co-cultures as well as mixtures of yeasts and lactobacilli showed to induce an anti-inflammatory response on the same immune cells in terms of cytokine profiles and activation surface markers, open new possibilities for their use in chronic inflammation conditions.

Workshops

Workshop 1 - Cell death, ageing, Telomeres

The Impact of V-ATPase Proton Pump Assembly and Function on Replicative Aging

Fiza Hashmi, [Patricia Kane](#)

SUNY Upstate Medical University, USA

Speaker: Patricia Kane

Lysosomes eliminate growing burdens of damaged proteins in aging cells and play a prominent role in theories of aging. V-ATPases are conserved proton pumps that acidify organelles in all eukaryotic cells. The pH of the yeast lysosome-like vacuole has been reported to rise with age, but it is unclear how V-ATPase activity changes. V-ATPases are regulated by reversible disassembly of the peripheral subcomplex from the integral membrane subcomplex. We find that after 5-6 cell divisions, the V-ATPase goes from being predominantly assembled to predominantly disassembled. Vacuolar pH measurements in vivo indicate higher vacuolar pH in populations enriched for older cells, demonstrating that disassembly compromises V-ATPase function. Caloric restriction is a highly conserved mechanism for extending lifespan across organisms. Fascinatingly, we find that, when grown in reduced glucose medium, older cells maintain V-ATPase assembly and vacuolar acidification. Three signaling arms of metabolism have been linked to acidification and aging: Ras/PKA, mTORC1/S6K (Sch9), and AMPK (Snf1). We are using non-essential nutrient pathway mutants targeting each of these pathways to address mechanisms controlling V-ATPase assembly state during replicative aging. We have also uncovered a potentially novel regulatory mechanism. The RAVE complex promotes V-ATPase assembly and activity. In older cells, where the V-ATPase is predominantly disassembled, levels of the RAVE subunit Rav2 are reduced. qPCR analysis reveals no change in RAV2 mRNA transcript levels in older cells; however, these cells show increased levels of a RAV2 antisense transcript. We hypothesize antisense RNA blocks Rav2 protein translation, resulting in V-ATPase disassembly with age.

Unraveling Metformin's Lifespan Extension in Aging Yeast Cells

Jimena Meneses-Plascencia¹, Ericka Moreno-Mendez², Alexander DeLuna¹

¹UGA - Langebio, Cinvestav Unidad Irapuato, Mexico. ²UGA - Langebio, Cinvestav Unidad Irapuato, Mexico

Speaker: Jimena Meneses-Plascencia

Metformin, a drug used for the treatment of Type 2 diabetes, has been found to have remarkable longevity benefits in a wide range of species, from yeast to nematodes and even mice. Furthermore, there is evidence to suggest that metformin could also be an effective means of increasing life expectancy in humans. Despite this, a comprehensive view of which genes are relevant for its lifespan-extension effect is still missing. In this study, we used a powerful functional genomics assay to scrutinize the genetic factors underlying lifespan extension by metformin in *Saccharomyces cerevisiae*. Specifically, we measured the chronological lifespan of 1,414 knockout strains, each selected for its lack of a single gene with a human ortholog. To obtain a broad picture of metformin's mechanisms of action, we also use high-resolution flow cytometry to assess changes in the proteome induced by the drug. We will present the relevant biological mechanisms and pathways that contribute to the lifespan extension property of the drug. Surprisingly, we find that inactivation of the Set3C deacetylation complex and metformin affect common pathways to promote longevity, providing unprecedented insight into the intricate mechanisms through which this drug works to extend lifespan. Our genome-wide analyses and proteome profiling offers a global, unbiased view of the genetic determinants and pathways that aging cells use to harness the life-extending power of metformin.

Yeast mutants in decapping-related proteins show autophagy induction defects

Benedetta Caraba, Mariarita Stirpe, Vanessa Palermo, Michele Maria Bianchi, Claudio Falcone, Cristina Mazzoni

Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Italy

Speaker: Benedetta Caraba

The LSM complexes are heteroheptamers involved in mRNA decapping in the cytoplasm and pre-mRNA splicing in the nucleus. LSM4 is an essential protein and a component of both complexes while LSM1 is resident only in the cytoplasmic complex. We previously reported that the expression of a truncated form of the homologous gene from the biotechnological yeast *Kluyveromyces lactis* KILSM4, named KILsm4 Δ 1, can restore cell growth in both *K. lactis* and *S. cerevisiae* not expressing the endogenous protein. However, cells showed premature loss of viability during the stationary phase due to regulated cell death (RCD) triggered by mRNAs accumulation in the cytoplasm. In this study we report that cells expressing Sclsm4 Δ 1, a truncated form of LSM4 also show the same RCD markers and increased sensitivity to acetic acid, hydrogen peroxide and caffeine, three inducers of cellular stress in yeast. Moreover, we previously reported that the overexpression of a protein involved in phospholipids homeostasis and autophagy activation, NEM1, restores the viability of the KILsm4 Δ 1 mutant strain, therefore we analysed the autophagy flux in different conditions. The Sclsm4 Δ 1 mutant strain shows high sensitivity to autophagy triggers such as nitrogen starvation and rapamycin treatments and defects in physiological autophagy induction during chronological life span. Similar sensitivities and defects are found in lsm1 Δ strains, suggesting a role of the cytoplasmic LSM complex in the proper activation of the autophagic process.

Arginyltransferase1 (Ate1) mediated cell death depends on mitochondrial permeabilization in yeast

Akhilesh Kumar¹, Vikas K. Yadav¹, Vikash Maurya¹, Manisha Malhotra¹, Fangliang Zhang²

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Speaker: Akhilesh Kumar

Arginylation is a protein post-translational modification, which is addition of an extra arginine to the amino terminus of target proteins. This reaction is carried out by a conserved enzyme arginyltransferase1 (Ate1) and usually leads to the degradation of the target protein by ubiquitin-proteasome pathway. Arginylation is an essential reaction and regulates several biological processes such as cardiovascular development, oxygen sensing, neuronal development, cell death and aging. Recently, it has been observed that Ate1 expression elevates under acute oxidative stress conditions, resulting in apoptosis of yeast and mammalian cells. However, how does Ate1 mediate cell death is still unknown. Here, by using budding yeast as a test model, we found that Ate1 which usually resides in cytosol, translocates into mitochondria under oxidative stress, and this translocation is essential for Ate1-induced apoptosis. We also found that the mutations which disrupt the arginylation activity also compromise the Ate1 overexpression-induced cell death, without affecting its mitochondrial localization. Furthermore, we found that Ate1-mediated cell death is not dependent on mitochondrial ETC activity or the caspase pathway. Rather, we found that Ate1-mediated cell death is dependent on the mitochondrial permeability transition pore (MPTP) and the apoptosis-inducing factor. Moreover, the knock-out of MPTP components and co-expression of anti-apoptotic protein Bcl2-xL, which antagonizes mitochondrial permeabilization, protect from Ate1-mediated cell death. Our finding suggests that mitochondrial permeabilization is a key event in Ate1-mediated cell death.

Ferroptosis-Protective Membrane Domains in Quiescence

Amalia H. Megarioti^{1,2}, Alexandros Athanasopoulos¹, Dimitrios Koulouris¹, Bianca M. Esch³, Manousos Makridakis⁴, Vasiliki Lygirou⁴, Martina Samiotaki⁵, Jerome Zoidakis⁴, Vicky Sophianopoulou¹, Bruno André⁶, Florian Fröhlich^{3,7}, Christos Gournas¹

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Speaker: Christos Gournas

Quiescence is a common cellular state, required for stem-cell maintenance and microorganismal survival under stress conditions or starvation. However, the mechanisms promoting quiescence maintenance remain poorly known. Plasma membrane components segregate into distinct microdomains, yet the role of this compartmentalization in quiescence remains unexplored. Ferroptosis is an iron-dependent form of non-apoptotic cell death mediated by increased peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids. Yet, ferroptosis protective mechanisms have not been identified in yeast. In this work¹, we show that Flavodoxin-like proteins (FLPs), ubiquinone reductases of the yeast eisosome membrane compartment², protect quiescent cells from lipid peroxidation and ferroptosis. Eisosomes and FLPs expand specifically in respiratory-active quiescent cells^{2,3}, and mutants lacking either show accelerated aging, defective quiescence maintenance, and accumulate peroxidized PUFA-phospholipids. FLPs are essential for the extra-mitochondrial regeneration of the lipophilic antioxidant ubiquinol⁴. FLPs, alongside the Gpx1/2/3 glutathione peroxidases, prevent iron-driven, PUFA-dependent ferroptotic cell death. Our work is the first description of ferroptosis-protective mechanisms in yeast and introduces plasma membrane compartmentalization as an important factor for the long-term survival of quiescent cells.

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Unlocking the Power of the Yeast Chronological Lifespan Paradigm in Aging Research

Alexander DeLuna

CINVESTAV, Mexico

Speaker: Alexander DeLuna

A major challenge in aging research is to describe the way in which different genetic factors are interconnected to one another and the environment. I will present results of functional genomics screens using extensive collections of single- and double-gene knockouts coupled to automated chronological lifespan profiling in budding yeast. Our genetic assays and following meta analyses expose a consistent set of genes underlying survival of non-dividing stationary phase populations, indicating that genome instability and ribosome biogenesis are critical factors of cellular aging. We also find that limiting transition metals can extend lifespan by increasing autophagy activity. Moreover, epistasis, gene-nutrient, and gene-drug screens reveal new players orchestrating lifespan extension, including signaling pathways, chromatin-remodeling complexes, and non-coding RNAs. The chronological lifespan paradigm provides an unprecedented systems-level perspective on the genetic players involved in promoting longevity and their regulatory cross-talks in aging cells.

CONTROL OF TELOMERE LENGTH IN YEAST BY SUMOYLATED PCNA AND THE ELG1 PCNA UNLOADER

Pragyan Singh, [Martin KUPIEC](#)

Tel Aviv University, Israel

Speaker: Martin Kupiec

Telomeres protect the chromosomal ends and play central roles in aging and cancer. In cells expressing telomerase (such as stem cells, or cancer cells) telomere length is tightly regulated. Very little is known about the mechanisms that control telomere length. A systematic screen for yeast mutants that affect telomere length maintenance in the yeast *Saccharomyces cerevisiae* revealed that mutations in any of ~500 genes affects telomere length (shorter or longer than wild type). One of the genes that, when mutated, causes telomere elongation is *ELG1*, a conserved gene that encodes an unloader of PCNA, the processivity factor for replicative DNA polymerases. PCNA can undergo SUMOylation on two conserved residues, K164 and K127, or ubiquitination at lysine 164. These modifications have already been implicated in genome stability processes. We report that SUMOylated PCNA acts as a signal that positively regulates telomerase activity. We also uncovered physical interactions between Elg1 and the CST (Cdc13-Stn1-Ten) complex, and dissected the mechanism by which Elg1 and Stn1 negatively regulate telomere elongation, coordinated by SUMO. We will present a model that provides mechanistic insights on how chromosomal replication and telomere elongation are coordinated.

Workshop 2 - Yeast Sociobiology-Sensing and Signaling

Silver wedding between sequence markers and yeast taxonomy: insight in the current view of the yeast species from multi-copy rDNA to single copy selectable markers.

Gianluigi Cardinali, Angela Conti, Debora Casagrande Pierantoni, Laura Corte

University of Perugia, Italy

Speaker: Gianluigi Cardinali

Yeast species delimitation has moved from morphological and physiological traits to marker sequencing 25 years ago with a seminal work on LSU sequence, followed 11 years ago by another step forward with ITS. Whereas these markers were primarily selected for their relative ease of manipulation, the need for more efficacy and of more genetic significance of the used markers is moving the interest toward single copy genes. Moreover, the relatively high variability within rDNA tandem repeats produces a level of heterogeneity that adds noise to metagenomic and metabarcoding studies, making these markers virtually obsolete or calling for a set of bioinformatic pipelines necessary for their correct analysis at both the phylogenetic and taxonomic level. In this context, the search for low homoplasy markers is necessary not only for the ease of taxonomic classification and identification, but also to have a clearer genetic view of the yeast speciation. In this presentation, we will compare the efficacy of multi-copy vs. some single copy markers in discriminating among yeast species. Quantitative markers of taxonomic resolution will be introduced to decrease the subjectivity in marker choice and analysis and to give solid criteria of species delimitation. Taxonomic markers represent a tool for indexing species, but our argument is that they are even more important as a view on the complex yeast speciation phenomenon proceeding through significant vertical gene transfer and relatively frequent horizontal gene transfer, producing a situation that challenges our insight in the very nature of the microbial and of the eukaryotic species.

Characterisation and immunomodulatory potential of extracellular vesicles from non-pathogenic yeast strains isolated from a fermented product

Stefano Nenciarini¹, Roberta Amoriello², Giovanni Bacci¹, Benedetta Cerasuolo¹, Monica Di Paola¹, Patrizia Nardini², Siria Mucci¹, Alessio Papini¹, Clara Ballerini², Duccio Cavalieri¹

¹Department of Biology, University of Florence, Italy, Italy. ²Department of Clinical and Experimental Medicine, University of Florence, Italy, Italy

Speaker: Stefano Nenciarini

Extracellular vesicles (EVs) are lipid-bilayered particles, containing various biomolecules, including nucleic acids, lipids and proteins, released by cells from all the domains of life. They perform multiple communication functions, exerting them either intra- or inter-kingdom. Evidence suggests that the interaction between host immune cells and small RNAs carried by fungal EVs induce modulation of the host immune system. To date, most of the studies on fungal EVs immunomodulation have been conducted in the context of fungal infections, therefore there is still a knowledge gap regarding EVs produced by non-pathogenic yeasts. In this work, we characterised EVs obtained by *Saccharomyces cerevisiae* and *Pichia fermentans* strains isolated from a fermented milk product with probiotic properties. Immunomodulation abilities of EVs produced by these strains have been studied in vitro through immune assays after internalisation from human monocyte-derived dendritic cells. Results showed a significant reduction of antigen presentation activity of dendritic cells treated with EVs derived by yeast strains from the fermented milk. The small RNA fraction of EVs contained a vast majority of yeast mRNA sequences with molecular functions shared between strains of different species isolated from the same matrix. Our results suggest that one of the mechanisms behind the anti-inflammatory properties of probiotic foods could be mediated by the interactions of human immune cells with yeast EVs. These insights are preliminaries to further investigations on clinical applications of fungal EVs as suitable candidates for immunomodulatory therapy delivery in several human conditions.

Investigate the Molecular Basis of Adaptive Evolution in *S.pombe* to the Deletion of the Fusion Gene Prm1

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Speaker: Gaowen Liu

The deletion of Prm1, a transmembrane protein, in pombe cells results in a 95% failure rate of cell-cell fusion. To investigate the molecular basis of cell-cell fusion efficiency evolution, we identified a fusion-specific marker and developed a high-throughput FACS-based method to measure the fusion efficiency. An evolutionary approach was employed by manually selecting the 5% of prm1 Δ cells that fused successful and cycled the process for 18 generations in 12 independent lines, with 6 biological replicates in each line.

We showed that the fusion efficiency of prm1 Δ cells evolved from 5% to an average of ~30% across the 12 lines. A 127kb chunk deletion was discovered by examining the sequence of one of the evolved high fusion efficiency genomes. However, when this chunk deletion was reconstructed in the unevolved prm1 Δ strain, only 10% of the fusion efficiency was recovered, suggesting that alternative fusion mechanisms maybe involved.

Furthermore, antifungal drug and anaerobic treatment were found to significantly increase fusion efficiency, particularly in the unevolved strain. Scanning the ergosterol synthesis pathway genes, we identified that of erg1, 9 and ste7 overexpression can be responsible for the rescue. These findings provide insights into the molecular basis of cell-cell fusion efficiency evolution and identify potential targets for further research in this field.

To kiss or to kill - sensing and signalling in predator yeasts

Jürgen Wendland, Davies Kaimenyi, Mareike Rij

Hochschule Geisenheim University, Germany

Speaker: Jürgen Wendland

Predator yeasts are either homothallic or heterothallic *Saccharomyces* yeasts as determined by the presence of either one or two mating type loci per haploid genome and their sporulation behavior. We determined several predator yeast genomes and identified their mating type loci. *Saccharomyces schoenii* is a homothallic species that encodes homologs of key components of the *Saccharomyces cerevisiae* pheromone signal transduction cascade. We have found that this cascade also regulates penetration peg formation. Here, we characterized upstream and downstream components of this signaling pathway. Upstream components include the pheromone receptor genes, which are encoded by *STE2* and *STE3*. Mutants in these genes are sterile, yet they appear to be virulent in predation spot assays suggesting that pathogenic development is not triggered by pheromones. One of the downstream effector genes is *FAR1* in *S. cerevisiae*. Blast searches of the *S. schoenii* translated ORFs indicated only a weak hit to ScFar1. However, better matches were found with *Wickerhamomyces anomalus* and *Ascoidea rubescens*, which belong to more closely related genera to *Saccharomyces*. Domain searches of the *S. schoenii* Far1 protein indicated a conserved RING domain and putative Pleckstrin Homology (PH)- and von Willebrand type A (vWA)-domains also found in ScFar1. Mutants carrying deletions of the *S. schoenii FAR1* gene show wild type-like virulence in predation spot assays. Microscopic observation of predation events, however, revealed a reduction in virulence compared to wild type. Here, we will present our current progress on the analysis of *S. schoenii FAR1* mutants with respect to penetration peg formation.

Exploring the mechanisms of exogenous DNA restriction in *Saccharomyces cerevisiae*

Sandra Bruderer, Hassan Mustapha, Yves Barral

Institute of Biochemistry, ETH Zürich, Switzerland

Speaker: Sandra Bruderer

How eukaryotic organisms outside of Animalia protect themselves against exogenous DNA is currently not well understood. To address this, in our lab, we use transformation of plasmid DNA into *Saccharomyces cerevisiae* to understand whether mechanisms of genetic immunity could be at play inside these cells. The presence of genetic immunity processes inside cells should lead to the discrimination and elimination of at least some of the plasmid DNA introduced by transformation. To capture these possible events, we have tracked the fate of pre-labelled plasmid DNA in yeast cells using live-cell fluorescence microscopy. From this, we observe that while ~24% of cells uptake plasmid DNA, only a small fraction of these cells (~0.5%) become stably transformed. By introducing pre-labelled TetO plasmids into TetR-expressing cells, we also observe that a large proportion of the entering plasmids are not accessible to cytoplasmic TetR binding. We hypothesise that this may be because the plasmid DNAs are being “packaged”. Related to this, we have identified an unannotated positively charged peptide, which intriguingly localises at the nuclear periphery in cells only after transformation. Together, these results from our ongoing work provide evidence for there being cell intrinsic mechanisms in *S. cerevisiae* to help to restrict the propagation of exogenous plasmid DNA.

Epigenetic rewiring of cellular morphology and physiology in meiotic individuals with impaired amino acid-sensing

Valentina Cappelletti¹, Mirko Moser², Irene Stefanini³, Elisa Asquini², Federico Vaggi⁴, Yuehan Feng⁵, Matteo Ramazzotti⁶, Luisa Berna⁷, Azeddine Si Ammour², Alessandro Cestaro², Yves Barral⁸, Attila Csikasz-Nagy⁹, Paola Picotti¹, Duccio Cavalieri¹⁰

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Speaker: Valentina Cappelletti

From prokaryotes to eukaryotes nutrient sensing plays a paramount role in living cells regulating cellular metabolism in response to sugars, amino acids, lipids and metabolites availability. Chromatin remodelling is emerging as an additional regulatory mechanism responding to nutritional sensing. The information on extracellular metabolites composition is transferred to the genome and "saved" at histone marks level leading to changes in metabolic pathways regulation. How and to which extent nutrients induce epigenetic modifications still remains elusive. In the present work we profiled histone acetylation and methylation as well as protein abundance levels in four meiotic individuals of a natural *S. cerevisiae* strain characterized by a Mendelian segregation of two nutrient-related traits: a recessive resistance to trifloroleucine (TFL) and a dominant filamentous morphotype. We found multiple histone mark modifications in regions associated with metabolic and transporter genes to segregate with the TFL trait as consequence of a loss-of-function mutation in the *SSY1* amino acid sensor. Protein abundance variations in the same metabolic pathways pointed to a regulatory role of chromatin remodelling in response to environmental cues. We finally reported the association of facultative morphotypes with histone mark modifications identifying in the acetylation of *NAB3* promoter a possible epigenetic hallmark of colony differentiation in response to nutritional stimuli.

Yeast cell wall genes and social interactions

Speaker: Florian Bauer

Workshop 3 - Yeast Evolution and Ecology

Nectar yeasts and their chemical footprints in agricultural settings

Andrey Yurkov¹, Moritz Mittelbach², Massimo Nepi³, Meina Neumann-Schaal¹, Benedetta Turchetti⁴

¹Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Germany. ²Plant Protection Agency Berlin, Germany. ³University of Siena, Italy. ⁴University of Perugia, Italy

Speaker: Andrey Yurkov

Yeasts inhabit nearly all terrestrial environments and are found in association with many plant and animal species. With a low species richness and diversity of yeasts, nectar stands apart of other plant-rated communities. Because plant species and visiting insects often have a limited influence on the composition of nectar yeasts, these few nectar-borne species are believed to enter in highly specialised relationships with insects and often referred to as nectar specialists. Although nectar yeasts can be found nearly everywhere, little is known about mechanisms of yeast interactions with plants and insects, including nectar alteration, signalling, and transmission. The vast body of knowledge is derived from traditional yeast physiological tests performed under conditions that are very different from those typical for the nectar.

Here, we present experiments directed towards the elucidation of the effects of yeasts on pollinators. The experimental design reflected typical near conditions, dominating sugars, amino acids and their concentration. Yeast growth and glucose fermentation substantially differed between the artificial nectar and commonly used casein-peptone media. To reveal the effects attributed to nectar yeasts, the experiments included several controls with yeasts associated with insects and plants. Nectar yeasts grew on plant amino acids and transformed sugars into sugar alcohols, acids and ethers. The products were not cross-assimilated by species naturally occurring together nectar suggesting chemical partitioning of the nectar ecological niche. The alteration of nectar and its potential nutritional value included quantitative and compositional changes in both carbon and nitrogen sources that can affect pollinators' preferences and visitation patterns.

142 telomere-to-telomere assemblies reveal the genome structural landscape in *Saccharomyces cerevisiae*

Samuel O'Donnell¹, Jia-Xing Yue², Matteo De Chiara³, Anne Friedrich⁴, Lorenzo Tattini³, Joseph Schacherer⁴, Gianni Liti⁵, Gilles Fischer⁶

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Speaker: Gilles Fischer

Pangenomes are composed of multiple contiguous telomere-to-telomere genome assemblies that provide access to an accurate representation of the genetic diversity of species, both in terms of sequence polymorphisms and structural variants (SVs). We generated the *Saccharomyces cerevisiae* Reference Assembly Panel (ScRAP) comprising reference-quality genomes for 142 strains representing the species' phylogenetic and ecological diversity. The ScRAP samples the genomic space of the species in terms of sequence divergence, heterozygosity and ploidy and includes phased haplotype assemblies for several heterozygous diploid and polyploid isolates. We identified a total of more than 36,000 SVs, representing over 4,800 non-redundant events comprising insertions, deletions, contractions, duplications and also balanced rearrangements such as inversions and translocations. The ScRAP provides a precise view of the genomic diversity of the species. We discovered that horizontally acquired regions insert at chromosome ends and can generate new telomeres. We found that SVs impact gene expression near the breakpoints and significantly contribute to gene repertoire evolution. We reconstructed the genealogy of Ty elements and of tRNA gene families and also uncovered complex aneuploidies where large chromosomes underwent large SVs, providing an alternative adaptive route that would be inaccessible to simple aneuploidies. Overall, the ScRAP demonstrates the benefit of a pangenome in understanding genome evolution at population-scale.

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

Michelle Hays¹, Angelina Chan¹, Angela Hickey¹, Magdalena Pieczynska², Arjan de Visser³, Gavin Sherlock¹

¹Stanford University, USA. ²Instytut Genetyki i Biotechnologii, Poland. ³Wageningen University, Netherlands

Speaker: Michelle Hays

Some yeasts are killers. They secrete toxins that kill neighboring cells, but protect themselves with an intracellular antidote. Killers have an advantage in populations that include sensitive cells, but toxin production confers a metabolic cost. *Saccharomyces cerevisiae* require viruses to be killers. With sensitive cells in the environment, this results in a complex genetic conflict: where yeast and viral genomes alike are capable of adaptation and fitness tradeoffs abound. Our research dissects the molecular basis of adaptation in the face of these competing selection pressures to understand evolutionary outcomes.

We identified beneficial mutations that arose in coevolved killer and sensitive yeast. We identified a putative gain-of-function missense mutation in the HOG osmoregulatory pathway component *SSK1* that confers toxin resistance. Preliminary data suggest this dominant-acting polymorphism is sufficient to protect yeast from the coevolved toxin, as well as other killer toxins. Ongoing experiments aim to determine the mechanism by which this polymorphism creates toxin-resistance and any host tradeoffs associated with the coevolved allele. This mutant is fit under laboratory coevolution conditions, but the mutation itself affects a residue that is strictly conserved across tens of millions of years of fungal evolution. *SSK1* is required for multiple drug resistance in *Candida auris* and our ongoing work will address whether this gain-of-function allele might be present in fungi associated with human pathogenicity and drug resistance. In future, we aim to understand how killer yeasts counter-adapt in the face of these resistant competitors, and how viral genomes evolve as competing host population composition changes.

Evolution at the point centromere interface

Jana Helsen^{1,2}, Gavin Sherlock², Gautam Dey¹

¹EMBL Heidelberg, Germany. ²Stanford University, USA

Speaker: Jana Helsen

During mitosis, the cell division machinery must rapidly and reproducibly partition the cell's chromosomes through a stable but dynamic interaction between the centromere DNA, kinetochore complex, and spindle microtubules. In most organisms, centromeres are defined epigenetically, and range in size from a few kilobases to several megabases. *Saccharomycetaceae* however, a clade that includes key model species such as *Saccharomyces cerevisiae*, *Candida glabrata*, and *Kluyveromyces lactis*, possess point centromeres, which are generally less than 200 bases long. Each point centromere is defined by an AT-rich region (*CDEII*), flanked by 2 DNA motives (*CDEI* and *CDEIII*), and is bound by centromere-binding proteins that are unique to the clade. While it is known that *CDE* regions vary across species, there is no systematic overview of how these DNA features vary across *Saccharomycetaceae*. Additionally, it is unknown if and how centromere-binding proteins coevolve with their associated centromeres. Here, we compiled an atlas of centromere sequences across 71 species, spanning the whole *Saccharomycetaceae* tree. We use this atlas to highlight novel centromere features, and show that it can also be used to provide estimates of chromosome numbers across the tree. Next, we looked for homologues of all centromere-binding and inner kinetochore proteins in each species. By testing for episodic diversifying selection, we show that only a small subset of centromere-associated proteins coevolve with specific centromere features. Specifically, we show that sudden jumps in *CDEII* length coincide with positive selection in *CBF1*. Together, our findings provide a unique example of coevolution at the centromere interface.

Structural Evolution of Yeast Metabolic Proteins

Benjamin Heineike^{1,2,3}, Oliver Lemke³, Sandra Viknander⁴, Nir Cohen³, Jacob Steenwyk^{5,6,7}, Leonard Spranger³, Feiran Li⁴, Federica Agostini³, Cory Lee³, Jens Nielsen⁴, Judith Berman⁷, Antonis Rokas⁶, Jurg Bahler², Toni Gossmann⁸, Markus Ralser^{3,1}

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Speaker: Benjamin Heineike

Understanding links between structural changes and protein evolution has been challenging due to the effort required to obtain high resolution protein structures. Alphafold 2 provides high-resolution predicted structures that allow the examination of structural evolution at a genome wide level. We gathered Alphafold structures from 426 orthogroups in 27 species of budding yeast spanning over 400M years of evolution, focusing primarily on orthogroups of metabolic enzymes in those species. We then built structural alignments to calculate structural metadata, evolution rate (DN/DS), and conducted an integrated analysis with various external datasets. We identify several factors that influence structural conservation such as surface accessibility, amino acid composition, protein expression, and presence in certain pathways. In particular we observe a positive correlation between conservation of an orthogroup and the presence of alanine and glycine as well as a negative correlation between conservation and the presence of leucine that is driven by strong relationships within specific structural features. We identify highly conserved regions within proteins that overlap with active sites and protein-protein interaction sites. We also analyze evolution rate and find that the Electron Transport Chain is enriched in orthogroups that are less conserved but have higher relative rates of positive selection. We find evidence of positive selection on the interface between subunits in the ETC, especially between nuclear encoded subunits and more quickly evolving mitochondrially encoded subunits. This study provides a framework for incorporating structural information into evolutionary analysis at a systems level using yeast metabolic evolution as a case study.

Molecular genetic analysis of the co-evolution of microalga *Chlorella sorokiniana* and the yeast *Saccharomyces cerevisiae* within a synthetic environment

Jennifer Oosthuizen, Rene Naidoo-Blassoples, Florian Bauer

Stellenbosch University, South Africa

Speaker: Jennifer Oosthuizen

Evolutionary strategies have proven effective in enhancing strains and generating desirable phenotypes. Furthermore, these experiments offer insights into the evolutionary path of microorganisms and illuminate adaptive mechanisms that are challenging to decipher within natural settings. Here we describe a synthetic ecology-based approach to evolve co-operative behaviour between winery wastewater isolates of the yeast *Saccharomyces cerevisiae* and microalga *Chlorella sorokiniana*.

Evolution of these species took place within an imposed obligate mutualism based upon cross-feeding of carbon and nitrogen sources in a continuous co-culture system. Strains isolated after 100 generations of co-evolution showed improved biomass production of both species in co-culture conditions. Co-evolved species accumulated less biomass compared to parental species in some monoculture and non-selective conditions, implying that a trade-off between cooperativity and individual growth has taken place during co-evolution.

Co-evolved strains were sequenced and analysed for genetic changes in order to characterise mechanisms that improve cooperative behaviour. Genes that carried SNPs predicted to impact gene function in evolved strains were further investigated using deletion mutants. This was followed by re-engineering the specific SNPs in the parental yeast strain using CRISPR. *EPT1* and *GAT1* are known to play roles in carbon and nitrogen catabolite repression respectively, suggesting that the elimination of some of the adaptations of *S. cerevisiae* to fermentative environments may improve the cooperativity of this species with other microorganisms. Taken together, these results demonstrate the importance of integrating biotic selection pressures in strain development projects and provide insights into molecular adaptations that favour cooperative behaviour between yeast and algae.

Using experimental evolution of hybrid genomes to identify genetic incompatibilities in yeast

Artemiza A. Martínez, Gregory I. Lang

Lehigh University, USA

Speaker: Artemiza A. Martínez

Saccharomyces yeast species exhibit low pre-zygotic barriers to mating, and interspecific hybridization readily occurs in nature and in the lab. Diploid hybrids, however, are sterile because high sequence divergence among *Saccharomyces* yeasts prevents proper chromosome segregation during meiosis. Although other factors such as chromosomal rearrangements and genetic incompatibilities may also contribute to hybrid sterility, there is no evidence of strong genetic incompatibilities outside of a few nuclear-mitochondrial interactions.

Here we are using experimental evolution to test for the presence of weak but pervasive negative-genetic interactions between nuclear genes in the genomes of the sibling species *S. cerevisiae* and *S. paradoxus*. We generated and sequenced 20 F1 haploid progeny from a cross between *S. cerevisiae* and *S. paradoxus* using the method developed by Bozdag, et al. (2021). Phenotyping of these interspecific hybrids show a wide range of growth rates at different temperatures and fitness defects in mating-type specification.

We evolved 320 independent populations of haploid and homozygous diploid hybrids for over 1,000 generations in rich glucose media. We find substantial fitness gains in the evolved populations and we are currently sequencing the genomes of these strains to identify the underlying beneficial mutations. We hypothesize that these mutations compensate for genetic incompatibilities in hybrid protein complexes.

Workshop 4 - Growth control and metabolism

Insights into the assembly pathway of mitochondrial respiratory chain supercomplexes

Lizeth Camacho-Lopez, Jack Sleeman, Tram Huynh, [Flavia Fontanesi](#)

University of Miami, USA

Speaker: Flavia Fontanesi

The mitochondrial respiratory chain (MRC) plays a central role in cellular energy conversion, a process essential for aerobic life. The MRC is comprised of multimeric enzymatic complexes that couple electron transfer from reducing equivalents to molecular oxygen with the generation of a proton gradient across the mitochondrial inner membrane. MRC biogenesis and function involve the dynamic organization of the single MRC complexes in ordered structures known as supercomplexes (SCs). Respiratory SCs have been identified in numerous organisms and it is currently accepted that they represent the MRC universal organizing principle. However, how SCs assemble remains poorly understood. In the yeast *Saccharomyces cerevisiae*, SCs are composed of a complex III obligatory dimer and one or two complex IV monomers.

Here, to elucidate the yeast SC assembly pathway, we generated a knock-out collection of yeast strains lacking one individual complex III or IV subunit and examined the accumulation of subassembly intermediates by sucrose gradient sedimentation and Tandem Mass Tag (TMT) mass-spectrometry analysis. We identified a subset of subunits whose absence does not preclude complex III and IV association and that we propose to be incorporated directly into SCs. Moreover, we detected potential SC assembly intermediates containing complex III subunits associating with complex IV most likely prior to complex III dimerization. Overall, our data support a working model in which the complete assembly of individual MRC holoenzymes is not a pre-requisite for their association, but rather complex III and IV subunits and assembly modules interact with each other during SC biogenesis.

RNA exonuclease Xrn1 regulates TORC1 signaling and autophagy in response to SAM availability

Madeline McGinnis¹, Benjamin Sutter¹, Samira Jahangiri¹, Benjamin Tu^{1,2}

¹University of Texas Southwestern Medical Center, USA. ²Howard Hughes Medical Institute, USA

Speaker: Madeline McGinnis

Autophagy is a conserved process of cellular self-digestion that promotes survival during nutrient stress. In yeast, methionine starvation is sufficient to induce autophagy. One pathway of autophagy induction is governed by the SEACIT complex, which regulates TORC1 activity in response to amino acids through the Rag GTPases Gtr1 and Gtr2. However, the precise mechanism by which SEACIT senses amino acids and regulates TORC1 signaling remains incompletely understood. We have identified the conserved 5'-3' RNA exonuclease Xrn1 as a surprising and novel regulator of TORC1 activity in response to methionine starvation. This role of Xrn1 is dependent on its catalytic activity, but not on degradation of any specific class of mRNAs. Instead, Xrn1 modulates the nucleotide-binding state of the Gtr1/2 complex, which is key for its interaction with and activation of TORC1. Our work identifies a critical role for Xrn1 in nutrient sensing and growth control that extends beyond its canonical housekeeping function in RNA degradation and indicates an avenue for RNA metabolism to function in amino acid signaling into TORC1.

The yeast Mkt1/Pbp1 complex promotes adaptive responses to respiratory growth

Daniel Caballero, Benjamin Sutter, Yu-san Yang, Yun Wang, Benjamin Tu

University of Texas Southwestern Medical Center, USA

Speaker: Daniel Caballero

Mkt1 (Maintenance of K2 Killer Toxin 1) is a poorly understood protein belonging to the Rad2/XPG family of DNA repair enzymes. Complex genetics studies have shown that a single Mkt1 amino acid polymorphism (Mkt1-G30D) underlies variation seen across many phenotypes in laboratory *S. cerevisiae* strains. These studies have suggested an important role for Mkt1 in promoting mitochondrial function, but this has not been thoroughly tested. Mkt1 is thought to form a complex with yeast ataxin-2, or Pbp1 (polyA binding protein-binding protein 1), which we have shown is required for the translation of Puf3-target mRNAs involved in mitochondrial biogenesis as well as the induction of autophagy during respiratory growth. We used CEN.PK yeast which naturally harbor the wild type Mkt1-30G allele to find that Mkt1 is required like Pbp1 in Puf3-target mRNA translation and autophagy. Notably, expression of the loss-of-function Mkt1-30D allele in CEN.PK yeast led to reduced Mkt1 and Pbp1 protein levels and disruption of both Puf3-target protein expression and autophagy. This result helps explain previously reported loss-of-function effects of the Mkt1-G30D polymorphism on mitochondrial function. Furthermore, we observed S288C yeast, which have evolved the Mkt1-30D allele, are deficient in Puf3-target mRNA translation and autophagy, and both of these processes were rescued by expression of the wild-type Mkt1-30G allele. Thus, Mkt1 and Pbp1 appear to function in a complex to promote vital processes during respiratory growth, and the Mkt1-G30D polymorphism may represent an adaptation by 20th century yeast to limit mitochondrial function for survival in an artificial, glucose-rich laboratory environment.

Branched-chain amino acid pathway regulation and its connection to carbon metabolism during yeast diauxic shift

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Speaker: Ximena Escalera-Fanjul

In *Saccharomyces cerevisiae* TORC1 controls the transition between exponential growth and stationary phase during the diauxic shift. This transition requires a balanced remodeling of metabolic fluxes through central carbon and amino acid metabolism, the appropriate distribution of the metabolites shared by these pathways ensures that energy and biomass production match cell growth during this transition. In yeast TORC1 pathway is regulated by leucine, but how this branched chain amino acid (BCAA) is regulated over the diauxic shift and to which extent the BCAA pathway plays a role in the crosstalk between central carbon and amino acid metabolism is only partially characterized. To clarify these issues, we generated a strain library of the pathway GFP-fusions, in wild type and the deletion strains that did not disrupt the biosynthesis of any BCAAs (isoleucine, valine and leucine). We assessed the protein and metabolite profile of the pathway, as well as the metabolite cell context throughout the diauxic shift, by high-throughput flow cytometry and untargeted LC-MS, respectively. We reveal that BCAA response to the diauxic shift matches TORC1 activity, the fraction of the pathway committed to leucine biosynthesis displays a fermentative profile, opposed to the respiratory signature of the portion of the pathway shared by the three BCAAs. We identify the key elements regulating the pathway, and how the misregulation of the BCAA pathway affects distant metabolic circuits including central carbon metabolism. Additionally, we show preliminary results of a constraint based model aiming to capture the dynamics of the pathway on a cellular context.

Stress Granules mediate metabolic adaptation by directing local remodelling of cellular architecture and resources

Daniel Kaganovich

University of Southampton, United Kingdom

Speaker: Daniel Kaganovich

“Persistence” in the face of antimicrobial drugs and other chemotherapeutics is the result of extreme adaptability on the part of a small sub-population of pathogenic cells to otherwise lethal concentrations of a drug, that does not rely on heritable genetic changes. Unlike heritable “resistance,” which manifests in cell growth despite drug presence, persistent populations arise stochastically, are metabolically and mitotically dormant during the presence of the drug, and resume growth after the drug is removed. Persistent populations can acquire adaptive features over time, thereby increasing their tolerance of recurring treatment. We have investigated an under-explored stress sensory/signaling pathway known to contribute to stress-tolerance and virulence in yeast and other fungi. We have shown that the eisosome, a fibrillar stress sensory structure on the membrane (where stress is initially perceived), communicates stress signals to the Stress Granule (SG), a response command center that facilitates adaptation on multiple post-transcriptional levels: translational, signaling, and metabolic. We discovered that SGs initiate a metabolic arrest sending cells into a state of partial dormancy. Importantly, the formation of SGs conferred a fitness advantage on yeast cells during stress suggesting that dormancy is coupled with metabolic rewiring and adaptation. In our preliminary work we uncovered mechanistic clues to how the eisosome-SG axis regulates metabolic adaptation and persistence.

A Coarse-Grained Model Integrating Metabolism, Growth and Cycle in *Saccharomyces cerevisiae*

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Speaker: Pasquale Palumbo

Whole-cell quantitative models are gaining interest to explain complex phenotypes and to accelerate biological discoveries (Karr et al, 2012). Towards this end, we recently proposed iMeGroCy (integrated Metabolism, Growth and Cycle, Palumbo et al, 2018) a model integrating the main cellular functions in *Saccharomyces cerevisiae*. Within the spirit of whole-cell models, iMeGroCy is conceived to be modular and hierarchical; its coarse-grained backbone is constituted by two building blocks: MeGro, combining metabolism and growth activities, and GroCy, linking growth to cell cycle and division. With respect to its preliminary version of 2018, iMeGroCy is now able to reproduce different quantitative and qualitative experimental findings both in single cells and in populations; besides, it offers a robust scaffold for plugging-in finer molecular details in each coarse module, so allowing to identify the molecular basis of mutant strains and to assign genotype-to-phenotype correlations.

1) Karr, J.R., Sanghvi, J.C., Macklin, D.N., Gutschow, M.V., Jacobs, J.M., Bolival, B. Jr, Assad-Garcia, N., Glass, J.I., Covert, M.W., A whole-cell computational model predicts phenotype from genotype, *Cell*, 2012

2) Palumbo, P., Vanoni, M., Papa, F., Busti, S., Wortel, M., Teusink, B., and Alberghina, L., An integrated model quantitatively describing metabolism, growth and cell cycle in budding yeast, *Communications in Computer and Information Science*, 2018

Yeast proteomic response to amino acid supplementation

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Speaker: Pauline Trebulle

Yeasts have evolved to optimize their use of environmentally available nutrients while retaining the capacity to adapt to potential adverse conditions such as nutrient depletion. Adaptation to different environments requires the simultaneous change of hundreds of metabolic reactions and the expression of a broad range of enzymes and proteins.

To deepen our understanding of how *S. cerevisiae* adapts its metabolism to nutrient availability, we first conducted metabolomic experiments to study the shift from production to uptake of 18 individual amino acids (AA) supplemented at different concentrations in the environment. We then used our high-throughput proteomics pipeline to measure the proteome of *S. cerevisiae* in 20 different amino acid-supplemented conditions to gain insights into the metabolic adaptation and change in resource allocation to the availability of the different AAs.

We identified a total of 3013 proteins across all conditions and used this dataset to identify differentially expressed proteins associated with media-specific and core response to supplementation, and to study the reorganization of metabolic fluxes, notably in AA biosynthesis and degradation pathways. We observed that supplementing leucine and methionine led to the strongest proteome reconfiguration for individual AA supplementation, while arginine metabolism was among the pathways most often perturbed. Interestingly, the proteome profiles displayed similarities independent of the supplemented AA structure or chemistry and seem instead driven by distinct metabolic and stress responses, thus reflecting the complex interplay between nutrient utilization, proteome reconfiguration, and environmental adaptation in yeast.

Mutations of *PRS1* and *PRS5* impair *Saccharomyces cerevisiae* response to cell wall integrity and rapamycin treatment

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Speaker: Michael Schweizer

The *Saccharomyces cerevisiae* genome contains five *PRS* genes (*PRS1–PRS5*). Theoretically, each of the Prs polypeptides is capable of synthesizing phosphoribosyl pyrophosphate (PRPP), required for the synthesis of purine and pyrimidine nucleotides and histidine and tryptophan, but yeast can only survive if one of the following functional entities – Prs1/Prs3, Prs2/Prs5 or Prs4/Prs5 – is present. Prs1 and Prs5 differ in length from Prs2, Prs3 and Prs4 since they contain non-homologous regions, designated NHR1-1, NHR5-1 and NHR5-2, respectively. Deletion of NHR1-1 causes a significant increase in PRPP synthetase activity and prevents the interaction with Slt2, allowing enzyme activity and maintenance of CWI to be separated.

Rapamycin inhibits TORC1 resulting in the activation of the cell wall integrity (CWI) pathway (Loewith & Hall, 2011). Phosphoproteome analyses indicated that Prs5 is phosphorylated upon rapamycin treatment (Huber et al., 2009), which we have now confirmed through gel-shift assays. Multiple alanine substitutions of the three phosphosites S₃₆₄, S₃₆₇ and S₃₆₉ in NHR5-2 compromise Rlm1 expression upon rapamycin treatment.

It appears that the *PRS* gene family has, as result of gene duplication and acquisition of NHRs, evolved to link two essential functions, PRPP synthesis and CWI, to regulation by TORC1.

Workshop 5 - Gene expression: from epigenetic regulation to mRNA stability and Cell Cycle Control

Targeting APEX2 to the mRNA encoding fatty acid synthase β in yeast identifies proteins that bind and control its translational efficiency in the cell cycle

Heidi Blank, [Michael Polymenis](#)

Texas A&M University, USA

Speaker: Michael Polymenis

Profiling the repertoire of proteins associated with a given mRNA during the cell cycle is unstudied. Furthermore, even from asynchronous cells, it is much easier to ask and answer what mRNAs a specific protein might bind to than the other way around. However, CRISPR-Cas and other technologies have recently been developed for proximity-labeling approaches engineered to target specific mRNAs. For the first time in any system, we implemented this technology at different points in the cell cycle in highly synchronous yeast cultures. We had previously shown that the translational efficiency of the *FAS1* mRNA, encoding fatty acid synthase, peaks late in the cell cycle, and translational upregulation of *FAS1* accelerates nuclear division. To understand how the translation of *FAS1* is cell cycle-regulated, we identified proteins that bind the *FAS1* transcript in a cell cycle-dependent manner. We used dCas13d-APEX2 fusions to target *FAS1* and label nearby proteins, which were then identified by mass spectrometry. Putative hits were independently validated by immunoprecipitation of the corresponding RNA-binding proteins and measuring the associated *FAS1* transcript. Surprisingly, the glycolytic enzyme Tdh3, a known RNA-binding protein, bound the *FAS1* mRNA, and it was necessary for the increased Fas1 expression late in the cell cycle. These results point to unexpected connections between major metabolic pathways. They also underscore the role of mRNA-protein interactions for gene expression during cell division. We will discuss the general uses of this novel, mRNA-centric technology for studies of mRNA-protein interactions.

Dissecting the genetic drivers of transcriptional heterogeneity under normal and stress conditions

Francesc Posas^{1,2}, Mariona Nadal-Ribelles¹, Carme sole¹, Eulàlia de Nadal^{1,2}

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Speaker: Francesc Posas

Cellular heterogeneity is a key determinant of disease outcome and therapeutic treatments across virtually all organisms. The various cellular responses that ultimately decide cell fate and phenotypic diversity are driven by variation within a population. The molecular basis of this cell-to-cell variation and how it affects the phenotypic spectrum that develops during adaptive responses to environmental changes are both poorly understood. To understand the contribution of each gene to the resulting adaptive transcriptome, we designed a gene-deletion strategy to combine genetic x environmental perturbation screens with single cell RNA-seq profiling (scRNA-seq). Here we profiled a total of 1.2M cells from more than 3000 different genotypes to generate high-resolution genotype-transcriptome maps under normal and osmostress conditions. We used transcriptional phenotype to identify distinct transcriptional architecture, variable gene usage, gene function associations, and uncover regulators of heterogeneity. Our results demonstrate that only a fraction of the core osmoresponsive programme is simultaneously co-expressed, including a preferential use of transcription factors. Harnessing intra- genotype heterogeneity led us to uncover and experimentally validate positive and negative universal or condition-specific regulators of cellular heterogeneity. Our findings expose the complexity of gene and genotype transcriptome layers.

Using Budding Yeast to Model and Characterize Human Oncohistone Mutants

Celina Jones, Maggie Zhang, Jennifer Spangle, Anita Corbett

Emory University, USA

Speaker: Celina Jone

Packaging genetic material into the nucleus is a major challenge that all eukaryotic organisms face. This packaging is accomplished by wrapping the genome around histone proteins. Hence, histones control chromatin accessibility and gene expression, which are largely regulated by histone post translational modifications (PTMs). Recently, recurrent missense mutations in genes encoding histones were found to drive oncogenesis in humans, creating oncohistones. Understanding how these pathogenic missense mutations alter histone function is difficult in humans as the four core histone proteins are each encoded by 12 – 15 genes, while budding yeast only contain two genes encoding each protein. Thus, engineering genetically homogenous strains that solely express the oncohistone mutant of interest is less technically challenging in yeast, facilitating clean characterization of altered PTMs and downstream mutant-driven molecular disruptions. Additionally, to more faithfully model the dominant effects observed in humans, yeast can be engineered to express both a mutant and wild type histone copy. Our group has leveraged the strengths of budding yeast to model and characterize oncohistone mutants. We previously performed a high copy suppressor screen to identify suppressors of growth defects in histone H3K36 mutant cells, which model the oncohistone H3K36M. This study revealed connections to histone modifying enzymes, with investigations underway to define the mechanisms of suppression. An added benefit to studying these mutations in yeast is how amenable this model organism is to undergraduate research, enabling us to prepare young scientists for careers in biological research. Employing yeast to investigate oncohistones promotes rapid discovery in disease-driving mutation research.

The spatiotemporal proteome of the yeast cell division cycle

Athanasios Litsios, Benjamin Gryss, Oren Kraus, Helena Friesen, Charlie Boone, Brenda Andrews

University of Toronto, Canada

Speaker: Athanasios Litsios

The successful execution of the cell cycle program relies on timely coordinated changes in protein localization and concentration. While cell population-level approaches were recently used to monitor changes in protein concentration during the yeast cell cycle, the systematic assessment of protein localization dynamics remains challenging. We have established an experimental and computational pipeline which allowed us to monitor the combined changes in subcellular localization and concentration of ≈ 3900 proteins in live cells, during the division cycle of budding yeast. We report that more than a quarter of the assessed proteome exhibits cell cycle-related periodicity. However, proteins tend to change in either concentration or localization, and not both, during the cell cycle. We show that protein movements span more than 15 distinct subcellular localization classes, and involve mostly physically large compartments and sites of polarized growth. By combining protein localization and concentration measurements, we show that intracompartamental changes in protein localization mediate changes in local protein concentration in a cell cycle-specific way, in the absence of changes in total protein levels. By integrating concentration measurements of all proteins, we observe that the total proteome content follows a global trend during the cell cycle, with most proteins displaying differential scaling with cell size during G1, and peaking in concentration around cell cycle commitment. Finally, by complementing our proteome data with cell cycle-resolved transcriptome and translational efficiency measurements, we highlight the underlying mechanisms that determine the periodicity in proteome concentration. Collectively, we present a high-resolution, proteome-level spatiotemporal map of the yeast cell cycle.

A prion-form of Cut4/Apc1 confers non-Mendelian inheritance of defective heterochromatin silencing in Fission Yeast

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⁴MS University, Baroda, India

Speaker: Jagmohan Singh

Prions represent epigenetic regulator proteins that can self-propagate their structure and confer their misfolded structure and function on normally folded proteins, which are inherited as cytoplasmic factors in a non-Mendelian fashion. Like the mammalian prion PrP^{Sc}, prions also occur in fungi. While a few prions, like Swi1, affect gene expression, none are shown to affect heterochromatin structure and function. In fission yeast and metazoans, histone methyltransferase Clr4/Suv39 causes H3-Lys9 methylation, which is bound by the chromodomain protein Swi6/HP1 to assemble heterochromatin. The heterochromatin structure is stably propagated as Mendelian epiallele during mitosis and meiosis. Earlier, we showed that sng2-1 mutation in the Cut4 subunit of Anaphase Promoting Complex abrogates heterochromatin structure due to defective binding and recruitment of Swi6. Here, we demonstrate that the Cut4p forms a non-canonical prion form, designated as [SNG2], which abrogates heterochromatin silencing. The heterochromatin defect of [SNG2] exhibits various prion-like properties, e.g., non-Mendelian inheritance, requirement of Hsp proteins for its propagation, de novo generation upon cut4 overexpression, reversible curing by guanidine, cytoplasmic inheritance, dominance and formation of infectious protein aggregates, which are dissolved upon overexpression of hsp genes. This is the first instance of non-Mendelian inheritance of heterochromatin structure in any species.

Post-transcriptional regulation of fungal cell walls by the conserved RNA-binding virulence factor Ssd1

Marah Jnied¹, Laura Tuck¹, Evelina Tutucci², Edward Wallace¹

¹The University of Edinburgh, United Kingdom. ²Vrije Universiteit Amsterdam, Netherlands

Speaker: Marah Jnied

Cells grow and divide while maintaining their spatial organisation, by controlling the subcellular localisation of their RNA and proteins. The asymmetric localisation of fungal cell wall biogenesis at the growth tip, yeast bud, and cell septum, raises the question of where cell wall proteins are synthesised. Ssd1 is a conserved RNA-binding protein, required for tolerance of cell wall stress in *Saccharomyces cerevisiae* and for virulence in *Candida albicans* and *Candida glabrata*. We hypothesise that Ssd1 affects cell walls and virulence through the localised control of cell wall protein synthesis.

Recent data from our lab show that Ssd1 binds near the start codons of mRNAs encoding specific cell wall proteins. We test the hypothesis that Ssd1 controls localised translation of target cell wall mRNAs near sites of cell wall synthesis. We employ single molecule fluorescence in situ hybridisation (smFISH) to understand Ssd1 regulation of target mRNAs in yeast. Using microscopy and flow cytometry, we also investigate the effect of Ssd1 on the production and localisation of cell wall proteins.

Our results support the hypothesis that Ssd1 represses the translation of cell wall proteins. We show that RNA targets of Ssd1 localise in a cell-cycle dependent manner. However, we do not find clear evidence that Ssd1 affects mRNA localisation, suggesting involvement of other RNA-binding proteins. Our findings in yeast can generate insight into post-transcriptional control of cell walls in pathogenic fungi, which pose major threats to human and crop health and rely on homologous systems of post-transcriptional regulation for growth and virulence.

Study of Cornelia de-Lange syndrome mimetics mutants in budding yeast (*Saccharomyces cerevisiae*).

Amparo Picard-Sánchez, Ethel Queralt

Institute of Biomedicine of Valencia (IBV-CSIC), Spain

Speaker: Amparo Picard-Sánchez

Cornelia de-Lange syndrome (CdLS) is a rare disease characterized by facial dysmorphism, upper limb reductions and digestive and cardiac problems. This affection is caused by mutations in structural maintenance chromosome genes (*SMC*) such as the cohesin complex and its regulators. Canonically, the cohesin complex has been described as a molecular glue that keeps the two sister chromatids together to avoid precocious sister chromatids' segregations. However recent studies showed that it may be also involved in the regulation of chromatin organization. Most of the CdLS patients (around 75%) present heterozygous mutations in *NIPBL* -described as the cohesin loader. Since *SMC* genes are well conserved through evolution, the aim of this work is to characterise the different *NIPBL* mutations in a budding yeast model (*hNIPBL – SCC2*), *Saccharomyces cerevisiae*. For that purpose, we have prepared different strains of *S. cerevisiae* in heterozygous diploid strains mimicking the mutations of CdLS patients where the control allele and the mutated allele have a different epitope-tagging to study the contribution of both alleles. We expect to get new information about the molecular basis of the CdLS. In addition, we want to characterize whether the CdLS phenotypes are due to a *NIPBL* haploinsufficiency or a negative dominance. These results will shade more light on the understanding of this rare disease and perhaps open the door to new personalized therapies.

Intron plays a role in releasing a gene from its repression state in yeast

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Speaker: Hiroki Kikuta

Higher eukaryotes have many introns in the genomes but the physiological importance is still unclear. One of the known intron functions is intron-mediated enhancement (IME), in which gene expression is enhanced by the presence of introns. The levels of enhancement in IME are affected by coding sequences (CDSs)¹, suggesting that nucleotide-sequences causing IME may be present in IME-sensitive CDSs. To reveal such sequences, we used codon-changed CDSs. In *Saccharomyces cerevisiae*, the expression of a yeast-codon-optimized luciferase yCLuc was enhanced by the presence of an intron, but another codon-changed luciferase hCLuc was not. In addition, the activity of hCLuc was similar to that of the intron-enhanced yCLuc. These results indicated that IME-sensitive CDSs were repressed. Chimeric CDSs consisting of yCLuc and hCLuc were constructed. As a result, N-terminal 100-150 bp region from the start codon was responsible for IME. Yeast-codon-optimized glucoamylase yRoGLU1 was also IME-sensitive¹. An IME-insensitive glucoamylase yRoGLU1-mc8 was obtained by random substitution of 5'-300 bp region of yRoGLU1 with synonymous codons. Finally, chimeric CDSs analysis and deletion analysis in 5'-UTR revealed that only the four-nucleotide sequence TCTT present at promoter-proximal positions caused repression, and the presence of an intron released such repressed-state genes. These results suggested that yeast may have the repression mechanism, which depends on nucleotides-sequence such as TCTT. Enhancing expression of such genes by getting introns may be advantage for the evolution of eukaryotic cells.

Reference:¹ Hoshida et al., 2017

Workshop 6 - Stress Response, aggregation and Misfolding

Nuclear and cytoplasmic spatial protein quality control is coordinated by nuclear-vacuolar junctions and perinuclear ESCRT

Fabian Morales-Polanco¹, Emily Sontag², Jin-hua Chen³, Gerry McDermott³, Patrick Dolan¹, Daniel Gestaut¹, Mark Le Gros³, Caroline Larabell³, Judith Frydman¹

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Speaker: Fabian Morales-Polanco

Effective Protein Quality Control (PQC), essential for cellular health, relies on spatial sequestration of misfolded proteins into defined inclusions. Here we reveal the coordination of nuclear and cytoplasmic spatial PQC. Cytoplasmic misfolded proteins concentrate in a cytoplasmic Juxta Nuclear Quality control compartment (JUNQ) while nuclear misfolded proteins sequester into an IntraNuclear Quality control compartment (INQ). Particle tracking reveals INQ and JUNQ converge to face each other across the nuclear envelope at a site proximal to the Nuclear-Vacuolar Junction (NVJ) marked by perinuclear ESCRT-II/-III protein Chm7. Strikingly, convergence at NVJ contacts facilitates VPS4-dependent vacuolar clearance of misfolded cytoplasmic and nuclear proteins, the latter entailing extrusion of nuclear INQ into the vacuole. Finding that nuclear-vacuolar contact sites are cellular hubs of spatial PQC to facilitate vacuolar clearance of nuclear and cytoplasmic inclusions highlights the role of cellular architecture in proteostasis maintenance.

A humanized yeast model to evaluate anti-aging effects of natural extracts

Farida Tripodi¹, Belém Sampaio-Marques^{2,3}, Elena Savino⁴, Massimo Labra¹, Paola Coccetti¹

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Speaker: Farida Tripodi

Age-related diseases are among the major challenges in modern medicine, because of the progressive aging of the world population. In this context, nutrition, with its long-term consequences for human health, can be useful to prevent diseases and achieve healthy aging.

The budding yeast *Saccharomyces cerevisiae* has been successfully used to model the aging of post-mitotic cells of higher eukaryotes. Many different models using yeast strains overexpressing human genes associated with diseases like Alzheimer's disease (AD), Parkinson's disease (PD) or Huntington's disease (HD) have also been exploited in the last decades. Among them, one of the most studied yeast models consists on the expression of α -synuclein (α -syn), a presynaptic protein whose alteration is associated with synucleinopathies, such as PD. In our laboratory, we exploit a humanized yeast model of PD to evaluate the beneficial effects of natural extracts from legumes, mushrooms, and other edible matrices. We have found that aqueous extract from *Vigna unguiculata* beans and from two edible mushrooms, *Grifola frondosa* and *Hericium erinaceus*, are able to delay senescence and to reduce α -syn toxicity and membrane localization. We have also confirmed most of these beneficial effects in more complex model organisms, such as human neuroblastoma cells, *Drosophila melanogaster* and *Caenorhabditis elegans*, supporting the use of the yeast model for the screening of bioactivities present in natural extracts.

RTG signalling in stress response and faster cellular adaptation in the presence of mitochondrial dysfunction

Maria Antonietta Di Noia¹, Pasquale Scarcia¹, Gennaro Agrimi¹, Ohiemi Benjamin Ocheja¹, Ehtisham Wahid², Isabella Pisano¹, Cataldo Guaragnella², Luigi Palmieri¹, Nicoletta Guaragnella¹

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Speaker: Nicoletta Guaragnella

Mitochondria act as cellular hub of exogenous and endogenous stressors and alterations in mitochondrial function are implicated in a wide variety of pathological processes. Due to its capacity to tolerate respiratory defects or the complete loss of mitochondrial DNA, yeast is an ideal model to study how mitochondrial (dys)function might affect stress response and cellular adaptation. We have been elucidating how mitochondrial (dys)functions can impact adaptation of *Saccharomyces cerevisiae* to osmostress. In particular, we focused on the long-term response, which follows the well established short-term response mediated by the High Osmolarity Glycerol pathway. We demonstrated the essential role of the RTG mitochondrial retrograde signalling in long-term osmoadaptation, as judged by the higher sensitivity of cells lacking RTG2, the upstream positive regulator of RTG pathway. Interestingly, in the same conditions, yeast mutants with distinct mitochondrial dysfunctions, including the lack of mitochondrial DNA or the inactivation of specific mitochondrial carriers or transcription factors, showed an advantage in the kinetics of stress response compared to wild type cells. The mechanisms of faster adaptation rely on gene expression reconfiguration and metabolic adjustment: CIT2 and TCA cycle genes, such as CIT1, ACO1 and IDH1, were up-regulated mostly by RTG pathway while fermentative metabolism was fostered in the cellular context of reduced or null respiration. Peroxisome-mitochondria-nucleus cross talk sustained metabolic reprogramming and first evidence suggests that specific metabolites might act as signalling molecules for cellular adaptation. Overall, our data shed light on the multifaceted role of mitochondria in stress response, emphasizing metabolic, bioenergetic and signalling functions.

Ire1 mediates protein quality control systems of the endoplasmic reticulum and cytosol in the methylotrophic yeast *Pichia pastoris*

Yasmin Nabilah Binti Mohd Fauzee, Yukio Kimata

Nara Institute of Science and Technology, Japan

Speaker: Yasmin Nabilah Binti Mohd Fauzee

In eukaryotic species, the accumulation of misfolded proteins in the endoplasmic reticulum (ER) leads to a condition called ER stress. This condition provokes a cytoprotective transcription program called the unfolded protein response (UPR). The UPR is triggered by transmembrane ER-stress sensors including Ire1, which in many fungal species, acts as an endoribonuclease to splice and mature the mRNA encoding the transcription factor Hac1. Through analyses of methylotrophic yeast *Pichia pastoris* (syn. *Komagataella phaffii*), we discovered previously unknown role of Ire1. In *P. pastoris* cells, the *IRE1* knockout mutation (*ire1* Δ) and the *HAC1* knockout mutation (*hac1* Δ) caused only partially overlapping gene-expression change and both led to slowed growth in *P. pastoris* cells. Interestingly, protein aggregation and the heat shock response (HSR) were induced in *ire1* Δ cells but not in *hac1* Δ cells. Moreover, Ire1 was further activated upon high-temperature culturing and seemed to confer heat-stress resistance to *P. pastoris* cell. Our findings shown here cumulatively demonstrate an intriguing case in which the UPR machinery through Ire1 controls cytosolic protein folding status and the HSR, which is known to be activated upon accumulation of unfolded proteins in the cytosol and/or the nuclei. Therefore, this research may give us insights on the benefit of multiple stress response mechanism for industrial usage through genetic manipulation of *P. pastoris* cells.

Calcineurin stimulation by Cnb1p overproduction mitigates protein aggregation and α -synuclein toxicity in a yeast model of synucleinopathy

Srishti Chawla

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Speaker: Srishti Chawla

The calcium-responsive phosphatase, calcineurin, senses changes in Ca^{2+} concentrations in a calmodulin-dependent manner. Here we report that under non-stress conditions, inactivation of calcineurin signaling or deleting the calcineurin-dependent transcription factor CRZ1 triggered the formation of chaperone Hsp100p (Hsp104p)-associated protein aggregates in *Saccharomyces cerevisiae*. Furthermore, calcineurin inactivation aggravated α -Synuclein-related cytotoxicity. Conversely, elevating production of the calcineurin activator, Cnb1p, suppressed protein aggregation and cytotoxicity associated with the familial Parkinson's disease-related mutant α -Synuclein A53T in a partly CRZ1-dependent manner. Activation of calcineurin boosted normal localization of both α -synuclein and the A53T allele to the plasma membrane, an intervention previously shown to mitigate its toxicity in Parkinson's disease and disease models. The findings demonstrate that calcineurin signaling, and Ca^{2+} influx to the vacuole, limit protein quality control in non-stressed cells and may have implications for elucidating to which extent aberrant calcineurin signaling contributes to the progression of Parkinson's disease(s) and other synucleinopathies.

Urm1 and Sulfur: An Ancient Partnership Against Oxidative Stress and Aging?

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Speaker: Lars Kaduhr

Ubiquitin-like proteins (Ubls) are present in all domains of life. Like ubiquitin, they are conjugated to their substrates, such as proteins or lipids, by specific enzymatic cascades. Defects in these sophisticated networks are linked to human diseases and thus require extensive research. In 2000, the team of Nobel Laureate Yoshinori Ohsumi discovered a novel Ubl named Ubiquitin-related modifier 1 (Urm1) in *Saccharomyces cerevisiae*. This unique Ubl was later found to deliver sulfur for tRNA anticodon thiolation, important to prevent ribosomal errors during translation and hence assigned as noncanonical Ubl. However, despite two decades of research into Urm1, its initially identified function as a protein modifier and the molecular mechanism behind its conjugation (urmylation), remained largely unclear. In 2022, Ravichandran, Kaduhr et al. (*EMBO J.* 41(20):e111318) found that urmylation triggered by oxidative stress is directly linked to cysteine persulfidation of target proteins. This post-translational modification of cysteine thiols is proposed to be a redox-switch for protein modulation and was found to decrease with aging. The main function behind protein persulfidation is thought to be protection of cysteine thiols that are prone to oxidation by reactive oxygen species. Thus, the attachment of Urm1 to target proteins might represent a remnant of the actual process of selective cysteine thiol protection.

Plasma membrane tension regulates the condensation of Whi3 into super assemblies during cellular memory acquisition.

Fabrice Caudron

IGMM, CNRS, France

Speaker: Fabrice Caudron

Asymmetric cell division allows cells to diversify and acquire distinct cell fates. For example stem cells divide into a differentiating cell and a cell that keeps its stemness. Moreover, cells can acquire memories of past stresses. We discovered that the yeast *Saccharomyces cerevisiae* can maintain a memory of deceptive mating attempts. This memory is maintained by a novel type of prion-like assembly of the protein Whi3, that we termed a mnemon. Mnemons can change their conformation, in a self-templating manner, yet distinctly from prions, they are confined into only one cell during cell division. How is the mnemon form of Whi3 induced during prolonged mating pheromone response remains unknown.

We have tested the idea that Whi3 mnemon form is induced by an increase in plasma membrane tension when cells form long shmoos. I will present our data that are consistent with a role for eisosomes and the TORC2 complex in initiating the conformational change of Whi3 and therefore promoting the establishment of the memory of past deceptive mating attempts.

RNA recoding induced by the adenosine deaminase acting on RNA enzymes (ADAR) may result in proteotoxic stress

Adi Avram-Shperling¹, Amit Ben-David¹, Itamar Twersky¹, Eli Kopel¹, Joshua J.C. Rosenthal², Erez Y. Levanon¹, Eli Eisenberg³, Shay Ben Aroya¹

¹Bar Ilan University, Israel. ²The University of Chicago, USA. ³Tel Aviv University, Israel

Speaker: Adi Avram-Shperling

Traditionally, DNA mutations are considered to bear the sole responsibility for alterations in genomic information. However, A-to-I RNA editing, in which genomically encoded adenosines are transformed and recognized as guanosines in the RNA sequence, is an endogenous and powerful mean of creating inner transcriptome diversity. The magnitude of A-to-I RNA editing is unprecedented, with millions of sites already identified in the human genome. Most of these editing events in human are located within the noncoding *Alu*-elements. An interesting predication is that similarly to DNA mutations, RNA editing can also serve as a source for proteotoxic stress. To test this possibility, we exogenously expressed heterologous ADARs in the yeast *Saccharomyces Cerevisiae*, whose origins precede the emergence of ADAR, but can express ADARs originated from different organisms. Exogenically expressed ADARs had different effect on yeast viability and showed a diverse editing spectrum, demonstrating that the expression in a selection-neutral environment can reveal their true editing potential. Furthermore, mass-spectrometry analysis revealed that many of the RNA editing events resulted in increased protein diversity, which enhanced their ubiquitination and tendency to aggregate. Hence, our results suggest that RNA editing events induced by ADAR dysregulation are manifested at the proteomic level, and are an overlooked source of proteotoxic stress. Since RNA secondary structure is affected by temperature, the enzymes most likely adapted distinctive mechanisms prompted among others by differences in body temperature, to better recognize their substrates. Elucidating such mechanisms could help to better understand ADARs evolution and the harmful effects of its dysregulation.

Hsp42: The Hidden Architect Behind Stress Granule Dynamics

Alice Lippi, Anita Kriško.

Department of Experimental Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany

Speaker: Alice Lippi

Protein homeostasis (proteostasis) involves complex pathways of maintenance and repair to preserve cellular functioning. Failure of proteostasis contributes to the accumulation of toxic protein aggregates in cells and has long been linked to aging and neurodegenerative diseases. Hsp42 chaperone is a yeast aggregase essential for nucleation and growth of protein aggregates, Q bodies. Moreover, when cells experience stress, they shut down the translation of housekeeping genes in order to prioritize survival and stress response genes. This event manifests in the formation of cytoplasmatic stress granules (SGs).

Our study reveals a novel role of Hsp42 in stress granule assembly and dissolution. Our findings demonstrate that SG formation and dissolution are stepwise processes in wild-type cells, with SGs undergoing fusion and fission events. However, these events do not occur in the absence of Hsp42. In addition, in the absence of Hsp42, the disaggregation machinery (Hsp104-Hsp70) fails to colocalize with Pab1, a well-known marker of SGs. This observation suggests that Hsp42 may play a significant role in influencing the compactness of SGs.

Furthermore, our results demonstrate that Hsp42 plays a crucial role in modulating the fluidity and organisation of SGs, contributing to the dynamic sorting and arrangement of molecules within these granules, both in live cells and in vitro. In live cells, the *hsp42*Δ mutant exhibited a notable reduction in the mobility of molecules within the SGs.

Taken together, these findings suggest that Hsp42 physical and chemical properties may influence the interactions and organisation of various components within SGs.

Workshop 7 - DNA replication & recombination; DNA Damage & Repair

Sequence and chromatin features guide resection initiation at DNA double-strand breaks

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Speaker: Robert Gnügge

DNA double-strand breaks (DSBs) are common genomic lesions that must be accurately and efficiently repaired to ensure genome stability and organism survival. One major DSB repair pathway, called homologous recombination (HR), relies on the nucleolytic degradation of DSB ends in a process termed end resection. The Mre11-Rad50-Xrs2 (MRX) complex initiates end resection by nicking the 5'-terminated DNA strands internal to DSBs. Previous studies established how cofactors and protein blocks at DSBs stimulate MRX nicking *in vitro*. However, it is unknown how MRX cleavage is influenced by the sequence and chromatin landscape surrounding a DSB *in vivo*. Here we show that both DNA sequence and chromatin features impact on MRX nicking. We mapped MRX nicks quantitatively and at single-nucleotide resolution next to multiple defined DSBs in the yeast genome. Consistent with previous *in vitro* assays, we observed that the Ku70-Ku80 complex directed DSB-proximal nicks. Repetitive MRX nicking then extended the resection tracts. MRX cleavage was enhanced in linker DNA between nucleosomes and reduced in highly transcribed regions. Consistently, MRX nicking was influenced by chromatin remodelers that are involved in nucleosome dynamics and promoter regulation. Additionally, we observed that MRX preferentially cleaved DNA containing a specific sequence motif and a related DNA meltability and bending profile, and these findings are consistent with recent structural studies. Our results provide fundamental mechanistic insights into the initial step of DSB repair by HR.

Role of the PP2A phosphatase regulatory subunits in the DNA damage response

Erika Casari, Maria Pia Longhese

University of Milano-Bicocca, Italy

Speaker: Erika Casari

DNA double strand breaks (DSBs) are cytotoxic lesions that must be repaired to ensure genomic stability. One way to repair DSBs is the homologous recombination (HR) mechanism, which uses intact homologous DNA sequence as template for the repair. HR is initiated by nucleolytic degradation (resection) of DSBs, which is a two-step process in which an initial short-range phase is catalyzed by the MRX complex. Then the two long-range resection Exo1 and Dna2-Sgs1 nucleases extend the resected DNA tracts. We have previously shown that the lack of both Exo1 and Sgs1 activates a checkpoint in response to genotoxic treatments that is stronger than wild-type cells and that depends primarily on the 9-1-1 complex and the scaffold proteins Dpb11 and Rad9 (referred to as the 9-1-1 axis). This persistent checkpoint activation contributes to the DNA damage sensitivity of cells lacking both Exo1 and Sgs1. To better understand how this pathway is regulated, we performed genetic screens in *Saccharomyces cerevisiae* to search for extragenic mutations that suppress the DNA damage sensitivity of *exo1* Δ *sgs1* Δ cells. Genome wide-sequencing and genetic analyses allowed to establish that the suppressing mutations hit two different genes: *CDC55* and *TPD3*, encoding the regulatory and the scaffolding subunits of the protein phosphatase PP2A. We demonstrated that Cdc55 and Tpd3 downregulate the checkpoint dependent on the 9-1-1 axis by interacting with the Ddc1 protein and counteracting Ddc1-Dpb11 interaction.

Mitochondrial DNA homeostasis in budding yeast is achieved through cell-volume-dependent machinery

Alissa Finster, Anika Seel, Francesco Padovani, Kurt Schmoller

Helmholtz Munich, Germany

Speaker: Alissa Finster

In contrast to cell-cycle-dependent replication of nuclear DNA, the mechanism of mitochondrial DNA (mtDNA) homeostasis is poorly understood. Recently, we have shown in budding yeast that mtDNA copy number increases with cell volume, maintaining constant mtDNA concentrations during cell growth. Based on our results, we propose that this coupling of mtDNA to cell volume is controlled by nuclear encoded limiting factors for mtDNA replication and maintenance, which increase in amount as cell volume rises, leading to a corresponding increase of mtDNA copy number. We find that the major components of this limiting machinery for mtDNA maintenance are the mitochondrial DNA polymerase Mip1 and the packaging factor Abf2, with additional contribution of the mitochondrial ssDNA-binding protein Rim1. Taken together, we show that cell-volume-dependent mtDNA homeostasis can be achieved without a need for cell-cycle-dependent regulation. Currently, we aim to test our model by putting the limiting proteins under the control of histone promoters. Thereby, we aim to decouple protein production from cell volume, which will allow us to analyze the impact of cell volume on mtDNA independently of the nuclear encoded machinery.

Regulation of DNA damage tolerance pathways in *Saccharomyces cerevisiae*

Matan Arbel-Groissman, Martin Kupiec

tel aviv university, Israel

Speaker: Matan Arbel-Groissman

The timely replication of genomes is a daunting challenge, especially in the presence of DNA damage. We are interested in understanding how cells cope with DNA lesions during DNA replication, using the yeast *Saccharomyces cerevisiae* as a model.

When encountering lesions in the DNA the replication fork is faced with a 'decision' to make: how and when to bypass the damage in order to finish the replication. There are at least four competing pathways to be chosen from; two are mutagenic in nature, while the two others are 'error-free'. This 'decision' is made primarily by PCNA, a protein complex that lies at the heart of the replication and DNA damage repair processes. PCNA is a ring-shaped processivity factor for DNA polymerases and also serves as a signaling hub for the different replication and DNA damage repair proteins. PCNA undergoes many post-translational modifications; however, the "PCNA code" for decision-making is still unsolved. We have created a novel tool, Uni-PCNA, to decipher the PCNA code. Our studies aim to understand the mechanism that creates mutations, and has implications for evolution, biology in general, and the development of cancer.

The role of DNA repair proteins in formation of circular DNA from chromosomes in *Saccharomyces cerevisiae*

Rasmus Alexander Behrend Eugen-Olsen, Sefa Alizadeh, Birgitte Regenberg

Copenhagen University, Denmark

Speaker: Rasmus Alexander Behrend Eugen-Olsen

Extrachromosomal circular DNA (eccDNA) has emerged as an important driver of evolution and genetic heterogeneity in eukaryotic cells. Genes can exit their chromosomal configuration to form eccDNA, where their copy number will vary from no to hundreds of copies through unequal segregation during cell division. This generates genetically heterogeneous cell populations, increasing the phenotypic diversity on which natural selection can act. We have previously shown that eccDNA is commonly found in *Saccharomyces cerevisiae* and other tested species and that eccDNA-mediated copy number variations can provide rapid adaptive evolution to the host cell. However, little is known about how eccDNA forms. In yeast, eccDNA can form from the entire genome in an apparently stochastic process, while specific genes in direct repeat seem to form eccDNA with high frequency. In our current study, we investigate the role of DNA repair in the formation of eccDNA. To this end, we purify, amplify and sequence eccDNA from yeast mutants deficient in DNA repair proteins. Our results indicate that microhomology-mediated end joining plays an important role in eccDNA formation from non-repetitive chromosomal regions, while homologous recombination drives high rate formation of eccDNA from genes in direct repeat such as *HXT6/HXT7*, *ENA1/ENA2/ENA5* and *CUP1-1/CUP1-2*. Identifying the DNA repair mechanisms responsible for eccDNA generation could have implications in the understanding of human diseases such as cancer, where eccDNA plays a role.

Effect of inversion size on recombination landscape, and its impact on selection for recombination suppression

Cristina Berenguer Millanes, Bart Nieuwenhuis, Chantal Krüger, Sophia Horner, Pia Landskron, Aatreyi Roy

Ludwig-Maximilians Universität (LMU), Germany

Speaker: Cristina Berenguer Millanes

The canonical theory for sex chromosome evolution says that sex chromosomes evolved from a pair of homologous chromosomes with a sex-determining locus, followed by incorporation of sexually antagonistic alleles through recombination arrest. Inversions are assumed to be the main mechanism of recombination suppression. Successive incorporation of loci with sexual antagonistic alleles will lead to divergence of the sex chromosomes over time, however little experimental evidence for this process exists. We used the fission yeast *Schizosaccharomyces pombe* as a model species to study the evolution of the first steps of sex chromosome evolution. Artificially introduced inversions of different sizes around the mating-type locus are used to study how such inversions affect suppression of recombination, within and outside of the inversion. Suppression of recombination has been measured by tetrad dissections, showing that bigger inversions present lower germination rates, and an effect of size in both the recombination within the inversion and its flanks. Decay of Linkage Disequilibrium has been measured by bulk segregant re-sequencing, followed by SNP calling. We have detected a significant effect of inversion size in germination rates, as well as the pattern of recombination within and outside the inversions.

Mapping disordered interactions in the astral microtubule plus-end condensates in *S. cerevisiae*

Madhurima Choudhury, Sandro Meier, Ana-Maria Farcas, Yves Barral

ETH Zurich, Switzerland

Speaker: Madhurima Choudhury

Microtubule plus-end binding proteins (+TIPs), Kar9, Bik1, and Bim1 phase-separate to form a +TIP body at the plus-end of one astral microtubule (MT) in *Saccharomyces cerevisiae* (Meier et al.,2023). During metaphase, this body promotes spindle positioning through functionally specializing the MT and controlling its dynamics. Kar9, Bik1 and Bim1 are interwoven by redundant and multivalent interactions. At MT plus-end, Kar9 is recruited through self-interactions, and interactions with Bim1 and Bik1. Kar9-Kar9 and Kar9-Bim1 interactions have been characterized. However, functionality of the +TIP body also depends on the Kar9-Bik1 interactions, molecular basis of which is unknown. To identify these interactions, we developed an assay that relies on partitioning of fluorophore-tagged peptides into Bik1 droplets. With this, we have identified two alternatively charged KDK patches in Kar9, that interact with Bik1. Abrogating these patches results in significant loss of Kar9 recruitment at the plus-end in-vivo. Upon analysing sequences of other +TIPs, we found similarly charged patches. Kip2, a plus-end directed kinesin, contains RDR and KDK motives, similarly spaced as in Kar9. Kip2 is stabilized at the MT plus-end with help from Bik1 (Chen et al.,2023). We hypothesize that Kar9 and Kip2 use similar interactions to interact with Bik1. Such interactions could give rise to molecular competition, which might have a role in contributing to fluidity of the droplet. Fluidity would be an important parameter to couple forces required for spindle positioning. Testing whether abrogating these patches have an effect on spindle positioning during budding yeast division will be interesting to explore next.

Workshop 8 - Systems & Synthetic Biology of Yeasts

The Magnificent Seven: Mandatory Genes and Minimised Metabolisms

Giorgio Jansen¹, Grigoris Amoutzias², Vito Latora³, Giuseppe Nicosia⁴, [Stephen Oliver](#)¹

¹University of Cambridge, United Kingdom. ²University of Thessaly, Greece. ³Queen Mary University of London, United Kingdom. ⁴University of Catania, Italy

Speaker: Stephen Oliver

We have devised a new computational synthetic biology pipeline that infers the set of minimal metabolic networks (MMNs) of an organism by removing redundant genes from the genome-scale metabolic network. The resulting minimal set of genes still ensures viability and high growth rates. The composition of these MMNs defines a new functional class of genes that we call mandatory. Mandatory genes, while not essential, are very rarely eliminated in the construction of an MMN for a given organism, suggesting that it is difficult for metabolism to work without them. Moreover, the removal of mandatory genes from the minimized metabolic network significantly reduces its overall efficiency. We find that these genes have more gene interactions than most metabolic genes, but also their protein products show more protein-protein interactions. In *S. cerevisiae*, there are just seven mandatory genes that are found in all MMNs, irrespective of the growth conditions. We dub these genes the Magnificent Seven and find that three of them encode components of multiprotein complexes, four encode enzymes that participate in multiple pathways, and two catalyse multiple reactions. The simultaneous removal of all seven of these genes has a profound effect on the efficiency of the metabolic network. These genes are thus prime candidates for pathway engineering and their protein products represent important targets for new antimicrobial or anticancer agents.

Plastic eating yeast: The enhancement of *Saccharomyces cerevisiae* as tool for recombinant protein production

Charlotte Bilsby¹, Jack Davis¹, Samuel Jones¹, Andy Pickford², Tobias von der Haar¹

¹University of Kent, United Kingdom. ²University of Portsmouth, United Kingdom

Speaker: Charlotte Bilsby

Plastic is the most common single-use synthetic material in the world. Since only 9% of globally produced plastic is recycled, large amounts end up in the natural environment, with adverse consequences for plants, animals and humans. It is essential to explore means of addressing this existing pollution, as well as preventing new one. PETase, a novel enzyme discovered in 2016, has prompted an expansion in the research of the potential bio-degradation of PET (one of the most common single use plastics). A number of strategies for improving PETase activity through engineering and for developing efficient expression systems have been published, but most applications rely on the use of purified PETase, which involves costly and time-consuming downstream processing steps.

As an alternative lower-cost approach we have developed an *S. cerevisiae*-based expression system that exploits the ability of engineered, PET-secreting yeast cells to form biofilms in situ on waste PET. Our results indicate that yeast is able to secrete active PETase into the extracellular environment, and that PETase secreting biofilms direct PETase efficiently towards the degradation substrate. Yeast biofilm-based systems are in principle effective against types of waste that are difficult to address by other means (for example environmental plastic waste mixed with earth and other contaminants), and the PETase based system is easily adaptable to incorporate improved engineered PETase versions, alternative PETases, or enzymes that degrade other types of plastics.

Engineering *Kluyveromyces marxianus* for production of aromatic molecules

John Morrissey, Joel Akinola

University College Cork, Ireland

Speaker: John Morrissey

Phenylpropanoids are a broad class of secondary metabolites that are synthesised from aromatic amino acids. Many have useful bioactive properties or applications in food, cosmetics or other sectors. Different yeast species have been engineered to produce flavonoids, and other phenylpropanoid molecules but our work has focused on *Kluyveromyces marxianus*, which is a food grade budding yeast. A natural high flux through the pentose phosphate pathway, which is the source of building blocks for aromatic amino acids, rapid growth, and thermotolerance make this yeast an attractive host. We developed a synthetic biology toolkit based on CRISPRCas9 engineering and the YTK Golden Gate standard that enables rapid construction of engineered strains. By removing feedback inhibition of shikimate pathway enzymes, and increasing precursor supply, we were able to elevate flux to aromatic amino acids, which are then substrates for further conversion into phenylpropanoid molecules. More recently, we focused on adding a module to convert phenylalanine and tyrosine to coumaric acid, a building block for flavonoids and other aromatics. A number of different strategies that include expression of biosynthetic genes, abolition of competing reactions, and optimisation of growth conditions were employed. This resulted in strains of *K. marxianus* that produced coumaric acid de novo from minimal medium in small-scale bioreactors. These data and the next steps to achieve production of higher value metabolites from sustainable feedstocks will be presented.

Protein dynamics of whole-genome duplicates reveals mechanisms of gene retention

Elena Kuzmin

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Speaker: Elena Kuzmin

Gene duplication is pervasive in eukaryotes and is thought to contribute to genomic robustness. In this study, we measured protein localization and abundance dynamics for 90 paralog pairs derived from the whole-genome duplication event. Using an automated form of yeast genetics called Synthetic Genetic Array (SGA) analysis, we generated a total of 360 strains harboring paralogs tagged with GFP in wild-type and deletion backgrounds of their sister paralogs. We used high-content screening and machine learning-based automated image analysis to quantify how the subcellular localization and abundance of paralogs changed in response to the perturbation of the sister paralog. Our findings revealed that ~20% of paralogs exhibited relative abundance and relocalization changes. Compensatory changes were less common than cases of dependency. Evolutionary and physiological features, such as sequence similarity, shared protein interactions, and negative genetic interactions correlate with the redistribution, suggesting that functional redundant paralogs compensate for each other's loss through protein abundance and localization changes. Redistributed paralogs show private protein-protein interactors in the subcellular compartment of the sister paralog, suggesting that the rewiring of protein-protein interactions might act as a requirement for redistribution. We uncovered noncanonical isoform expression as a novel mechanism of compensation of paralogs. Specifically, CUE4 is predicted to carry out ubiquitination, and it changes localization from ER to the cytoplasm to compensate for the loss of its cytoplasmic paralog CUE1 due to expression of a CUE4 noncanonical isoform. Overall, our study uncovers novel insights into the retention of paralogs in evolution and forces that shape genomes.

Genome-scale analysis unveils the eukaryotic metal-responsive biochemical network

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Speaker: Simran Aulakh

Metal ions are essential for cells and function as cofactors in ~80% of all metabolic pathways. Although metal ion concentration can exhibit substantial variability in natural environments, metal ion composition has, so far, been explicitly defined and altered only in a small subset of laboratory experiments. This limited investigation into the involvement of metal ions across all biological processes has resulted in a relatively incomplete understanding of the networks that regulate and mediate their crucial biological functions. Herein, we systematically varied the concentration of essential metals over several orders of magnitude to determine the buffering capacity for each metal, interactions between metals and quantified cellular responses to perturbations in metal availability. Using a combination of metallomics, proteomics and phenotypic screening of a genome-scale knock-out collection in budding yeast, we discovered that over half the coding genome is involved in the cellular network that regulates and responds to essential metal availability. By identifying protein-level associations for 67 previously uncharacterised proteins that are part of this network, we exemplify how exploring the metal homeostasis network can unveil novel gene functions. Our data revealed previously unknown interactions between metal ion biology and the metabolic network, corroborating prior evidence for the critical role of metals in metabolism. By demonstrating the profound influence of essential metal ions on the biochemical network, our work advocates for a revision of current laboratory practices and the study of all biological processes in the context of essential metal ion availability.

Synthetic closed-loop, in vivo evolution of ligand receptors in *S. cerevisiae*.

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Speaker: Eline Bijman

To generate proteins with desired functionality, laboratory evolution relies on serial labor-intensive rounds of *in vitro* mutagenesis and selection. By contrast, *in vivo* continuous mutagenesis can enable continual generation of diversity and continuous selection when the desired phenotype, such as the ability to grow without a particular nutrient, is directly linked to cell growth. However, this type of selection does not work for protein classes such as ligand receptors that are not naturally linked to growth. To address this limitation, we designed a complex synthetic genetic system (named the Evolverator) that diversifies a target receptor and links that receptor's affinity to a ligand to the growth rate of the host cell. To evaluate the feasibility of such a closed-loop system in *Saccharomyces cerevisiae* and to guide its use, we developed a mathematical model. This model bridges molecular, cellular, and population level effects. By modelling the introduction of mutations in an isogenic starting population and the subsequent rise of cells that differ by their affinity within a nutrient constrained environment, we can predict the rate of enrichment of mutants with improved binding within a population. In an experimental proof-of-principle, we evolved the human estrogen receptor and isolated mutant proteins that bound chemically related estrogen derivatives with increased affinity. To better understand the evolution results, we parametrized our model with independent experiments on the individual Evolverator subsystems. Our model captures the experimentally observed behavior of an evolution system and suggests a path toward synthetic designs for evolving other receptors and binding interactions.

Multidimensional single-cell characterization of inducible promoters enabled by deep-learning tools for microscopy image analysis

Vojislav Gligorovski, Sahand Rahi

EPFL, Switzerland

Speaker: Vojislav Gligorovski

For quantitative systems and synthetic biology, independent control over different genes' activities is essential. In contrast to readout systems such as fluorescent proteins, control systems such as inducible transcription-factor-promoter systems have only been characterized in an ad hoc fashion, impeding precise manipulations of biological systems and reliable modeling.

To enable high-throughput analysis of time-lapse microscopy data, we developed YeaZ, a deep-learning tool for segmenting and tracking budding and fission yeast cells. Our method is highly accurate, including for buds, septa, and shape mutants, and outperforms existing methods on benchmark images.

Next, we built a comprehensive library of inducible systems controlling standardized fluorescent protein expression in budding yeast, including GAL1pr, GALL, MET3pr, CUP1pr, PHO5pr, tetOpr, Z3EV system, the blue-light optogenetic systems EI222-LIP, EI222-GLIP, and the red-light PhyB-PIF3 system. To characterize each system, we estimated its basal activity, induction speed, time-delays, degradation rate, level of noise, and effect on cellular growth from > 200000 single cells tracked using YeaZ. This multidimensional benchmarking uncovered unexpected disadvantages of widely used tools, e.g., the nonmonotonic activity of the MET3 and GALL promoters, slow off-kinetics of the doxycycline- and estradiol-inducible systems tetOpr and Z3EV, and high variability of PHO5pr and the PhyB-PIF3 system. Finally, we introduced two new tools for controlling gene expression: strongLOV, a more light-sensitive EI222 mutant, and ARG3pr which functions as an OR gate induced by the lack of arginine or the presence of methionine.

The presented characterization defines the compromises that need to be made for quantitative experiments in systems and synthetic biology.

Systematic mapping of natural variants that bypass essential genes

Amandine Batté¹, Núria Bosch¹, Carles Pons², Marina Ota¹, Maykel Lopes¹, Jolanda van Leeuwen¹

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Speaker: Amandine Batté

Mutations often show phenotypic differences across genetically distinct individuals. In the most extreme case, a gene can be essential in one genetic background but have no effect on viability in another. Here, we investigated the frequency and underlying causes of differences in gene essentiality across 19 genetically diverse natural yeast strains. We identified 77 context-dependent essential genes that were not required for viability in at least one genetic background, suggesting that the strain contained variants that could bypass the requirement for the essential gene. Although some genes appeared to be essential in the reference background only, the vast majority of genes were nonessential in only one or two genetic backgrounds. We identified and validated the causal bypass suppressor variants for all 77 context-dependent essential genes using bulk segregant analysis and allele replacements. Bypass suppression was generally driven by a single, strong modifier gene, and involved both genes directly counteracting the molecular defect caused by loss of the context-dependent essential gene, as well as general modifiers bypassing the requirement of many genes. For example, a variant in the karyopherin MSN5 could bypass the essentiality of the checkpoint kinase RAD53 by modulating histone levels. Our findings highlight the frequency of genetic background effects and range of underlying mechanisms, providing insight on how genetic variance accumulates during evolution and affects genetic traits.

Workshop 9 - Yeast Biotechnology

Repurposing *Ogataea polymorpha* into a superior microbial platform for bio-productions

Jiaoqi Gao, Yongjin Zhou

Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China

Speaker: Jiaoqi Gao

Ogataea (Hansenula) polymorpha represents a promising microbial host for bio-manufacturing from inexpensive feedstocks like methanol and lignocellulose owing to the advantages in wide substrate spectrum, thermal-tolerance and high-density fermentation. However, limited to the inefficient genetic manipulation and unclear metabolic characteristics, *O. polymorpha* has only been applied in productions of protein and ethanol. Here, we proposed to repurpose *O. polymorpha* into a superior microbial platform for bio-productions. Firstly, recombination machinery engineering (rME) was explored for enhancing homologous recombination (HR) together with establishing an efficient CRISPR/Cas9 system in *O. polymorpha*, achieving up to 90% of editing rate and 70% of HR rate (iScience, 2021, 24(3), 102168). On this basis, we, for the first time, achieved the efficient fatty acid production from sole methanol, an ideal feedstock that can be beneficial for global carbon neutrality. The engineered cells were rescued from necrosis by adaptive laboratory evolution, and the relationship between phospholipids homeostasis and methanol toxicity was firstly identified for methanol-based bio-manufacturing (Nature Metabolism, 2022, 4, 932-943). Finally, we engineered the *O. polymorpha* for lignocellulose bio-refinery by engineering the co-utilization of glucose and xylose, which enabled the highest production of free fatty acids (38.2 g/L) and 3-hydroxypropionic acid (79.6 g/L) from lignocellulose. Consequently, our results demonstrated the great potential of *O. polymorpha* in bio-refinery for industrial biotechnology applications.

A multi-pronged strategy to boost muconic acid yield in *S. cerevisiae* by metabolic funneling, shikimate pathway promotion, and toxicity mitigation.

Paul Vandecruys, Ilse Palmans, Patrick Van Dijck

KU Leuven Laboratory of Molecular Cell Biology, Belgium

Speaker: Paul Vandecruys

The need to shift from our current CO₂-intensive, fossil-resource-based economy toward a sustainable bioeconomy is high. Biobased platform chemicals can assist in making this transition. Muconic acid, for example, is an attractive platform chemical that can be transformed into industrially important commodity chemicals that are currently produced from non-renewable petrochemical feedstocks.

We have further optimized a *S. cerevisiae* strain that can convert glucose and xylose into muconic acid [1]. Muconic acid (MA) synthesis in this strain starts from the shikimate pathway-derived molecule 3-dehydroshikimate (DHS) and is enabled by the expression of a DHS dehydratase, protocatechuic acid (PCA) decarboxylase (PCAD), and a catechol 1,2-dioxygenase (CDO) [1].

In the presented work, metabolic funneling was applied to maximize the MA yield, i.e., by heterologous expression of an additional pathway able to convert chorismate to MA. This approach diverged a larger fraction of the flux towards MA production while retaining the strains' prototrophy for aromatic amino acids. Moreover, the shikimate pathway was strengthened, and MA toxicity was addressed by promoting its export, finally delivering a strain with a yield over two-fold higher compared to the starting strain. With this work, we strive to develop a robust yeast cell factory that efficiently produces muconic acid from glucose and xylose-containing feedstocks.

References:

[1] T. Nicolai, et al., *Microb Cell Fact.* 2021, 114

Pushing and pulling proteins into the yeast secretory pathway enhances recombinant protein secretion

Richard J Zahrl^{1,2}, Roland Prielhofer^{1,2}, Özge Ata^{1,2}, Kristin Baumann^{1,2}, Jonas Burgard^{1,2}, Diethard Mattanovich^{2,1}, [Brigitte Gasser](#)^{2,1}

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Speaker: Brigitte Gasser

Yeasts including *Komagataella phaffii* (syn *Pichia pastoris*) are popular hosts for the production of recombinant proteins. One of their key advantages is the ability to secrete proteins into the culture media. However, secretion of some recombinant proteins is less efficient. We recently identified translocation of nascent antibody Fab fragments from the cytosol into the endoplasmic reticulum (ER) as one major bottleneck.

Conceptually, this bottleneck requires engineering to increase the flux at the translocation step by pushing on the cytosolic side and pulling on the ER side. To apply the push-and-pull strategy to recombinant protein secretion, we modulated the cytosolic and ER Hsp70 cycles, which have key impact on the translocation process. After identifying the relevant candidate factors of the Hsp70 cycles, we combined the push-and-pull modules in a single strain and achieved synergistic effects for antibody fragment secretion, reaching up to 5-fold higher titers.

To further simplify the combinatorial possibilities for creating new production strains, we exchanged push-and-pull factors by synthetic activation of whole transcriptional programs. Indeed, the artificial activation of the general stress response transcription factor Msn4, mimicked the secretion enhancing effect of the push-factors, while pull-factors could be replaced by the UPR transcription factor Hac1. Altogether, we were able to successfully engineer strains reaching titers of more than 2.5 g/L scFv and 8 g/L VHH in bioreactor cultivations.

The increased secretion capacity of different industrially relevant protein classes indicates that the push-and-pull strategy represents a general concept to improve recombinant protein production in yeast.

Yeast Adaptive Evolution towards the Cellulosic Ethanol

Ana Paula Jacobus^{1,2}, Yasmine Alves Menegon¹, Lucas Souza de Bem³, Gabriel Rodrigues Alves Margarido⁴, Leandro Vieira dos Santos⁵, Jeferson Gross¹

¹UNESP, Brazil. ²SENAI ISI Biotech, Brazil. ³UNICAMP, Brazil. ⁴Bayer, Brazil. ⁵University of Manchester, United Kingdom

Speaker: Ana Paula Jacobus

Saccharomyces cerevisiae fermentation of biomass-derived sugars for ethanol production depends on two technical innovations: (1) the establishment of metabolic pathways for xylose consumption, a C5 sugar rich in lignocellulosic hydrolysates (LCHs); (2) and the development of robust yeasts capable of tolerating lignocellulosic inhibitors (weak acids, phenols, and furfurals). Through a vigorous research program involving metabolic engineering, QTL mapping, and adaptive laboratory evolution (ALE), we are developing strains suitable for biomass hydrolysate fermentation. An innovative QTL mapping approach, based on reiterated selections and backcrosses, allowed the charting, in the Brazilian bioethanol strain PE-2_H4, QTLs for LCH tolerance related to the genes VPS70, MKT1, HAP1, PHO84, and HTA1. Swapping these alleles into the S288C conferred LCH tolerance to the sensitive lab strain. Three ALEs in sugarcane LCH were performed with 8 replicate populations of CEN.PK113-7D and PE-2_H4. After 550-750 generations, about 70 alleles were identified associated with the LCH tolerance. Common adaptive pathways involve protein SUMOylation/ubiquitination (SIZ1), chromatin modification (CHD1), rRNA synthesis (RET1), and protein stability (SSB2). An evolutionary parallelism is found for alleles putatively downregulating the plasma membrane H⁺-ATPase (PMA1 and HRK1), suggesting that energy conservation (i.e., high ATP/ADP ratios) might be a driving force for LCH tolerance. A different metabolic / evolutionary engineering approach resulted in yeasts with 12-18 copies heterologous expressed xylose isomerase and mutations in genes regulating the iron-sulfur cluster homeostasis. A xylose-consuming yeast population has been crossed with a pool of LCH-tolerant ALE strains generating robust yeasts selected for efficient cellulosic ethanol fermentation.

High quality de novo genome assembly of non-conventional yeast *Kazachstania bulderi* a new potential low pH production host for biorefineries.

Laura Balarezo-Cisneros¹, Soukaina Timouma¹, Alistair Hanak¹, Andrew Currin¹, Fernando Valle², Daniela Delneri¹

¹University of Manchester, United Kingdom. ²BP Biosciences Centre, USA

Speaker: Laura Balarezo-Cisneros

Kazachstania bulderi is a non-conventional yeast species able to grow efficiently on glucose and D-gluconolactone at low pH. These unique traits make *K. bulderi* an ideal candidate for use in sustainable biotechnology processes including low pH fermentations and the production of green chemicals including organic acids. To accelerate strain development with this species, detailed information of its genetics is needed. Here, by employing high accuracy long read sequencing we report a high-quality phased genome assembly for three strains of *K. bulderi* species, including the type strain. The sequences were assembled into 12 chromosomes with a total length of 14Mb, and the genome was fully annotated at structural and functional levels, including allelic and structural variants, ribosomal array, centromeres and mating type locus. This high-quality reference genome provides an essential resource to advance our fundamental knowledge of biotechnologically relevant non-conventional yeasts and to support the development of genetic tools for manipulating such strains towards their use as production hosts in biotechnological processes.

Social wasps as a tool for restoring yeast diversity in vineyards and wineries.

Agnese Gori¹, Alessandro Russo¹, Jennifer Badura², Jurgen Wendland², David Baracchi¹, Stefano Turillazzi¹, Duccio Cavalieri¹

¹Università degli studi di Firenze, Italy. ²Hochschule GEISENHEIM University, Germany

Speaker: Agnese Gori

In the last few years our knowledge of the existing ecological connection between social wasps, *Saccharomyces cerevisiae* yeasts, and the vineyard has been deepened. Social wasps are able to host *S. cerevisiae* in their gut, allowing it to survive the winter and be released into the environment during the following spring. Inside wasp's gut, yeasts belonging to the genus *Saccharomyces* mate with an increased fitness for inter-specific hybrids and enriching the yeast genetic heritage. On the other hand, strains of the *S. cerevisiae* can increase the immune competence of foundresses of *Polistes* wasps. In this study, we are going to use *Polistes dominula*, as vector for the controlled dispersion of specific *S. cerevisiae* strains inside the vineyard, demonstrating their biotechnological potential in organic wine farming. The experiment will be divided into two parts. A standard experiment in which we will verify the ability of our farmed wasps of transporting a laboratory-transformed strain (resistant to Geneticin, G418) onto grapes, in a controlled environment. The second part will take place in the field, at the Laura Casadei winery (in Romena, AR) where we will exploit the wasps, fed with a strain isolated in the vineyard itself, *Metschnikowia pulcherrima*, to spread the latter on the grapes, and go to test the different organoleptic properties of the must given by the greater presence of the strain, after the harvest.

Conversion of CO₂ into organic acids by synthetic autotrophic yeast

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Speaker: Özge Ata

One of the biggest challenges humankind is facing these days is the climate crisis. One clear cause is the increasing atmospheric CO₂ level due to human activity. Anthropogenic CO₂ emission is more than can be captured by plants and microorganisms which consequently causes an imbalance in the carbon cycle. Towards a more sustainable future, we need to restore this balance.

To address this problem, we aim to enable microbial assimilation of CO₂ as a carbon sink by converting it into value-added, bio-based polymers. Previously, *Komagataella phaffii* was converted from a methylotroph into an autotroph that can grow solely on CO₂ for biomass formation while using methanol to harvest energy. Here, we further engineer this CO₂ fixing *K. phaffii* and use it as a platform to produce value-added organic molecules. Using synthetic biology tools and CRISPR-Cas9, we generated an autotrophic *K. phaffii* strain that can produce itaconic acid by fixing CO₂. Balancing the synthetic itaconic acid metabolism, identifying and engineering targets in the central carbon pathway and CBB cycle, and optimizing the process parameters resulted in a final titer of 3 g/L itaconic acid in lab-scale bioreactors. ¹³C-labelling experiments confirmed the incorporation of the captured CO₂ into itaconic acid. To demonstrate broader applicability, the same metabolic engineering strategy was also applied to the production of lactic acid. In the light of our results, we show that the synthetic autotrophic yeast *K. phaffii* can be a platform for the production of value-added chemicals by the microbial conversion of CO₂ for sustainable bioprocesses.

High-potency yeasts for production of glycolipids from waste to valuable biosurfactants

Nitnipa Soontorngun

King Mongkut's University of Technology Thonburi, Thailand

Speaker: Nitnipa Soontorngun

Sophorolipids (SLs) produced by *Candida* yeasts have a unique structure consisting of ω -hydroxy fatty acids, which can be used as important substrates in the polymer industry including bioplastics, as well as food, pharmaceuticals and other high-value substances such as perfumes. In this research, we employed sequencing technology to uncover potential enzymes of the yeast *C. riidocensis*. Data obtained from the next generation sequencing was used to compare with genes of *C. bombicola* that is widely used in both research and industrial development for sophorolipids production. Waste materials or by-products from the agricultural and food industry were used in culture media to reduce production cost. The results indicated that sophorolipids production from yeasts can be enhanced by optimizing the media composition and fermentation method in flask and bio. The produced sophorolipids displayed good applications as antihyphal and antibiofilm agent against the opportunistic yeasts. Furthermore, sophorolipids could be used as the bioplastic precursors such as polyhydroxy alkanate, thus reducing negative environmental impact and adding value to biological waste.

Posters

The ID “Stand” reported on top of each poster’s abstract indicates the location and timing of presentation. The code is composed by a letter (corresponding to the session) and a number (corresponding to the stand number shown on site).

Poster session codes:

A: “Cell death, Ageing, Telomeres”, “Yeast Sociobiology-Sensing and Signaling”, “Yeast Evolution & Ecology”, “Stress response; Aggregation and mis-folding” - Monday 21st August, 12:30-13:00

B: “Growth Control & Metabolism”, “Gene expression: from epigenetic regulation and cell cycle control” - Tuesday 22nd August, 13:00-15:00

C: “DNA replication & recombination; DNA damage & repair”, “Synthetic and Systems Biology of Yeasts”, “Yeast biotechnology” - Wednesday 23rd August, 13:30-15:15

D: “Yeast biotechnology” - Thursday 24th August, 12:30-13:00

E: “Satellite: Yeast ecology and fermentations: the intimate relationship of *S. cerevisiae* and fermented foods”, “Yeast Commensalism & Pathogenesis” - Friday 25th August, 13:30-15:30

Posters should be hung (with adhesive tape, provided) on the session’s morning and removed at the end of the session.

**Poster session: “Cell death, Ageing, Telomeres”,
“Yeast Sociobiology-Sensing and Signaling”,
“Yeast Evolution & Ecology”, “Stress response;
Aggregation and mis-folding”**

Monday 21st August, 12:30-13:00

Investigation of the role of Hog1 in longevity regulation in *Saccharomyces cerevisiae*

[Sujin Hong](#)^{1,2}, Won-Ki Huh^{1,2}

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Presenter: Sujin Hong

In *Saccharomyces cerevisiae*, replicative life span (RLS) is primarily affected by the stability of ribosomal DNA (rDNA). Longevity is promoted by increased stability of the highly repetitive rDNA array, which is maintained through transcriptional silencing facilitated by the NAD⁺-dependent histone deacetylase Sir2. The high osmolarity glycerol (HOG) pathway is a mitogen-activated protein kinase signaling pathway that is activated in response to osmotic stress and mediated by Hog1 kinase. Upon exposure to hyper-osmotic stress, Hog1 undergoes activation through phosphorylation and subsequently translocates into the nucleus to initiate stress responses. Our previous study revealed that Hog1 is required for increased rDNA stability and longevity induced by Smi1 deficiency. Nevertheless, it remains unclear whether Hog1 functions as a general regulator of rDNA stability and RLS regulation. Here, we demonstrate that Hog1 activation promotes rDNA stability under various conditions. Deletion of the Type2C protein phosphatase Ptc1 or the adapter protein Nbp2 increased rDNA stability via activation of Hog1. In addition, hyper-osmotic stress and ER stress also increased rDNA stability in a Hog1-dependent manner. Among several conditions tested, increased rDNA stability led to RLS extension in *nbp2Δ* cells and sorbitol- or dithiothreitol-treated cells. Furthermore, the impact of anchoring Hog1 at the plasma membrane on the regulation of rDNA stability varied depending on the activation condition of Hog1. Collectively, our findings uncover new roles of Hog1 in the regulation of rDNA stability and RLS, indicating its significance in mild external stress-induced longevity mechanisms.

ID: 84; Stand: A02

Resumption of cell division during stationary phase in *Saccharomyces cerevisiae*

Yuko Kishi¹, Akira Matsuura²

¹Chiba university Graduate School of Science and Engineering, Japan. ²Chiba university Graduate School of Science, Japan

Presenter: Yuko Kishi

When cultured in glucose-rich medium, the budding yeast *Saccharomyces cerevisiae* undergoes a log phase followed by a diauxic shift with a metabolic switch to a stationary phase (SP). The SP is a state of arrested cell growth due to nutrient depletion and can be divided into early-SP and late-SP, although the state of the cells in each period has not been well understood. Here, we report the details of the cell state specific to each period. We found that cells which resumed division appeared in the population several days after cell proliferation has ceased in YPD medium without subculture. Therefore, we divided SP into two periods, early-SP as the state before cell division resumed and late-SP as that after cell division resumed. Analysis of the medium composition revealed that cells stop their growth before carbon source is exhausted in early-SP. In late-SP, the cell number is increased approximately twice as much as that in early-SP, associated with the frequent appearance of cells with a non-axial budding pattern. In addition, we found that the pH of the medium is decreased in early-SP and turns to increase in accordance with the resumption of division. These findings suggest that non-proliferating cells in early-SP, which look like just to endure starvation, change their metabolic state to resume cell division, causing an increase in survival rate. We are currently in the process of analyzing the molecular mechanisms that induce the resumption of division during the stationary phase.

ID: 190; Stand: A03

Revisiting the role of the spindle assembly checkpoint in the formation of gross chromosomal rearrangements

Yue Yao

University Medical Center Groningen, Netherlands

Presenter: Yue Yao

Chromosome instability is a characteristic of most cancer cells. Gross chromosomal rearrangements (GCRs), including translocations, deletions, and amplifications, have been observed in cancers. Multiple pathways are known to suppress the formation of GCRs. In contrast, much less is known about pathways that promote their formation. The spindle assembly checkpoint (SAC), which ensures the proper separation of chromosomes, has been implicated in the formation of GCRs (Myung et al., 2004) using the classic GCR assay developed by the Kolodner lab where GCRs can be selected via simultaneous loss of two genetic markers, *CAN1* and *URA3*, on the left arm of chromosome V. We modified this assay by inserting a 50-bp interstitial telomeric sequence (ITS) upstream the *CAN1* and *URA3* markers, which causes a dramatic increase in GCR rate. We performed a genome-wide screen and identified approximately 200 genes that promote ITS-induced GCRs, including many important for the SAC. However, rather than confirm a role for the SAC in the formation of GCRs, we found that the previous and our findings can be explained by the synthetic lethality caused by combining the deletion of a SAC gene with loss of *CIN8*, which is also located on the left arm of chromosome V and is often co-deleted with *CAN1* and *URA3* when selecting for GCRs. We demonstrate that defects in the SAC cannot suppress GCRs in strains carrying an extra copy of *CIN8* located elsewhere in the genome. Thus, our data show that the SAC is actually not important for the formation GCRs.

ID: 191; Stand: [A04](#)

Interstitial telomeric sequences promote de novo telomere addition via multiple mechanisms

[Ziqing Yin](#)

University Medical Center Groningen, Netherlands

Presenter: Ziqing Yin

Telomeric sequences are not found exclusively at the ends of chromosomes, but are also present at internal sites where they are called interstitial telomeric sequences (ITSs). ITSs are difficult regions for the conventional DNA replication machinery due to their repetitive nature, stable association with specialized proteins, and ability to form DNA secondary structure. These obstacles can lead to replication problems that trigger gross chromosomal rearrangements (GCRs), which can cause cancer and congenital diseases. We find that GCR rates increase exponentially with ITS length. This increase can be attributed to the presence of the telomere repeat binding protein Rap1, which is known to impede replication fork progression, and a bias of repairing DNA breaks at or distal to the ITS via de novo telomere addition. We performed a genome-wide screen for genes that modulate ITS-induced GCRs using a genetic assay in *Saccharomyces cerevisiae*. We identified 9 genes that suppress ITS-induced GCR, 8 of which have known roles in DNA replication. Surprisingly, many mutants known to increase GCR rate in the absence of an ITS do not show any change in GCR rate when an ITS is present. In addition, we identified many genes that promote ITS-induced GCR, including genes involved in nucleotide excision repair and transcription.

ID: 353; Stand: A06

Conserved Apoptotic Machinery: Insights from Orthologous Proteins Function in Yeast

Vandana Kaushal, Joanna Klim, Anna Kurlandzka, Urszula Zielenkiewicz, Szymon Kaczanowski

Institute of Biochemistry and Biophysics, PAS, Poland

Presenter: Vandana Kaushal

Apoptosis, a prevalent form of programmed cell death, serves as a mechanism for cellular self-destruction upon specific stimuli and is documented in multicellular organisms, unicellular eukaryotes and even prokaryotes. Our objective was to experimentally confirm that the apoptotic machinery is an ancient adaptation acquired during mitochondrial domestication. We identified four key apoptotic proteins in yeast namely Mca1, Nuc1, Ndi1, and Nma111 through SGD and literature search, all known for their roles in yeast apoptosis. To investigate the retention of ancient functions in gene orthologs across different kingdoms, we replaced yeast apoptotic factors with codon-optimized orthologs from bacteria, protists, plants, and animals. The orthologs were artificially synthesized and subsequently inserted into common vector upstream of CPS1 terminator. Yeast mutants with ortholog replacements were then generated using homologous recombination. Additionally, GFP-tagged versions of these orthologs were created to assess their expression and localization in yeast. Fluorescence microscopy confirmed correct localization, and western blot analysis validated proper expression and size of chimeric GFP-tagged orthologous proteins of yeast apoptotic factors. Recombination mutants were tested for functional complementation using drop tests, Annexin-V/PI co-staining and ROS production assay after inducing apoptosis with acetic acid. We found that Mca1, Ndi1, and Nma111 exhibited pro-apoptotic properties, while Nuc1 showed anti-apoptotic properties. Moreover, most of the studied orthologous proteins from distantly related eukaryotic and even eubacterial proteins can induce apoptosis in yeast under oxidative stress. This suggests that apoptotic mechanisms have been conserved since mitochondrial domestication and supports hypothesis that certain apoptotic factors might be modified eubacterial toxins.

ID: 127; Stand: A07

The microtubule +TIP-body and its role in nuclear positioning during yeast mating

Michaela Remisova, Yves Barral

ETH, Switzerland

Presenter: Michaela Remisova

In budding yeast, proper function of the microtubules in division and mating relies on the ability of the cell to harvest forces generated through microtubule dynamics. In all these processes, microtubule plus-end tracking proteins (+TIPs) are essential for regulation of microtubule dynamics and allow plus-ends to interact specifically with other cellular components. To function, +TIPs need to track not only growing but also shrinking microtubules, where their binding sites rapidly disassemble, constantly forming new interactions with the microtubule. In addition, despite their transient nature, these interactions possess the ability to transduce sufficient forces to pull the nucleus through the cytoplasm. This is pivotal for nuclear congression in mating, which is facilitated by the interaction of microtubules emanating from the spindle pole bodies of the mating partners. We hypothesize that the interaction of the partner microtubules is secured through the +TIP-body condensate, by keeping the microtubule tips together. The +TIP-body then might act as a sensor for reciprocal microtubule attachment and for inducing a switch in their behavior from growth to shrinkage, bringing the two partner SPBs close to each other and enabling them to fuse. Supporting this notion, our preliminary data indicate that reducing multivalency of the +TIP phase separated condensate has a negative impact on the efficiency of nuclear congression. Additionally, we have observed an effect of reducing +TIP body multivalency on zygotic budding. Our results show an exciting possibility of regulation of microtubule dynamics and perhaps even later events in karyogamy through tension sensing by a liquid droplet.

Endogenous selfish yeast plasmid utilizes condensed chromosomal sites to ensure its stable propagation.

Deepanshu Kumar¹, Hemant Kumar Prajapati², Santanu Kumar Ghosh¹

¹Indian Institute of Technology Bombay, India. ²National Institute of Health Bethesda, USA

Presenter: Deepanshu Kumar

The 2-micron plasmid, a multicopy selfish genetic element (~40-100 copies), is a ubiquitous resident of the budding yeast nuclei with high mitotic stability. Interestingly, the plasmid appears to challenge the evolutionary logic by being present in a high copy number irrespective of its any fitness advantage conferred to the host. It imposes a selective growth disadvantage of 1-3 % on yeast cells over plasmid-free cells. More mechanistic insight has revealed that the plasmids couple their segregation with the host chromosome via host-coded proteins involved in chromosome segregation by hitchhiking themselves at the chromosomal sites. We have demonstrated that these sites are not random. Instead, they are the condensed and repetitive regions of chromosomes: Telomeres (TEs), rDNA, and tRNA gene clusters. To pinpoint the host proteins involved in the hitchhiking process, we have shown that condensin and RSC chromatin remodeling complexes contribute to this. Our findings suggest that these proteins, along with the plasmid-encoded Rep proteins, are required for the plasmid-chromosome association. Thus, our work unravels the mechanistic insight of the remarkable stability of the 2-micron plasmids in budding yeast. This knowledge will turn out to be crucial in understanding the analogous strategies utilized by other episomal elements, including episomes of certain mammalian viruses, and how hitchhiking of selfish genetic elements plays a role in the course of evolution.

ID: 75; Stand: A09

The impact of wooded areas on yeast populations vectored by social insects into vineyards

Beatrice Valentini, Francesca Barbero, Luca Pietro Casacci, Anna Luganini, Irene Stefanini

Department of Life Sciences and Systems Biology, University of Turin, Italy

Presenter: Beatrice Valentini

Yeasts are widespread in nature and given that most are not airborne, they must rely on natural vectors to spread and colonize new environments. It is currently acknowledged that forests host several yeast species, and insects, particularly social wasps, can vector and maintain throughout the year the yeasts populating the vineyard when grapes ripen. In this study, we aimed to assess the existence of links between a potential natural source of yeasts (woods), the vectors (social wasps), and the composition of the vineyard mycobiota. Adult *Vespa crabro* and *Polistes* spp. were captured over two vintages (2020 and 2021) in vineyards far and close to woods in three areas in the Piedmont region (Italy). The results obtained through culturomic analyses on the insects' gut mycobiota highlighted that wasps caught in vineyards near wooded areas bear in their intestines a higher number of yeast cells and species diversity than insects caught in vineyards far from woods. Moreover, unlike insects caught in vineyards far from woods, those caught close to woods harbor oenologically interesting species, first and foremost *Saccharomyces cerevisiae*, but also *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, and *Lachancea thermotolerans*. Overall, our work provides fundamental insights into the ecology of the vineyard mycobiota, highlights the relevance of social wasps in natural yeast ecology, and calls for the urgency of promoting the sustainable use of terrestrial ecosystems and preserving wooded areas to halt biodiversity loss.

CRE-zy Buddy: a sensitive molecular system to quantify yeast mating strategies

Chiara Vischioni¹, Benjamin Barré², Sakshi Khaiwal¹, Simon Laugier¹, Marco Cosentino Lagomarsino³, Bertrand Llorente⁴, Gilles Fischer⁵, Gianni Liti¹

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Presenter: Chiara Vischioni

Given our ability to control its sexual cycle under fixed laboratory conditions, the mating pathway of *S. cerevisiae* is one of the best characterized processes in yeast biology. In yeast, sexual reproduction is normally triggered by nutrient starvation, which lead to the production of four meiotic spores enveloped in a well-protected ascus. These haploid gametes restore their diploid state by mating with their daughter upon a first round of mitotic division followed by mating-type switching (haplo-selfing), fusing with another spore within the same ascus (intra-tetrad mating), or mating with an unrelated individual (outcrossing). To date, the relative frequency of the different breeding strategies has mostly been inferred from population genomics and in-silico analyses, often resulting in contradictory outcomes. Moreover, little experimental evidence is available, often limited to a few heterothallic strains. Here, we present the first sensitive molecular system based on a multicolor flow cytometry design, able to experimentally quantify different patterns of yeast mating strategies, applicable under ecologically relevant environmental variations. This allowed us to measure and compare at different molecular scale levels the mating behavior of multiple homothallic yeast genetic backgrounds. Understanding the impact of environmental variables on the different mating routes will help us to identify their effects on the species evolution, as well as to understand how they may affect the general level of heterozygosity and genetic diversity within the population.

Ancient and recent origins of shared polymorphisms in yeast

Nicolò Tellini¹, Matteo De Chiara¹, Simone Mozzachiodi¹, Lorenzo Tattini¹, Chiara Vischioni², Elena Naumova³, Jonas Warringer⁴, Anders Bergström⁵, Gianni Liti⁶

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Presenter: Nicolò Tellini

Shared alleles between populations can be ascribed to ancestral polymorphisms or underlie more recent gene flows. These mechanisms occur at fundamentally different timescales but they can be hard to disentangle in recently diverged species. Here, we developed a computational framework to map shared polymorphisms in *Saccharomyces cerevisiae* and its closest relative *S. paradoxus*, which have diverged 4.0-5.8 million years ago. We mapped shared polymorphisms across 1,673 sequenced *S. cerevisiae* isolates and we catalogued 11,863 introgression blocks, with the majority being recent and clade-specific, while wild Chinese lineages harboured a large number of individual ancestral polymorphisms that could be explained by incomplete lineage sorting. We propose that the most divergent Chinese lineage has retained an excess of ancestral polymorphisms, perhaps because of fewer or less dramatic bottlenecks during its history, and these polymorphisms contribute to inflating its genetic distance. In the non-Chinese lineages, we inferred introgression ancestry and revealed multiple pulses of introgressive hybridization that accumulated at different timescales. We reconstructed the major hybridization events and detected cases of overlapping introgression due to either shared histories or convergent events. We set up a sensitive assay based on reconstructing introgression blocks in isogenic backgrounds tagged with distinct fluorescent proteins that enabled us to measure direct growth competition. We revealed that the introgression of the PAD1-FDC1 gene-pair potentiates stress response to organic acid and antifungal drugs. These results highlight the functional role of introgressions and provide an overview of the mechanisms underlying the phylogenetic relationships between populations of *S. cerevisiae* and *S. paradoxus*.

ID: 201; Stand: A12

Investigating ecological interactions and toxin-mediated competition in mixed populations of *Saccharomyces cerevisiae*

Bíborka Pillér, Tünde Gaizer, Nóra Görög, Eszter Szintai-Major, Csaba István Pongor, Attila Csikász-Nagy

Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Hungary

Presenter: Bíborka Pillér

Microbial populations in nature form intricate communities consisting of multiple strains, which possess the ability to mutually influence one another. In these mixed communities, a dynamic interplay of cooperation and competition emerges among the strains, leading to the flourishing of certain strains and the suppression of others. One specific form of competition arises from the production of toxins by killer active yeast strains. This toxin production has been proven to be significant in the fermentation of food and beverages, as well as in medical research.

Our research aims to investigate the distinct growth characteristics of various strains of *Saccharomyces cerevisiae* by studying the growth of yeast cultures, both in isolation and in mixed communities. The primary objective is to examine the ecological interactions that may arise within these mixed populations and identify the key factors that impact cell proliferation within the culture. Furthermore, another goal is to explore the impact of various toxin-producing strains on the growth of other laboratory strains.

To conduct the laboratory experiments, several strains of *Saccharomyces cerevisiae* were carefully selected and labeled with fluorescent proteins. Then to identify the specific types of toxins produced by each strain, we employed a PCR-based approach. Subsequently, we tested the toxin-producing ability of each selected strain on different types of solid media and in liquid culture. Our preliminary results show that the growth conditions greatly impact the efficiency of toxin production and the sensitivity of the strains to given toxins.

ID: 225; Stand: A13

Characterising interaction specific effects between yeast and microalgae

Rene Naidoo-Blassoples, Jennifer Oosthuizen, Florian Bauer

Stellenbosch University, South Africa

Presenter: Rene Naidoo-Blassoples

Yeast and microalgae are microorganisms with widely diverging physiological and biotechnological properties. Yeasts have been primarily applied in fermentation driven processes, while microalgae are used to produce high value metabolites and for wastewater bioremediation. Given their broad applicability, there has been sustained interest in developing functional yeast and microalgae consortia. However, molecular insights into how these autotroph-heterotroph mutualisms are established, as well as interaction specific effects are poorly understood.

In this study, a multi-compartment membrane bioreactor system which allows for the co-culturing of microorganisms with and without physical (cell-to-cell) contact was used to investigate the effects of physical contact on co-cultured populations of *Saccharomyces cerevisiae* and the microalga, *Chlorella sorokiniana*. The results demonstrate that physical contact results in reduced biomass for individual species, while metabolic contact led to increased biomass levels for each individual species under the same conditions. Furthermore, gene expression analysis using RNA sequencing unveiled distinct transcriptomic responses in *S. cerevisiae* in response to the presence of *C. sorokiniana*, leading to the activation of specific cellular pathways associated with cooperative interactions, particularly sporulation and cell wall associated genes.

These insights for the first time demonstrate interaction specific responses between yeast and microalgae due to physical contact and may be the key to unlocking the potential of these ecosystems for biotechnological applications, while allowing a more in depth understanding of the interactions that shape these mutualisms.

ID: 238; Stand: A14

Role of hornet (*Vespa crabro*) associated yeasts as environmental bioindicators

Sonia Renzi, Alessandro Russo, Niccolò Meriggi, Giovanni Bacci, Monica Di Paola, Giulia Marino, Duccio Cavalieri

University of Florence, Italy

Presenter: Alessandro Russo

Social wasps and hornets are known to represent an important natural reservoir for Saccharomycetes, and yeasts of the genus *Saccharomyces* are able to survive and mate in the intestinal tract of these insects, representing an environment capable of enhancing fungal biodiversity. This peculiar host-microbe interaction between wasps and microorganism, and in particular Ascomycetes, hold the potential to develop novel microbiome-based strategies for environmental management. In fact, they could serve as an ecological bioindicator, being sensible to temperature changes and environmental pollutants, and capable of detecting climatic and environmental changes that characterize the Anthropocene Epoch. In addition, social wasps and hornets are known as ideal vectors able for inoculating yeast in ripe grape berries, biting them to obtain sugar, and consequently enhancing the fermentation of the grape must. On this basis, we have therefore described the effects of different environmental stimuli, inside a vineyard framework belonging to the Tuscan Coast, on the intestinal bacterial and fungal communities of European hornet *Vespa crabro*, using targeted-metagenomic sequencing with MiSeq Illumina platform.

It is interesting that different environmental and temporal patterns evaluated in this study showed different enrichment of yeasts in the gut of European hornets, and in particular, for Saccharomycetes, confirming the potential of Ascomycetes yeast to provide information about the impact of environmental changes on specific ecosystems.

ID: 245; Stand: A15

Understanding and simulating the effects of environmental factors on colony growth dynamics

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Presenter: Tünde Gaizer

Complex communities formed by microbial populations containing more strains can affect each other. To determine the unique growth characteristics of different strains we study the development of yeast colonies in isolation, in close proximity or in mixed communities.

A systems biological approach is used to get a better understanding of these local interactions and growth limiting factors. The experimental data serve as parameterizing factors for an agent-based mathematical model that is able to capture the growth differences between various strains. Several strains of *Saccharomyces cerevisiae* were selected and labelled with fluorescent proteins to conduct the laboratory experiments. Preliminary results show that the size and the structure of the colony is greatly influenced by growth conditions. Effectors like initial inoculation parameters of colonies (drop size, initial cell number), agar wetness, nutrient level and proximity of other colonies were tested, simulated and predicted with our agent-based model. Current efforts are focusing on extending the capabilities to high-throughput screening and analysis.

ID: 263; Stand: A16

A genomic introgression drives sexual selection in yeasts

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Presenter: Simone Mozzachiodi

Gene flow across populations can result in genomic introgressions, which can contribute to the fitness of a species. Signatures of positive selection detected by population genomics can indicate adaptive introgressions. However, collecting experimental evidence that confirm the adaptive role is often challenging or unfeasible. Here, we developed a recombined outbred population approach to systematically test the fitness consequences of introgressions in budding yeast. We generated two parallel advanced intercross lines by crossing representatives of *Saccharomyces cerevisiae* populations with a strain of the Alpechin clade, which represents the most introgressed lineage with up to 5% of its genome derived from the sister species *S. paradoxus*. First, we generated the F2 populations and then we further propagated those either through four cycles of sexual reproduction, or in stressful conditions and measured genome-wide allele frequency variation. We found that only a few introgressed alleles were selected, suggesting that introgressions are mostly neutral under the conditions tested. However, an introgression region harbouring the gene *SST2* involved in pheromone signalling showed a strong selection during sexual reproduction. We engineered the *SST2* allele from *S. paradoxus* into different *S. cerevisiae* strains and validated its effect on mating. This result, together with the *SST2* introgression being fixed in the Alpechin population indicates that it has been selected throughout the recent history of the lineage. Our results provide new insights into the functional consequence of genomic introgressions in the Alpechin clade and show an unexpected role in driving sexual selection in a facultative sexual microbe.

Investigating Factors involved in the Regulation of Mitochondrial Inheritance in *S.cerevisiae*

Nourah Nayef, Ewald Hettema, Kathryn Ayscough

University of Sheffield, United Kingdom

Presenter: Nourah Nayef

Mitochondria are organelles involved in cellular energetics in all eukaryotes and defects in their dynamics, fission, fusion or localization can lead to disease. *S. cerevisiae* is a model organism that has been used to identify factors regulating mitochondrial dynamics.

In this study, strains overexpressing kinases, phosphatases and ubiquitin ligases were crossed with a strain expressing a mitochondrial marker. Resulting diploids were screened microscopically for strains showing changes in their mitochondrial distribution or appearance.

One of the most striking mitochondrial phenotypes was observed on overexpression of the gene encoding the PAK family kinase Cla4. Cells overexpressing CLA4 showed a delay in mitochondrial transport to the bud. Previous work showed Cla4 phosphorylates Vac17 which is required for myosin transport of vacuoles to the yeast bud. Vac17 is phosphorylated by Cla4 which targets it for degradation. This mechanism ensures one-way traffic to the bud. We hypothesised that Cla4 could regulate mitochondrial inheritance in a similar way through phosphoregulation and degradation of the mitochondrial adaptor Mmr1. Our research showed Mmr1 protein level is decreased upon CLA4 overexpression and also, small buds are devoid of Mmr1 accumulation. Using a range of genetic, biochemical approaches we investigated the interaction of Mmr1 with myosin and generated mutants disrupted in this interaction. Mutant Mmr1 was not impacted by CLA4 overexpression. Intriguingly, mutant Mmr1 was still trafficked to the bud suggesting that resistance to the effects of Cla4 overexpression is not solely due to Mmr1 being retained in the mother and that additional mechanisms are responsible.

The diversity of biocidal yeasts in natural and cultural ecosystems

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Presenter: Živilė Strazdaitė-Žielienė

Yeasts found in the environment are among the most abundant and diverse forms of life on Earth, and their biodiversity is linked to human and animal health. One of the mechanisms for the regulation of microbial population in the ecosystem is the production of biocidal agents capable to kill microorganisms or inhibit their growth. The biocidal phenomenon is species- and strain-dependent. The objective of the present work was to isolate and identify yeast from natural and cultural environment and evaluate their biocidal properties. Samples were collected from water (lakes, rivers, sea, and swamps), fodder (silage, hay, grass) and soil (pastures, gardens). Over a hundred strains of yeast-like morphology, representing each of mentioned above habitat, were isolated and applied for molecular identification and killer assay. Seventeen biocidal strains were isolated from water, fourteen - from silage and five - from soil. It was shown that the most common yeast genera found in water were *Rhodotorula*, *Cryptococcus*, *Candida* and *Aureobasidium*, from silage – *Wickerhamomyces*, *Candida*, and *Debaryomyces*. Predominated yeasts in soil were representatives of the genera *Rhodotorula* and *Cryptococcus*. This study showed that biocidal phenotype is moderately prevalent in the environment. 36 of 112 yeast strains isolated from environmental samples demonstrated antagonistic activity against different yeast species. The obtained data offer new insights on biocidal systems, distributed in natural and cultural ecosystems, potential in food industry, human health, and therapy. This work was funded by the Research Council of Lithuania (grant number S-MIP-23-7).

Tight integration of the yeast killer systems and host metabolism

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Presenter: Elena Servienė

The yeast killer systems confer the hosts with the capability to contend for resources, enabling it to dominate in a certain environmental niche by outcompeting other microorganisms. To date, more than a hundred killer yeast species of *Saccharomyces*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Torulaspota*, etc. genera were described. Yeast killer trait is often determined by the viral killer system, consisting of two *Totiviridae* dsRNA viruses. Yeasts and their innate dsRNA viruses provide a convenient model system for studying host-virus interactions. In this study, transcriptomics and proteomics techniques were exploited for elucidation of the functioning of yeast killer systems. Transcriptomics analysis of *S. cerevisiae* and *S. paradoxus* yeasts performed by high-throughput RNA-Seq revealed a moderate response to viral dsRNA, suggesting the long-term co-adaptation of killer viruses and host cells. The distinct impact of dsRNA viruses was observed in the regulation of gene transcription. In *S. cerevisiae*, differently expressed genes were related to stress response, carbohydrate metabolism, mitochondrial functions, cellular amino acid, and lipid biosynthesis. In *S. paradoxus*, numerous changes were identified in the transcription profile of genes linked to ribosome biogenesis, and mitochondrial functions. To uncover the principal targets of killer viruses, a proteomic analysis was performed. The essential pathways of protein metabolism, from biosynthesis and folding to degradation, were found substantially enriched in virus-linked subsets. In agreement with transcriptomics data, proteomics analysis revealed a moderate response of the cells to the viral content and so further substantiated the tight integration of the killer system with the essential pathways of the host cells.

ID: 287; Stand: A20

Understanding Sub-Antarctic yeast community response to temperature changes

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Presenter: Pablo Villarreal

Sub-Antarctic regions face significant impacts from global warming, causing a worrisome temperature increase. Thus, understanding the genetic and phenotypic traits affecting local adaptation and the composition of communities in evolving environments becomes crucial. Yeasts are vital for biogeochemical processes and valuable indicators of ecosystem health. They contribute to essential functions like nutrient cycling, and plant growth promotion, making them particularly insightful for studying biological responses to temperature environmental changes. This study aims to investigate the effects of temperature variation on fungal biodiversity in isolated yeast inhabiting the Karukinka Natural Park, a Sub-Antarctic region in Patagonia. For this, we collected soil samples and sequenced an ITS amplicon library to identify the entire fungal community in the forest rhizosphere. Our data shows that the three most abundant families were Aspergillaceae, Pseudeurotiaceae, and Myxotirichaceae related to Saprotroph and Ericoid mycorrhizal metabolic function. To determine the impact of temperature changes on the community composition, we carried out a yeast isolation procedure from each sample using a species-sorting technique at different temperatures (4-40 °C). Before sorting, we identified at least 5 different species per sample. After sorting species count decreased to two per sample/treatment, with species belonging to the *Cryptococcus* genus being the most frequent across all temperatures, showing a wide thermal range of tolerance and pre-adaptation to temperature changes. Our work contributes to the characterization and understanding of the genetic diversity of yeasts in sub-Antarctic regions, providing a valuable resource to study the genetic and phenotypic traits of pre-adapted species to temperature changes.

Mating partner selection and species separation in budding yeast

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Presenter: Lina Heisteringer

As unicellular eukaryotes, yeasts can reproduce both asexually, by budding or fission, and sexually by mating between two cells of opposite mating type. Although much rarer than mitotic cell division, mating allows the exchange of genetic information between related cell populations. During evolution, speciation occurs by the formation of reproductive barriers that prevent gene flow between such populations. In their natural environment, closely related yeast species often inhabit the same niche, where they can also hybridize. Such hybridization events result in sterile diploid progeny, representing an evolutionary disadvantage. Currently, our understanding about the significance of yeast mating partner discrimination as a mechanism to prevent the formation of sterile hybrids in the wild is limited.

We use natural isolates and established laboratory strains of *Saccharomyces* species to study mechanisms of mating partner discrimination. The different species produce identical or almost identical pheromones for mating signaling and easily hybridize in laboratory and industrial conditions. Time-laps microscopy of mating mixtures allows us to assess pheromone signaling, mate choice and zygote viability on a single cell level. While we only see a weak, condition dependent partner discrimination between independent isolates of *S. cerevisiae* and *S. uvarum*, our data indicates that differences in pheromone signaling dynamics between genetically distinct strains could facilitate permanent escape from pheromone-induced arrest. Such adaptation to unfruitful mating encounters could act as a mechanism to distinguish mating partners in mixed species environments.

ID: 291; Stand: A22

Multicellularity of yeast

Valentina Madár

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Presenter: Valentina Madár

Plethora of evolutionary innovation created the world we know today. Several lifestyle strategies have given advantage to organisms to adopt to continuously changing environment. One of these is the emergence of multicellularity, which provided an opportunity for more complex organisms to evolve. The way from unicellularity to multicellularity was a long journey. In this study we want to explore this process with the widely used unicellular model organism: baker's yeast (*Saccharomyces cerevisiae*).

Previous studies have demonstrated that knockout of the ACE2 (Activator of CUP1 Expression 2) gene leads to significant phenotypic changes. This activator is a DNA-binding transcription factor involved in the positive regulation of post-division septum digestion. In its absence, the daughter cell fails to fully separate from the mother cell, resulting in their remaining together in a clonal "snowflake" structure.

Due to starvation or in insufficient environmental conditions yeasts can form facultative multicellular secondary structures (e.g. pseudohyphae, flocs), that helps the colony to gain nutrients and reduce the deficient environmental impact. Our goal is to mutate this facultative multicellularity to obligate multicellularity by knocking out ACE2 gene and evolve a stay-together strategy in yeast.

Large-scale structural variations and extrachromosomal circular DNA in an industrial strain of fission yeast revealed by whole genome sequencing

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Presenter: Chengyuan Li

Chinese Moutai, a famous style of baijiu liquor, is produced through fermentation by microbiota under complex conditions. Strains isolated from these industrial microbiota are often genetically distinct from their laboratory counterparts and vary significantly in their biological phenotypes, including different cell morphology and mating behaviors that hinder their genetic manipulation and analysis. Here, we employed a combination of Next-generation Sequencing (NGS) and Long-read Sequencing (LRS) to analyze the genomic sequences of 11 strains of *Schizosaccharomyces pombe* isolated from the fermentation microbiota of Chinese Moutai. We first identified an extrachromosomal circular DNA (eccDNA) that carries approximately 77 kb of the sequence of chromosome I genome in some of the Moutai strains using CNV detection and genome assembly. Moreover, our assembly revealed a 500bp sequence shared between *S. pombe* chromosome I and the Moutai-specific mitochondrial genome. Additionally, we found a universally present, ~2 Mb long inversion that spans the second half of chromosome I genome and 102 commonly found structural variations with lengths ranging from 54 bp to 38 kb in Moutai. Further investigation showed that these structural mutations were enriched around the *pfl+* gene family, which encodes cell surface flocculins and thus suggests a connection between genomic rearrangement and abnormal cell morphology. These findings provide insights into the characterization of eccDNA in yeast genome and its contribution to the genomic diversity and complexity of the industrial variants of *S. pombe*, which can be useful for future exploration of the genetic mechanisms underlying the production of Moutai and other fermented beverages.

Revealing the genome instability of *Saccharomyces pastorianus* by transfer of *Saccharomyces eubayanus* chromosome into *Saccharomyces cerevisiae*

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Presenter: Terumi Nakanishi

Saccharomyces pastorianus, used in lager beer brewing, is an allopolyploid with subgenomes derived from *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. The genome structure of *S. pastorianus* has been altered through selection and acclimation in the brewing environment, resulting in complex genome structures composed of these two species. The impact of the subgenomes from these two species on the physiology of *S. pastorianus* remains unclear. Our study aimed to investigate the evolutionary process through which *S. pastorianus* acquired its current genome structures and to enhance our understanding of chromosomal aneuploidy and interspecific hybridization, which are considered driving forces of evolution.

We initially generated “interspecific disomic strains” by transferring each *S. eubayanus* chromosome into *S. cerevisiae*. We observed that some of these strains exhibited growth retardation. Additionally, certain chromosomes from *S. eubayanus* in these strains were highly unstable and prone to loss. We hypothesized that this instability could be attributed to malfunctioning of *S. eubayanus* centromeres in *S. cerevisiae*. Since the precise centromeric regions of *S. eubayanus* were unknown, we estimated them from the consensus sequences of *S. cerevisiae* centromeres. Subsequently, we analyzed the function of *S. eubayanus* centromeres in *S. cerevisiae* using plasmids containing the inferred *S. eubayanus* centromeric regions. Our results indicate that all deduced *S. eubayanus* centromeres might be functional in *S. cerevisiae*.

ID: 309; Stand: A25

Homoplasy: an auxiliary criterion for species delimitation

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Presenter: Angela Conti

Species have been created by a series of changes occurred over time. At DNA level these changes appear as variations of the nucleotide sequences that are passed from a generation to the following one. This process of inheritance creates relationships among the different species that can be represented by phylogenetic trees which can be inferred according to the Hennig's Auxiliary Principle. It affirms that shared sequences should be considered homologous characters, and it is evidence of common ancestor between the two. Despite homology, there can be also similarities not due to evolutionary relationships but because of other processes such as convergence, parallel evolution and horizontal gene transfer (HGT). The latter is probably the most important component of homoplasy, and it is extremely frequent in both prokaryotes and some eukaryotes such as fungi. Thus, an extensive HGT could be reflected in increasing the level of homoplastic characters. This means that the Hennig's Auxiliary Principle could be too restrictive for those species. The rationale behind this paper was to explore the possibilities offered by the study of homoplasy in species delimitation, using four partial taxonomic models, characterized by different distances among species. We calculated the level of homoplasy both in ITS and LSU, which are the standard barcodes used in fungal taxonomy and in the emerging secondary barcodes used to overcome rDNA limitations. We evaluated the levels of homoplasy as indirect measure of the species semipermeable boundaries concluding that it could be seen as evidence that species barriers are semiporous.

Genome-scaled phylogeny of *Saccharomyces cerevisiae* from spontaneous must fermentations

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Presenter: Alessia Tatti

Modern winemakers commonly inoculate selected *S. cerevisiae* strains in must to obtain controlled fermentations and reproducible products. However, wine has been produced for thousands of years using spontaneous fermentations from wild strains, a practice that is experiencing a revival among small wine producers. Despite the widespread usage of such strains in the past, there is much to know about their ecology, evolution and functional potential. For example, the reciprocal affinities of these strains within the *S. cerevisiae* phylogeny have yet to be discovered, as well as the degree of their biodiversity and their impact on wine terroir. To fill this knowledge gap, we aim at characterising at strain level the *S. cerevisiae* present in spontaneously fermented must sampled across Italy. We set up a protocol based on polyphenols-removing prewashes, followed by whole-genome shotgun sequencing. We performed both an assembly-free analysis to reconstruct the strain-level phylogeny of *S. cerevisiae* strains using the species-specific-marker based StrainPhlAn, and the reconstruction of Metagenome-Assembled Genomes of these strains for downstream functional analyses. To plan conservation acts in a scenario of continuous climate change, we aim at isolating and maintaining strains of interest. We will present preliminary results from the analysis of spontaneous musts sampled at different fermenting stages.

The isoprenyl chain length of coenzyme Q mediates the nutritional resistance of fungi to a predatory amoeba

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Presenter: Nauman Saeed

Prey-predator interactions among microorganisms play a significant role in microbial evolution and the diversity of microbial communities. *Protostelium aurantium*, a fungivorous amoeba, coexists with various fungal species in nature and feeds on a wide range of fungal species. Interestingly, it has been observed that members of the *Saccharomyces* clade, which includes certain yeast species, exhibit resistance to predation by these amoebae despite being readily taken up by them (1, 2).

One of the very few fundamental molecular differences of this clade in comparison to other edible fungi is the different lengths of the side chain of the major mitochondrial electron carrier, ubiquinone, or coenzyme Q. While most fungi use coenzyme Q with chain lengths of 8-10 isoprenyl units (Q8 to Q10), *Saccharomyces* sp. generally use only Q6. The biological reason for this evolutionary switch to shorter isoprenyl chain lengths is currently unknown. Genome analyses of *P. aurantium* suggested the absence of a functional biosynthetic pathway for the coenzyme and indicated that this vital cofactor is supplied only via predatory feeding on its fungal prey. Exogenous supplementation of Q9 or the introduction of a functional pathway for Q9 biosynthesis restored amoeba predation on *Saccharomyces cerevisiae*. Hence, the inability of *P. aurantium* to feed on members of the *Saccharomyces* clade indicates that a nutritional resistance (switch to Q6) can function as an efficient strategy to escape environmental predation.

1) Radosa et al., 2019; *Environ microbiol*, 21, 1809-1820.
Radosa et al., 2021; *Cell microbiol*, 23, e13389.

2)

Evolution of cheaters displacing the specific subpopulation of aging yeast colonies

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Presenter: Jana Marsikova

Saccharomyces cerevisiae laboratory strains form complex, smooth colonies whose fitness depends on cell-cell interactions, division of labor, and ability of cell subpopulations to secrete public goods (e.g., nutrients, metabolites, and signaling molecules). Two main cell subpopulations are formed in differentiated colonies: U-cells in the upper layers and L-cells in the middle/lower part of the colony. U-cells activate a unique adaptive metabolism that allows them to survive longer compared to L-cells. Starving L-cells activate various hydrolytic pathways and release nutrient components (public goods) that are predominantly utilized by U-cells (Mol Cell 46:436, 2012). We showed that the colony structure is disrupted later in development by proliferating "cheaters" that escape colony regulatory rules. These cells arise by mutations or heritable epigenetic changes, proliferate exclusively in the U-cell layer, and form small papillae visible on the surface of developing colonies (PNAS 117:26, 2020). Papillae formation increases the genetic heterogeneity of this subpopulation and provides the altered cells with a numerical advantage after dispersal from colonies, forming the basis for subsequent competitive success under changing conditions. Analysis of chimeric colonies of papillae clones mixed with the GFP-labeled parent strain showed that competitive advantage of these clones becomes apparent only at the later stages of colony development, after colony differentiation has occurred. At this stage, the cells of papillae clones displace the subpopulation of U-cells of the parent strain. This suggests that cheater proliferation is enabled by a specific microenvironment of U-cells with unique metabolic and regulatory properties. The work is supported by GACR23-06368S.

Genome annotation of two thousand hemiascomycete yeast strains: towards the development of an Information System dedicated to Yeast Comparative Genomics and Pangenomics

Paulo Jorge Dias

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Presenter: Paulo Jorge Dias

A decade ago, our research group started an initiative dedicated to the field of Comparative Genomics focusing on model-organism *S. cerevisiae* and the Hemiascomycetes. This initiative led to the development of two databases, the GenomeDB and BlastDB. The GenomeDB compiles genomic and biological information on the genes encoded in yeast species with publicly available genome sequences. Presently, the SaccharomycotinaDB spans 2074 hemiascomycete strains, corresponding to 528 yeast species. A SaccharomycesDB has also been compiled, spanning 1024 *Saccharomyces cerevisiae* strains. Funannotate software was used to annotate these genome sequences, allowing the identification of approximately 13 million genes. A wide range of assembly and annotation metrics on these yeast genomes were obtained, including number of contigs, total nucleotide length, GC content, gene number, tRNA number, average gene length, complete CDSs, no start CDSs, no stop CDSs, no start and no stop CDSs, single exon transcripts, multiple exon transcripts, average exon length, average protein length.

An online Information System is being developed to make available the genome annotation, the functional information on each gene and the Gene and Graph Pangenomes of yeast species to the research community. A pipeline developed in-house will also allow performing advanced Comparative Genomics approaches based on the gene family codes, including pairwise genome alignments and synteny analysis. To exemplify the power of this type of analysis, we have identified 20454 homologs of the *S. cerevisiae* *FLR1* gene and *C. albicans* *MDR1* gene in the sub-phylum Saccharomycotina and made the phylogenetic analysis of this Drug:H⁺ Antiporter (DHA1) subfamily.

Interactions of mutagenic and amyloidogenic mechanisms in yeast *Saccharomyces cerevisiae*

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Presenter: Yulia Andreychuk

Amyloids are proteins with altered spatial structure, that form highly organized aggregates with cross- β structure. Amyloid-forming proteins are associated with more than 30 diseases in mammals, including humans. There are numerous evidence for association between amyloidogenesis and genome instability but the exact mechanism of this interaction has not been investigated.

To study the nature of the interactions between amyloidogenesis and genome instability we measured the frequency of simultaneous occurrence of mutations in the *CAN1* gene and the prion [*PSI*⁺], the inherited amyloid form of Sup35 protein in yeast *S. cerevisiae*. If the *can1* mutations and the prion [*PSI*⁺] appear independently, the frequency of their simultaneous appearance (*can*^r [*PSI*⁺] colonies) should be equal to the product of frequencies of spontaneous *can*^r colonies and the [*PSI*⁺] colonies. We showed that experimental value of *can*^r [*PSI*⁺] colonies frequency exceeds the theoretically expected one. Moreover, the *de novo* occurring [*PSI*⁺] strains frequently have altered mutagenesis rate and bear changes in their genome such as whole genome duplication, aneuploidy and point mutations. Summarizing the results of the experiments, we hypothesized that amyloids and changes in the genome appear in cells concomitantly, probably due to the unknown pleotropic factor affecting both genome and proteome.

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ID: 92; Stand: A31

An alternative route of arsenate detoxification by the yeast *Saccharomyces cerevisiae*

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Instituto de Tecnologia Química e Biológica António Xavier, Portugal

Presenter: Teresa Pissarro

The development of sustainable and environmentally friendly processes able to mitigate arsenic contamination, a major public health concern, relies on the comprehensive knowledge of arsenic detoxification pathways. In *Saccharomyces cerevisiae*, a powerful eukaryotic model organism, the arsenate (As^{V}) reductase Acr2, the arsenite (As^{III}) permease Acr3 and the aquaglyceroporin Fps1 are key players in arsenic detoxification, and their mode of action is well documented. However, our recent data indicate that arsenic detoxification is far more complex than generally assumed. We found that the deletion of both *ACR2* and *ACR3* genes increased *S. cerevisiae* sensitivity to As^{V} and led to a massive cellular accumulation of arsenic, which does not occur in Δacr2 single mutant. These results challenge the accepted model for As^{V} detoxification in yeasts, and suggest the direct involvement of the Acr3 transporter in As^{V} detoxification. On the other hand, although the deletion of *FPS1* from Δacr2 background also renders the cells more sensitive to As^{V} , it does not lead to arsenic accumulation. Instead, it triggers the accumulation of large amounts of glycerol in the presence of As^{V} , suggesting that osmolyte export is required to avoid As^{V} toxicity.

In order to determine if As^{V} is indeed a substrate of the Acr3 permease we are currently expressing a recombinant form of the protein on yeast $\Delta\text{acr2}\Delta\text{acr3}$ cells. The ability to transport As^{V} by the recombinant Acr3 will be evaluated using membrane vesicles.

Glycoengineering of gluten protein intensifies the unfolded protein response in *Komagataella phaffii*

Kai Büchner, Roland Kerpes, Thomas Becker

Technical University of Munich, Chair of Brewing and Beverage Technology, Germany

Presenter: Kai Büchner

The unfolded protein response (UPR) is a central, highly conserved eukaryotic pathway to maintain endoplasmic reticulum (ER) homeostasis. Upon protein accumulation in the ER, the model organism *Saccharomyces cerevisiae* induces the UPR mediator *HAC1* through nonconventional cytosolic mRNA splicing. Induced HAC1p then upregulates genes to help alleviate ER stress, thereby enhancing protein processing in the early secretory pathway. Unlike *S. cerevisiae*, the popular protein production host *Komagataella phaffii* (*Pichia pastoris*) shows high basal UPR activity, a proposed reason for its high secretory capacity. This study aimed at exploiting *K. phaffii*'s secretory capacity to express the gluten protein 75k γ -Secalin. Gluten, the storage protein family of cereal grains, is highly interesting as a primary quality determinant of cereal products and the trigger of Europe's second most prevalent food allergy. Native gluten proteins are non-glycosylated and accumulate in the ER when expressed in plants or yeast cells. To recruit chaperone proteins preventing gluten protein aggregation and associated ER stress, an N-glycosylation site was introduced into the 75k γ -Secalin N-terminus. Previous studies reported increased protein secretion by *K. phaffii* through such glycoengineering of the target protein. Unexpectedly, the N-glycosylated 75k γ -Secalin triggered a significantly intensified stress response, measured as the abundance of the total, induced, and uninduced *HAC1* mRNA. Expression of the N-glycosylated protein also affected genes with central roles in ER-associated degradation and genes supporting glycoprotein synthesis. These findings hint at UPR induction above the base level as a possible mechanism behind the effect of glycoengineering on *K. phaffii* protein secretion.

ID: 122; Stand: A33

Cells couple assembly and function of a disordered translational repressor with nutrient conditions

Diana Silvia Marina Ottoz, Annie Dyatel, Luke Berchowitz

Columbia University, USA

Presenter: Diana Silvia Marina Ottoz

Amyloid-like assemblies have been associated with toxic phenotypes because of their repetitive and stable structure. However, evidence that cells exploit these assemblies to activate protein functions in response to stimuli has questioned this paradigm. Understanding how assembly can confer an emergent function and how cells couple assembly with environmental conditions remains a challenge. Here, we study Rim4, an RNA-binding protein which forms amyloid-like assemblies that repress translation of critical transcripts during yeast meiosis. We establish that assembly is necessary for translational repression and stimulates binding with the 5' UTR of target RNAs. Moving the 5' UTR to the 3' end of the transcript abolishes translational repression, although the RNA-Rim4 interaction is preserved. We propose that Rim4 prevents translation by burying the 5' region of its target transcripts into its own assemblies, making their start codon inaccessible to ribosomes. Finally, we demonstrate that starvation stimulates both Rim4 assembly and its ability to bind its targets. Rim4 assembly depends exclusively on its intrinsically disordered region and is prevented by the Ras/protein kinase A signaling pathway, which promotes growth and suppresses meiotic entry in yeast. Our results elucidate a mechanism where cells couple a functional protein assembly with a stimulus to enforce a cell fate decision.

ID: 144; Stand: A34

ATP synthase new subunit / has a role in permeability transition pore in yeast

Chiranjit Panja, [Aneta Wiesyk](#), Roza Kucharczyk

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland

Presenter: Aneta Wiesyk

In *Saccharomyces cerevisiae*, the uncharacterized protein Mco10 was previously found to be associated with mitochondrial ATP synthase and referred to as a new 'subunit /'. However, recent cryo-EM structures of *S. cerevisiae* ATP synthase could not ascertain Mco10 as a structural subunit of the enzyme making questionable its role as a structural subunit. The N-terminal part of Mco10 is very similar to Atp19 (subunit *k*) of ATP synthase. The subunit *k*/Atp19, along with the subunits *g*/Atp20 and *e*/Atp21 plays a major role in stabilization of the ATP synthase dimers. We investigated the impact of Mco10 on ATP synthase functioning. Biochemical analysis revealed in spite of similarity in sequence and evolutionary lineage that Mco10 and Atp19 differ significantly in function. The absence of Mco10 delays the induction of PTP, while the deletion of Atp19 does not. Conversely, lack of Atp19 reduces slightly ATP synthase activities, while deletion of Mco10 has no effect. This is the first work to show Mco10 is an auxiliary ATP synthase subunit that only functions in permeability transition.

Financed by National Science Center of Poland: 2018/31/B/NZ3/01117

URM1* gene shuffles between archaea and *Saccharomyces cerevisiae

Katharina Zupfer¹, Lars Kaduhr¹, Larissa Bessler², Mark Helm², Raffael Schaffrath¹

¹Department for Microbiology, Institute for Biology, University of Kassel, Kassel, Germany. ²Institute of Pharmaceutical and Biomedical Science (IPBS), Johannes Gutenberg University Mainz, Mainz, Germany

Presenter: Katharina Zupfer

Cells often respond to stress with post-translational modifications to react appropriately to changes in their environment. One such post-translational modification in eukaryotes is the covalent attachment of ubiquitin to target proteins, usually resulting in their degradation. There are also ubiquitin-like proteins (Ubls) that share a β -grasp fold with ubiquitin, collectively known as members of the ubiquitin-fold superfamily. Ubls are found in all domains of life. Some prokaryotic Ubls such as CysO and ThiS only act as sulfur-carrier proteins (SCP) for thiolation reactions while others also attach to target proteins like ubiquitin. Mechanistically, these conjugations differ from the classical ubiquitination pathway. The evolutionary origin of the eukaryotic ubiquitin-superfamily is unclear. There is a bifunctional Ubl discovered in yeast and termed ubiquitin-related modifier 1 (Urm1). It has SCP function for tRNA-thiolation and conjugates to proteins in ubiquitin-like fashion. Thus, Urm1 combines both Ubl functions potentially important with regards to superfamily evolution. To get further insights into Ubl evolution and functional conservation, we currently investigate prokaryotic Urm1-like counterparts in the eukaryotic model organism *Saccharomyces cerevisiae*. We found that an Urm1-like protein from an archaeon can be transferred to yeast using gene-shuffle techniques and successfully expressed. Preliminary inter-species complementation analysis shows that the post-translational Ubl conjugation function is rescued in yeast. The modification targets a yeast protein previously identified and characterized in our group as a substrate for urmylation. Together, our findings may provide new insights into the origins of ubiquitin-like proteins and evolution of the ubiquitin-superfamily.

ID: 161; Stand: A36

The effect of glutathione on the accumulation and toxicity of antimony compounds in yeast *Saccharomyces cerevisiae*

Jacek Staszewski, Ewa Maciaszczyk-Dziubinska

Department of Genetics and Cell Physiology, Faculty of Biological Sciences, University of Wrocław, Poland

Presenter: Jacek Staszewski

Antimony (Sb) is a trace element occurring in the environment as trivalent Sb(III) and pentavalent Sb(V) compounds. Alike poisonous arsenic, majority of antimony forms pose a global health hazard due to their harmful effects on living organisms. One mechanism of Sb toxicity involves thiol association which leads to inhibition of thiol-containing enzymes, protein aggregation and redox instability, thus radicals formation and oxidative stress. Pentavalent antimoniacals like stibogluconate are also used in treatment of parasitic disease leishmaniosis.

In yeast, main detoxication pathway of antimony involves vacuolar sequestration of Sb-glutathione conjugates. Reduced glutathione (GSH) is a thiol containing tripeptide (Glu-Cys-Gly) which beside cytoplasmic metal chelator is serving as a major buffering molecule, maintaining cellular redox homeostasis by forming reversible disulphide (GSSG) upon oxidation.

While the effect of intracellular glutathione on metalloid noxiousness alleviation is relatively well established, the knowledge about possible role of extracellular GSH and its influence on toxicity of different antimony species is scarce. Here we report that medium supplementation with GSH dissimilarly alters the toxicity of Sb(III) and Sb(V). Additionally exposure to antimony cause a change in glutathione export of *Saccharomyces cerevisiae* cells and extracellular GSH influences trivalent antimony accumulation by reducing its uptake.

Summing up, this research gives insight into toxicity of different antimony compounds in context of extracellular or environmental glutathione.

ID: 163; Stand: A37

Unique properties of plant metalloid transporters revealed by heterologous expression in budding yeast

Kacper Zbieralski, Katarzyna Mizio, Jacek Staszewski, Ewa Maciasczyk-Dziubińska, Robert Wysocki, Donata Wawrzycka

Department of Genetics and Cell Physiology, University of Wrocław, Kanonia 6/8, Wrocław, Poland, Poland

Presenter: Kacper Zbieralski

The Acr3 family is one of the best studied group of transporters involved in high-level resistance to arsenic compounds in microorganisms. Recently, a plethora of putative Acr3 orthologues have been identified in plants from unicellular green algae to gymnosperms. We employed budding yeast as a heterologous expression system to functionally characterize plant Acr3 proteins from green microalgae *Coccomyxa subellipsoidea*, *Raphidocelis subcapitata*, *Chlamydomonas eustigma*, liverwort *Marchantia polymorpha*, moss *Physcomitrella patens*, fern *Pteris vitatta*, clubmoss *Diphasiastrum complanatum* and gymnosperm *Picea sitchensis*. We observed diverse levels of resistance to arsenic and antimony provided by these transporters in the yeast *acr3* deletion mutant; thus, we further investigated their substrate specificity. Most notably, exceptional tolerance to pentavalent arsenate was observed in yeast cells producing some of the studied transporters. Furthermore, fluorescent microscopy revealed that the subcellular distribution of the investigated plant Acr3 proteins varies and in some cases is directly regulated by metalloids. Our results obtained using *Saccharomyces cerevisiae* as a heterologous expression system prove the versatility of this model organism and provide novel insight into the properties of the plant members of the Acr3 family.

This work was supported by the National Science Centre, Poland, grant No. 2019/35/B/NZ3/00379.

ID: 235; Stand: A38

Role of Ynl320w – *S. cerevisiae* serine hydrolase – in the OXPHOS and mitochondrial redox homeostasis

Marta Kasabuła, Chiranjit Panja, Aneta Więsyk Więsyk, Róża Kucharczyk

Institute of Biochemistry and Biophysics, Polish Academy of Sciences; Warsaw, Poland

Presenter: Marta Kasabuła

Ynl320w is an uncharacterized protein in the yeast *S. cerevisiae*. It belongs to the serine hydrolase family and is highly similar to human ABHD17 and ABHD13 hydrolases. Deletion of the YNL320w gene (similarly to deletion of FMP40) allows cells with deletion of the GSH1 gene to tolerate the lack of glutathione (GSH) in the medium and restores their growth on media without supplementation of this tripeptide (Yadav et al. 2020). In addition, *gsh1*Δ cells grow more slowly in medium with the non-fermentable carbon source glycerol, and this phenotype was restored by deletion of the YNL320w gene as well. The complementation of *gsh1*Δ cells phenotypes by lack of Ynl320w depends on the activity of the redoxins Prx1, Grx2, Trx3 and structurally similar to flavodoxin: Ycp4, indicating on the Ynl320w function within mitochondria. We hypothesize that Ynl320w is a depalmitoylase, as ABHD17, and these redox proteins can be palmitoylated and regulated (i.e. their cellular localization) by Ynl320w by depalmitoylation. Lack of Gsh1 dramatically reduces the respiration and ATP synthesis in cells grown at elevated temperature, activities are restored by lack of Ynl320w. Levels of Fmp40, Prx1, Trx3 and Grx2 redoxins are increased in Gsh1 lacking cells as well, what depends on the Ynl320w. The data suggests that Ynl320w has a function in regulating oxidative phosphorylation in mitochondria in function of the redox homeostasis.

The Fmp40 AMPylase regulates mitochondrial redoxin cycle in *Saccharomyces cerevisiae*

Suchismita Masanta, Aneta Więsyk, Chiranjit Panja, Róża Kucharczyk

Institute Biochemistry and Biophysics, PAN, Poland

Presenter: Suchismita Masanta

AMPylation (adenylation) is one of the post-translational protein modifications (PTM) leading to the diversification of protein functions and activity, in which the AMP is attached through its α phosphate to the serine, threonine or tyrosine residues via a phosphodiester linkage. Recently with our collaborators, we discovered that the SelO family members of human, yeast and *E. coli* have the ampylase activity. The yeast SelO – Fmp40 – was identified in the proteome of inter-membrane space of mitochondria. We have shown that Fmp40 is involved in response of cells to hydrogen peroxide and menadione treatment: cells lacking the Fmp40 ampylase died faster than the wild type cells upon hydrogen peroxide and menadione treatment. *E. coli* SelO ampylates glutaredoxin GrxA, and the glutathionylation level of proteins is reduced in bacterial and yeast cells lacking SelO (1). Here we show that *fmp40* Δ cells are resistant upon exposure to high concentrations of the hydrogen peroxide - phenotype dependent on the presence of the Grx2, Trr2, Aif1 and Oxr1. We found the genetic interaction of FMP40 with SOD1 gene - the double mutant *sod1* Δ *fmp40* Δ grows very weakly on nonfermentable carbon source glycerol, indicating on Fmp40 role on oxidative phosphorylation activity of yeast mitochondria. Sod1 is superoxide dismutase, neutralizing the superoxide anion ($O_2^{\bullet-}$). Fmp40 ampylates Prx1, Trx3 and Grx2 in vitro and impacts the redox cycle of Prx1 peroxiredoxin. Furthermore, we observed that Fmp40 has a matrix-localized echoform whose pool is increased upon higher oxidative stress. Our results shade light on the Fmp40 mechanism of regulation the mitochondrial redox homeostasis.

Analysis of disease-associated protein Mdm38/LETM1 identifies new proteostatic pathway in the inner mitochondrial membrane

Iryna Bohovych¹, Gabriella Menezes da Silva¹, Jonathan Dietz¹, James Wohlschlegel², Patricia Kane-Popp³, Oleh Khalimonchuk^{1,4,5}

¹University of Nebraska-Lincoln, USA. ²University of California Los Angeles, USA. ³SUNY Upstate Medical University, USA. ⁴Nebraska Redox Biology Center, USA. ⁵Fred and Pamela Buffett Cancer Center, USA

Presenter: Oleh Khalimonchuk

Mitochondria are complex and dynamic organelles that are vital for cellular physiology. Many mitochondrial activities are localized to the inner mitochondrial membrane (IM) – the most protein-rich membrane in the cell, whose tightly controlled integrity is crucial for mitochondrial functions. However, many aspects of the IM homeostasis are incompletely understood. The IM protein Mdm38/LETM1, has been implicated in a variety of functions including mitochondrial morphology, protein homeostasis, and osmotic balance. Mutations or altered expression of LETM1 have been linked to a variety of human diseases including neurodevelopmental Wolf-Hirschhorn syndrome, tumor development, and seizures. Despite its significance, many aspects of the Mdm38 function remain elusive and contradicting reports exist regarding the protein's role in mitochondrial physiology. Using the genetic, biochemical, and multi-omics analyses, we systematically characterized molecular and physiological consequences of Mdm38 loss in the budding yeast model. We show that loss of Mdm38 leads to a profound respiratory defect due to impacted mitochondrial translation machinery and protein integration into the IM and—consequently—impaired IM protein homeostasis. One major group of affected IM proteins is represented by IM transporters and IM-shaping factors, leading to altered IM permeability, which appears to be the root cause of secondary pleiotropic phenotypes previously reported in the *mdm38Δ* mutant. Our analyses identify a series of genetic interactors for Mdm38, further supporting this notion. Collectively, our data provide new insights into Mdm38/LETM1 functions and clarify its complex role in the IM homeostasis, thereby deepening our understanding of relevant pathophysiological mechanisms in humans.

ID: 249; Stand: A41

Regulation of Cdc42 Protein Levels Impacts a Cell Differentiation Program In Yeast

Beatriz González, Paul Cullen

State University of New York at Buffalo, USA

Presenter: Paul Cullen

Rho GTPases are central regulators of cell polarity and signaling. Many aspects of Rho GTPase regulation are understood, yet how these proteins function in specific contexts remains unclear. Our work centers around the ubiquitous Rho GTPase Cdc42p, which regulates bud emergence and signal transduction pathways, such as evolutionarily conserved MAPK pathways. Cdc42p specifically controls pathways that govern mating and filamentous growth in yeast, where cells change their shape in response to external signals. We recently discovered that Cdc42p is modified by ubiquitin, and the active conformation of the protein is turned over by an E3 ubiquitin ligase Rsp5p and heat shock protein (HSP) chaperones in the 26S proteasome. A GTP-locked (Q61L) and turnover-defective (TD) version of Cdc42p, Cdc42p^{Q61L+TD}, hyper-activated the MAPK pathway that regulates filamentous growth (fMAPK). Unexpectedly, Cdc42p^{Q61L+TD} did not impact the activity of the MAPK pathway that controls mating. An adaptor protein for the fMAPK pathway, called Bem4p, stabilized Cdc42p protein levels resulting in sustained fMAPK pathway signaling. A residue in Cdc42p (K16) was also identified that when changed to arginine led to misfolding and aggregation of the protein. We also show that the main effector for Cdc42p in the MAPK pathway, Ste20p, was also turned over, which occurred presumably to modulate MAPK pathway activity. Quality control pathways may play a general role in regulating Rho GTPase folding and protein levels, and may broadly regulate the activity of regulatory pathways to promote the execution of specific developmental programs.

Deleting *ATG11* causes changes in lipid composition and sensitivity to α -Synuclein expression

Jana Schepers, Timo Löser, Christian Behl

The Autophagy Lab, Institute of Pathobiochemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Presenter: Jana Schepers

The Parkinson's Disease (PD)-associated protein α -Synuclein (α -Syn) is known to interact with negatively charged phospholipids and, when it is expressed in the yeast *S. cerevisiae*, to localise to the plasma membrane. It was also shown that expression of α -Syn changes cellular lipid composition and increases lipid droplet numbers. We investigated the effect of several autophagy-related gene (*ATG*)-deletions and identified the *atg11 Δ* deletion strain as having increased LD-numbers. Lipidomic analyses showed that the lipid composition of the *atg11 Δ* strain is changed, including a stark reduction of phosphatidylinositol (PI). As some of the changes in the cellular lipid composition of *atg11 Δ* are similar to the changes after expression of α -Syn constructs, we expressed α -Syn-WT-GFP, -A30P-GFP, and -A53T-GFP constructs to test for the strain's sensitivity. Indeed, the *atg11 Δ* strain is more sensitive to expression of all α -Syn constructs than the WT or the *atg1 Δ* strains. Quantitative PCR analyses of the PI-Synthase *PIS1* support the reduction of PI in the cells. In accordance with this, expression of cellular PIP-kinases is significantly reduced in the *atg11 Δ* strain. Expression of α -Syn-GFP constructs in cellular PIP-Kinase-mutants suggests that especially a lack of PI(3)P and a reduction of PI(4)P seems to play a role in sensitivity and also localisation of α -Syn. Thus, we propose that a deletion of *atg11* is likely to cause a PIP-imbalance, which, in turn, affects cellular sensitivity to α -Syn expression. However, the exact mechanisms still need to be investigated.

ID: 284; Stand: A43

The role of the small GTPase Rho5 and its GEF in the oxidative stress response of *Saccharomyces cerevisiae*

Linneth Bischof, Jürgen J. Heinisch

Osnabrück University, Germany

Presenter: Linneth Bischof

Rho5 is a small GTPase, homologous to mammalian Rac1, which has been associated with several health problems such as cancer, cardiac function and neurodegenerative diseases. This GTPase and its dimeric GEF (composed of the subunits Dck1 and Lmo1) have been shown to rapidly translocate from the plasma membrane and the cytoplasm to the mitochondria under oxidative stress conditions. Using fluorescence microscopy and an ALFA-nanobody, we trapped each of the GEF components to different intracellular compartments and assessed the influence on the distribution of the other one. We thus demonstrated an interdependence for the localization of the two GEF proteins to mitochondria, as trapping one is sufficient to induce the translocation of the other, as well as that of its target Rho5.

Furthermore, a previously detected genetic interaction of Rho5 with the yeast VDAC Por1 was studied in more detail. Using an ALFA-tag in combination with an ALFA-nanobody-GFP construct, we intended to detect a proposed Rho5 – Por1 colocalization, ensuring that the in vivo function of either of the two proteins remained intact.

ID: 285; Stand: A44

Phosphatidylinositol 3-Phosphate Metabolism Affects Cellular α Synuclein Localization in *Saccharomyces cerevisiae*

Timo Löser, Jana Schepers, Christian Behl

The Autophagy Lab, Institute of Pathobiochemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Presenter: Timo Löser

Alpha-Synuclein (α -Syn) is a key protein implicated in the pathogenesis of the neurodegenerative Parkinson's disease (PD). Although its specific function remains elusive, past studies revealed its involvement in synaptic vesicle secretion in presynaptic neurons. Recently, α -Syn was also shown to specifically bind to phosphorylated phosphoinositides (PI(x)Ps), which are important for membrane signalling processes. Interestingly, in the baker's yeast *Saccharomyces cerevisiae*, human α -Syn was found to target the plasma membrane via the secretory machinery. Through expression of α -Syn in various yeast single deletion mutants, we discovered that this plasma membrane localization of α -Syn is highly dependent on the abundance of the signalling phospholipid PI(3)P. Fluorescence microscopy analysis showed that *VPS15* and *VPS34* deletion mutants, which are deficient in PI(3)P, exhibit a cytoplasmic localization of α -Syn, partially accumulating near the vacuole. By including two familial PD-related variants α -Syn A30P and A53T, we also observed that these accumulations were formed specifically by lipid-binding variants of α -Syn. Furthermore, α -Syn expression strongly increased the number of PI(3)P containing vesicles in wild type cells. Based on these findings, we suggest that PI(3)P functions as a specific entry point for α -Syn targeting to the cell's budding-site via the secretory pathway. These insights contribute to our understanding of the relationship between vesicular trafficking pathways and PD, providing potential avenues for future research.

ID: 311; Stand: A45

The role of the small GTPase Rho5 in the nutrient signalling of *Saccharomyces cerevisiae*

Franziska Schweitzer, Jürgen Heinisch

University Osnabrück, Germany

Presenter: Franziska Schweitzer

Rho5 is a small GTPase, homologous to mammalian Rac1, whose malfunctions have been associated with several health problems such as cancer, cardiac function and neurodegenerative diseases. In yeasts, Rho5 and its dimeric GEF have been shown to rapidly translocate from the plasma membrane and/or the cytoplasm to mitochondria upon oxidative stress or glucose starvation. We investigated the epistatic relationships between a *rho5* deletion and strains lacking various components in known stress and glucose signal transduction pathways (e.g. Ras2, Rim15, Msn2/4, Hxk1/2, Reg1 etc.). For this purpose, growth behaviour of different mutant combinations was assessed both from colony sizes after tetrad analyses and by monitoring growth curves of selected strains. Mass spectrometry was employed to investigate changes in the proteome, comparing a *rho5* deletion and a hyperactive *RHO5*^{G12V} variant to the isogenic wild type strain.

We will present a working hypothesis on the crosstalk of Rho5 with the yeast glucose signalling network.

Systematic analysis of temperature-sensitive alleles uncovers new functions for essential genes in yeast filamentous growth

Atindra Pujari¹, Zhijian Li², Ankita Priyadarshini¹, Deanna Williams¹, Dale Climie², Sondra Bahr², Helena Friesen², Michelle Li¹, Joshua Oken¹, Brenda Andrews², Charles Boone², Paul Cullen¹

¹University at Buffalo, USA. ²University of Toronto, Canada

Presenter: Atindra Pujari

During cell differentiation, cells specialize into different types with distinctive shapes and functions. Many fungal species, including pathogens, undergo filamentous growth, where cells differentiate to pseudohyphal and hyphal cell types in response to environmental cues. Baker's yeast also undergoes filamentous growth. Genetic screens and nonessential gene-deletion collections have identified many regulators of the response. One group of genes yet to be systematically analyzed are those that perform essential functions. We transferred a collection of 325 temperature-sensitive (ts) alleles containing mutations in essential genes into a strain that undergoes filamentous growth. Ts strains were examined at semi-permissive temperatures for invasive growth, pseudohyphal growth, and biofilm/mat formation. Over half the alleles tested showed a phenotype in filamentous growth by at least one assay. New connections to filamentous growth and splicing, kinetochore assembly, and chromosome segregation were identified. We also found that the Wiskott-Aldrich Syndrome protein (WASp) homolog, Las17p, regulated the Cdc42p-dependent MAP kinase pathway (fMAPK) that controls filamentous growth. Las17p was required for the localization and levels of the tetraspan sensor protein Sho1p, which may account for its role in regulating the fMAPK pathway. Differences in ts phenotypes were also identified across individuals. Our results highlight the critical roles that essential genes play in cell differentiation and may provide a roadmap for studies in other organisms.

**Poster session: “Growth Control & Metabolism”,
“Gene expression: from epigenetic regulation and
cell cycle control”**

Tuesday 22nd August, 13.00-15.00

ATP synthase proton translocation mechanism: lesson from yeast models of MT-ATP6 pathogenic variants

Roza Kucharczyk

Institute of Biochemistry and Biophysics PAS, Poland

Presenter: Roza Kucharczyk

FOF1-ATP synthase is a rotary machine, located in the inner mitochondrial membrane, synthesizing ATP using the proton motive force created by the respiratory chain. The proton gradient across the inner membrane drives the rotation of the rotary part of the enzyme, what generates the conformational changes within the catalytic domain of the enzyme, F1, permitting binding of ADP and inorganic phosphate and ATP synthesis. The mechanism of proton translocation through the membrane part of the enzyme, FO, is still not well understood. The subunits forming the proton channel - Atp9 and Atp6 - are encoded in mitochondrial DNA. The list of MT-ATP6 gene variants detected in individuals suffering from neurodegenerative diseases constantly increases (86 reported in the MITOMAP database). We used yeast *Saccharomyces cerevisiae* to investigate the functional consequences of 24 MT-ATP6 variants. With the recent advent of complete structures of ATP synthase from various mitochondrial origins we better understand the mechanism of proton transport through the FO domain and we can analyze how amino acid substitutions disrupt the functioning of the enzyme. By selection and analysis of the genetic suppression mutations of the most severe atp6 variants, which were located within ATP6 gene, we could study in which extend they restore the functioning of ATP synthase and analyze in silico what role they have within the proton half-channels. These studies provided information on the importance of individual amino acid residues for the functioning of the proton channel and the mechanism of enzyme functioning.

ID: 29; Stand: B02

Translational regulation of glutamate metabolism in *Pichia pastoris* and development of a novel glutamate-inducible expression system

Neetu Rajak, Trishna Dey, P N Rangarajan

Indian Institute of Science, India

Presenter: Neetu Rajak

A methylotrophic yeast and a highly successful protein expression system host *Pichia pastoris* can utilize multiple amino acids (Proline, glutamate, and aspartate) as the sole source of carbon. Among these amino acids, glutamate metabolism is facilitated by a bHLH-LZ containing protein Rtg1p. In *Saccharomyces cerevisiae*, Rtg1p acts as a nuclear transcription factor. However, in *P. pastoris*, Rtg1p is a cytosolic regulator of glutamate catabolism. In *P. pastoris*, glutamate assimilation is facilitated by metabolic enzymes glutamate dehydrogenase 2 (GDH2) and phosphoenolpyruvate carboxykinase (PEPCK). This study focuses on the translational regulation of GDH2 and PEPCK by Rtg1p in the presence of glutamate. We demonstrate that Rtg1p regulates the translation of *PEPCK* mRNA via its 5' untranslated region (UTR). Furthermore, we show that only the bHLH domain of Rtg1p is essential for glutamate catabolism. We also report the development of a glutamate-inducible expression system as an alternative to the methanol-inducible expression in *P. pastoris*. To characterize the novel glutamate-inducible expression system, we demonstrate the expression of various heterologous proteins and the modification of induction media composition.

Clarifying modes of intercellular signalling in yeast

Michela Winters¹, Violetta Aru², Nils Arneborg², [Kate Howell](#)¹

¹University of Melbourne, Australia. ²University of Copenhagen, Denmark

Presenter: Kate Howell

Saccharomyces cerevisiae can alter its morphology to a filamentous form associated with unipolar budding in response to environmental stressors. Induction of filamentous growth is suggested under nitrogen deficiency in response to alcoholic signalling molecules through a quorum sensing mechanism. To investigate this claim, we analysed the budding pattern of *S. cerevisiae* cells over time under low nitrogen while concurrently measuring cell density and extracellular metabolite concentration. We found that the proportion of cells displaying unipolar budding increased between local cell densities of 4.8×10^6 and 5.3×10^7 cells/ml. However, the observed increase in unipolar budding could not be reproduced when cells were prepared at the critical cell density and in conditioned media. Removing the nutrient restriction by growth under high nitrogen conditions also resulted in an increase in unipolar budding between local cell densities of 5.2×10^6 and 8.2×10^7 cells/ml, but there were differences in metabolite concentration compared to the low nitrogen conditions. This suggests that neither cell density, metabolite concentration, nor nitrogen deficiency were necessary or sufficient to increase the proportion of unipolar budding cells. It is therefore unlikely that quorum sensing is the mechanism controlling the switch to filamentous growth in *S. cerevisiae*. Only a high concentration of the putative signalling molecule, 2-phenylethanol resulted in an increase in unipolar budding, but this concentration was not physiologically relevant. We suggest that the compound 2-phenylethanol acts through a toxicity mechanism, rather than quorum sensing, to induce filamentous growth.

ID: 41; Stand: B04

Study of the functional link between separase and Pah1 in *Saccharomyces cerevisiae*

MALINA CARMINA ILINCA, JOSÉ A. RODRÍGUEZ, DAVID VAQUERO, ETHEL QUERALT

BIOMEDICINE INSTITUTE OF VALENCIA (IBV-CSIC), Spain

Presenter: Malina Carmina Ilinca

The correct segregation of genetic material into the two new daughter cells is a crucial process during mitosis. This process, which is highly regulated and controlled, is initiated in eukaryotic cells by the caspase-like protein separase (Esp1 in budding yeast). The degradation of its inhibitor securin (Pds1 in budding yeast) in the metaphase-anaphase transition leads to cohesin cleavage thanks to its proteolytic activity, allowing the segregation of the sister chromatids. In addition, Esp1 induces PP2A^{Cdc55} inhibition, increasing Net1 phosphorylation and promoting the activation of Cdc14. Esp1 is also involved in promoting mitotic spindle elongation and rDNA segregation. The direct substrate of separase in these functions is still unknown. This work aims to discover new interactors of separase that help us learn more about its function as a key regulator of mitosis. For this reason, we isolated suppressors of the *esp1-2* mutant at restrictive temperatures. A mutation in Pah1 was found as a suppressor of the *esp1-2* mutant. Pah1 is a phosphatidate phosphatase that regulates the conversion of phosphatidic acid (PA) to diacylglycerol (DAG) in response to cell metabolic signals and has a fundamental role in the control of nuclear membrane synthesis. In order to analyze the possible role of separase in the regulation of the nuclear membrane, we are studying the genetic interactions with Pah1 and the effects in the mitosis phase of the cell cycle.

Gene *ACG1*, encoding β -1,6-N-acetylglucosaminyltransferase, as a new possible regulator of autophagy in the methylotrophic yeast *Komagataella phaffii*

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Presenter: Anastasiya Zazulya

Komagataella phaffii, a methylotrophic yeast, is a highly efficient producer of industrially important recombinant proteins. However, cytosolic recombinant proteins are prone to degradation, and the mechanisms involved in this process are not completely understood. To identify factors that influence this process, a developed system for the selection of recombinant strains of *K. phaffii* with impaired autophagic degradation of the heterologous model cytosolic protein (yeast β -galactosidase) was used for insertional tagging of the genes involved in cytosolic proteins degradation. We identified the gene *ACG1*, which encodes β -1,6-N-acetylglucosaminyltransferase, as a potential regulator of autophagy in *K. phaffii*. The insertion into the open reading frame of the *ACG1* gene and deletion of *ACG1* resulted in slower degradation of β -galactosidase. The rate of degradation of the β -galactosidase enzyme was two times slower in the insertion mutant and 1.5 times slower in the deletion strain as compared to the parental strain with native β -1,6-N-acetylglucosaminyltransferase. The rate of degradation of native *K. phaffii* cytosolic and peroxisomal enzymes, formaldehyde dehydrogenase, formate dehydrogenase, and alcohol oxidase, respectively, showed similar trends to that of β -galactosidase—slower degradation in the deletion and insertional mutants as compared to the wild-type strain, but faster degradation compared to strains completely defective in autophagy. Our findings suggest that *ACG1* plays a role in the autophagy of cytosolic and peroxisomal proteins in the methylotrophic yeast *K. phaffii*. Our results suggest that targeted manipulation of *ACG1* expression could lead to increased yields of recombinant proteins with improved stability.

ID: 51; Stand: B06

Regulatory events played by MIOREX proteins in mitochondrial gene expression in *Saccharomyces cerevisiae*

Jhulia Chagas, Camila Bomfim, Mario Barros

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Presenter: Jhulia Chagas

The mitochondrial genome provides a small set of subunits to the oxidative phosphorylation system (OXPHOS), which is responsible for ATP synthesis. In the baker's yeast *Saccharomyces cerevisiae*, the mitochondrial gene expression system has many regulatory steps that are required for proper expression of nuclear and mitochondrially encoded subunits of the OXPHOS complexes, including the presence of introns in the yeast mtDNA. Moreover, clusters formed by mitoribosomes and many proteins that regulate post-transcriptional steps have been called mitochondrial organization of gene expression (MIOREX) complexes. We aimed to identify nuclear genes that regulate mitochondrial translation depending on mtDNA intron composition. Through overexpression screening analyses we firstly identified that the excess of the *MRX9* product interferes in *COX1* and *COB* mRNAs intron processing, which are both mitochondrial. Here we show that the toxicity caused by the excess of Fmp30 and Aim34 proteins also depends on the presence of mitochondrial introns. Indeed, the overexpression of *FMP30* and *AIM34* genes under the control of *GAL10* promoter results in a reduction in translation of mitochondrial polypeptides and an elevated rate of petite formation. However, the excess of both proteins does not exhibit any deficiency on mitochondrial translation process and mtDNA stability in yeast strains devoid of mitochondrial introns. Ours results suggest that Fmp30 and Aim34 roles are associated with mtDNA transcription and RNA processing.

The dualsteric agonist for human M2 muscarinic receptor induces mitochondrial dysfunction in *S. cerevisiae*

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Presenter: Elena Passarini

Glioblastoma is a malignant human brain tumor of the astrocytic type. The activation of M2 muscarinic receptor (a G protein-coupled receptor, GPCR) by orthosteric agonist Arecaidine Propargyl Ester (APE) and dualsteric agonist N-8-Iper (N8) caused a significant decrease of cell proliferation and survival in Glioblastoma cancer stem cells and in Glioblastoma stable cell lines. Preliminary data showed the two agonists exerted a mitochondrial damage and an alteration of the lipid pattern in Glioblastoma cells. GPCRs are conserved in evolution from yeast to humans and the yeast system is considered a platform for human GPCR studies. To evaluate if the mitochondrial dysfunction is directly linked to the activation of the M2 muscarinic receptor, we tested the M2 agonists in yeast cells, elucidating the effects on mitochondria in a yeast model system. N8, but not APE, induces a mitochondrial dysfunction in yeast cells in a time and concentration-dependent manner. These results mimic the activity on glioblastoma cell cultures, suggesting a direct effect of the N8 agonist on mitochondrial function.

ID: 269; Stand: B08

Vps27 mediates the mitochondrial derepression and increased lifespan of yeast cells lacking the Sit4 protein phosphatase

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Presenter: Vitor Costa

Saccharomyces cerevisiae Sit4 is a catalytic subunit of a PP2A-like Ser/Thr protein phosphatase implicated in the regulation of several cellular processes such as cell cycle, carbohydrate and lipid metabolism, nutrient signalling, vacuolar and mitochondrial function, and chronological lifespan. In this study, we performed a phosphoproteomic analysis of vacuolar membranes from wild type and *sit4Δ* cells. Our results revealed changes in *sit4Δ* vacuolar proteins that are mostly associated with late endosome to vacuole transport, vacuole fusion, microautophagy, polyphosphate metabolism and metal ion transport. Notably, the levels of the endosomal sorting complex required for transport-0 complex (Vps27-Hse1) increased on *sit4Δ* vacuolar membranes and *SIT4* showed a negative genetic interaction with *VSP27*. The analysis of protein trafficking and vacuolar sorting pathways showed that autophagy was impaired in both *sit4Δ* and *sit4Δvps27Δ* cells and the Cvt pathway was induced in the *sit4Δ* mutant in a Vps27-independent manner. In contrast, the CPY pathway was not affected in *sit4Δ* cells, but it was impaired in the *sit4Δvps27Δ* double mutant, which exhibited an aberrant CPY secretion phenotype. Plus, *SIT4* deletion increased mitochondrial respiration and chronological lifespan in a Vps27-dependent manner and *sit4Δvps27Δ* cells exhibited a very low oxygen consumption rate and a shortened lifespan compared to *vps27Δ* cells. These results suggest that the CPY pathway, mitochondrial fitness and lifespan extension in *sit4Δ* cells are dependent on Vps27.

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MOLECULAR MECHANISM OF CONCENTRATION-REGULATED METHANOL INDUCTION IN METHYLOTROPHIC YEASTS

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Graduate School of Agriculture, Kyoto University, Japan

Presenter: Hiroya Yurimoto

Methylotrophic yeasts, such as *Komagataella phaffii* (*Pichia pastoris*) and *Candida boidinii*, can utilize methanol as the sole source of carbon and energy. These yeasts have strong methanol-induced gene promoters and have been used as hosts for heterologous gene expression systems. The expression of methanol-induced genes is regulated based on the environmental methanol concentration (concentration-regulated methanol induction: CRMI). CRMI is responsible for regulating the metabolic flux of a toxic intermediate formaldehyde generated from the oxidation of methanol. Several transcription factors have been identified for methanol-induced gene expression (1), however, how cells transmit the signal from the methanol-sensing machinery to transcription factors has not been elucidated.

Recently we revealed that the cell surface membrane-spanning sensor Wsc family proteins KpWsc1 and KpWsc3 function in sensing the environmental concentration of methanol and are responsible for CRMI in *K. phaffii* (2). Furthermore, we found that a transcription factor KpMxr1 receives the methanol signal and that the phosphoregulation of KpMxr1 plays a crucial role in CRMI (3). In this presentation, we propose a novel methanol signaling pathway (CRMI pathway) from KpWsc1/3 to KpMxr1 via KpPkc1 independent of MAPK cascade. We also demonstrate that CRMI-dependent expression of a transcription factor *C. boidinii* Mpp1 is important for the coordinated regulation of multiple methanol-induced genes to achieve a balanced methanol metabolism in methylotrophic yeasts.

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The mechanisms of degradation of methanol catabolism enzymes formaldehyde dehydrogenase and formate dehydrogenase in methylotrophic yeast *Komagataella phaffii*

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Presenter: Olena Dmytruk

The investigation of the mechanisms of cytosolic protein degradation is of great fundamental and applied importance. The decrease in the specific activity of formaldehyde dehydrogenase (Fld1) and formate dehydrogenase (Fdh1) in the wild type strain GS200, the strain with the deletion of the GSS1 hexose sensor gene, and the strain that is defective in autophagy pathway SMD1163 of *Komagataella phaffii* during short-term and long-term induction with methanol, with or without the addition of the MG132 (proteasome degradation inhibitor), was investigated. It was shown that the duration of cell incubation on methanol had no particular effect on the inactivation of enzymes. The effect of the proteasome inhibitor MG132 was insignificant. Catabolic inactivation of cytosolic and peroxisomal enzymes was damaged in the *gss1* mutant since glucose signaling was impaired. The strains with the deletion of three different ATG-genes (ATG1, ATG6, ATG15), involved in autophagy, were constructed to test the effect of these genes on the degradation of Fld1 and Fdh1 in *K. phaffii*. It was shown that Fld1 and Fdh1 are degraded via the vacuolar pathway regardless of the duration of methanol induction. Such a conclusion was confirmed by western blot analysis and fluorescence microscopy studies.

A crouton in a soup. How TORC1 signaling is fine-tuned by a swarm of small molecules

Raffaele Nicastro¹, Laura Brohée², Hélène Gaillard^{3,4}, Josephine Alba¹, Gianluca Figlia⁵, Ralf Wellinger^{3,4}, Stefano Vanni¹, Aurelio Teleman⁵, Constantinos Demetriades², Claudio De Virgilio¹

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Presenter: Raffaele Nicastro

In most eukaryotic organisms, the target of rapamycin complex 1 (TORC1) kinase regulates anabolic processes in response to nutritional and hormonal stimuli, transducing the signals of nutrient sufficiency to downstream effectors, promoting growth primarily through the activation of protein synthesis.

Several features of the regulation of the TORC1 signaling pathway have been extensively studied, and the role of specific amino acids on upstream regulators of the pathway has been reported in several organisms. However, virtually nothing was known about how metabolism impinges directly on the catalytic activity of the kinase. Recently, chemical-genetic screenings, proteomics and metabolomics pinpointed a series of small molecules and metabolic pathways that could directly regulate TORC1 in *Saccharomyces cerevisiae*, with remarkable evolutionary conservation in mammals. Our findings highlight that metabolites such as indole-3-acid (auxin) and malonyl-CoA and metals such as manganese regulate TORC1 activity by tuning its binding to ATP.

Altogether, these findings hint at a less simplistic picture of how a kinase is regulated, suggesting in particular that TORC1 is embedded in an environment of potentially catalytic activity-interfering small molecules. According to this view, the metabolic milieu may constrain TORC1 activity to ultimately tune down protein synthesis in accordance to a change in the nutritional status, fostering the idea of an unexplored layer of evolutionary ancient, non-enzymatic signaling.

On the illusion of auxotrophy

Nelson Castilho Coelho¹, Saurin Bipin Parikh¹, Branden Van Oss¹, Aaron Wacholder¹, Ivan Belashov², Sara Zadancewicz², Manuel Michaca², Jiazhen Xu², Yun Kang³, Nathan Ward³, Sang Yoon³, Katherine McCourt¹, Jake McKee¹, Trey Ideker⁴, Andrew VanDemark², Gina DeNicola³, Anne-Ruxandra Carvunis¹

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Presenter: Nelson Castilho Coelho

Since its discovery in the 1970s, the homocysteine synthase Met15 (also known as Met17 and Met25) has been considered essential for inorganic sulfur assimilation in yeast. The *MET15* gene has served as auxotrophic marker for hundreds of experiments carried out in yeast, especially those using large collections like the yeast deletion collection, which have been of extreme impact in the foundation of eukaryote genetics and systems biology.

Here, we demonstrate through structural and evolutionary modeling, in vitro kinetic assays, and genetic complementation, that an alternative homocysteine synthase encoded by the previously uncharacterized gene YLL058W enables cells lacking Met15 to assimilate enough inorganic sulfur for survival and proliferation. We show that toxic accumulation of the gas hydrogen sulfide explains the failure for these cells to grow in patches or liquid cultures unless provided with exogenous methionine or other organosulfurs. The addition of a hydrogen sulfide chelator to the culture media, and propagation as colony grids, allow cells without Met15 to assimilate inorganic sulfur and grow, and cells with Met15 to achieve even higher yields.

Contrary to what has been assumed for decades, Met15 is not essential for inorganic sulfur assimilation in yeast. Instead, *MET15* is the first example of a yeast gene whose loss conditionally prevents growth in a manner that depends on local gas exchange.

ID: 102; Stand: B13

Sfp1 integrates TORC1 and PKA activity towards ribosome biogenesis in budding yeast

Luc-Alban Vuillemenot, [Andreas Miliadis-Argeitis](#)

University of Groningen, Netherlands

Presenter: Andreas Miliadis-Argeitis

Target of Rapamycin Complex 1 (TORC1) and Protein Kinase A (PKA) are two major regulators of cell growth in *Saccharomyces cerevisiae*, coupling nutrient availability with a plethora of anabolic and catabolic processes. Even though TORC1 and PKA signaling pathways converge on several shared targets, little is known on how these targets integrate signals from the two pathways. This is the case for Sfp1, a transcriptional activator of hundreds of ribosomal protein and ribosome biogenesis genes. Under favorable growth conditions, Sfp1 localizes in the nucleus, but it rapidly relocates to the cytoplasm upon nutrient limitation or exposure to various stresses. To disentangle the roles of PKA and TORC1 in Sfp1 regulation, we constructed a large collection of Sfp1 (phospho)mutants and monitored their intracellular localization and phosphorylation dynamics in response to acute TORC1 and PKA inhibition. Our findings show that Sfp1 is controlled independently by TORC1 and PKA, and that loss of activity from either pathway is sufficient to deactivate Sfp1. By using two independent signaling inputs, cells are able to adjust Sfp1 activity in response to a large number of intra- and extracellular inputs that alter TORC1 and/or PKA activity, and ensure that Sfp1 is maximally active only when both signaling pathways are active. Our work contributes to our understanding of how cells regulate their growth by monitoring the outputs of multiple growth-regulatory pathways.

Cymoxanil inhibits respiration through inhibition of mitochondrial complex IV

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Presenter: Filipa Mendes

Cymoxanil is a synthetic acetamide fungicide, used against oomycetes. It was first introduced in 1977 and can be used against downy mildew diseases induced by *Plasmopara viticola* in grapevine cultures and late blight diseases caused by *Phytophthora infestans*, in tomatoes and potatoes cultures. This fungicide is used in mixed formulations and its higher solubility enables a relatively widespread occurrence in toxic concentrations in aquatic environments. Although it has been used over the years, its biochemical mode of action is not yet known. Some studies reported that cymoxanil affects growth, respiration, DNA, RNA and protein synthesis and RNA polymerase activity of *Phytophthora infestans*, and it was reported to inhibit cell growth and biomass production and decrease the respiration rate of *S. cerevisiae*. Using yeast *S. cerevisiae* as model, we further characterized its effect on mitochondria. We found that whole cells treated with cymoxanil present a higher inhibition of oxygen consumption after 3 h of treatment that remains over time. Using isolated mitochondria, we observe that cymoxanil inhibits respiratory rate of yeast cells by inhibiting oxidative phosphorylation, through inhibition of complex IV activity. Although other targets cannot be excluded, our data provide new information about mode of action of cymoxanil that can be instrumental to drive informed management regarding the use of this fungicide.

ID: 131; Stand: B15

Transient septin sumoylation steers a Fir1-Skt5 protein complex between the split septin ring

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Institute of Molecular Genetics and Cell Biology, Department of Biology, Ulm University, Germany

Presenter: Monique Furlan

The five mitotic septins of *Saccharomyces cerevisiae* Cdc11, Cdc12, Cdc10, Cdc3 and Shs1 assemble into hetero-octameric rods that polymerize into apolar filaments. Septin filaments form a ring-like structure next to the previous division site that expands later into an hourglass structure. The splitting of the hourglass into two rings prominently marks the beginning of cytokinesis. The split septin double ring (SSDR) seals off the membrane of the cytokinesis compartment to keep the membrane-attached proteins within the SSDR, and it serves as a platform from which proteins are sequentially released into the cleavage furrow. Cdc11, Cdc3, and Shs1 get sumoylated during mitosis and de-sumoylated shortly before cell separation. Timing and location imply that septin-sumoylation plays a role during cytokinesis. We discovered that sumoylated septins recruit the checkpoint protein Fir1 to the peripheral side of the septin hourglass. Subsequent de-sumoylation and synchronized binding to the scaffold Spa2 relocate Fir1 in a seamless transition between the split septin rings. Fir1 binds and carries Skt5, the activator of the chitin synthase III, on its route to the division plane. We propose that the opposite positioning of the sumoylated septins and Spa2 creates a tension across the ring that upon de-sumoylation drives the membrane-bound Fir1-Skt5 complex through a transiently permeable septin ring into the space between the SSDR. Here the Fir1-Skt5 complex serves as receptor for Chs3 and facilitates its incorporation into the plasma membrane.

ID: 140; Stand: B16

Purine depletion in budding yeast evoke stress resistance by nutrient dependent pathway

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Presenter: Janis Liepins

Cells rely on intracellular metabolites: amino acids, purines, and pyrimidines to form essential macromolecules, but de novo synthesis of these metabolites can be energetically costly. In the absence of adequate external supply, prototrophic cells activate de novo synthesis while auxotrophs experience starvation for the necessary metabolite. In opposite to G1/G0 arrest that occurs during "natural starvations" - when nitrogen or carbon sources are scarce, auxotrophic starvation for different nutrients can have distinct outcomes. Uracil, leucine starvation etc. leads cells to glucose wasting and disregulation of cell metabolism.

Our group explored the effects of purine auxotrophic starvation on *Saccharomyces cerevisiae* metabolism and found that a decrease in AXP concentration led to cell cycle arrest at G0/G1 phase, altered central carbon fluxes and activation of stress resistance in *RIM15*, *MSN2/4* dependent manner. We think that the mechanism how purine depletion evokes stress resistance involves nutrient dependent signalling pathway/s which might be jammed due to low AXP content. Additionally, our work sheds light on the importance of mutation location-dependent response and the metabolic adaptations under nutrient-limited conditions.

This phenomenon may have ecological relevance, as many eukaryotic organisms unable to synthesise their own purine scavenge it from their surroundings, including intracellular parasites that exploit host cell purines. Some of these parasites are able to withstand prolonged periods of purine starvation, suggesting that the loss of purine synthesis capacity and development of stress-resilient phenotypes may confer selective advantages in certain ecological niches.

Acknowledgements

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5-fluorouracil induced tRNA decay in yeast

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Presenter: Katharina Goerlitz

The cancer drug 5-fluorouracil (5-FU) is incorporated into RNA via its metabolite 5-fluorouridine triphosphate. In *Saccharomyces cerevisiae*, defects in various tRNA modification genes enhance 5-FU sensitivity at elevated temperatures. Rapid tRNA decay of specific tRNAs lacking a combination of modifications is known to be triggered at elevated temperatures and can be suppressed by deletion of *MET22* or overexpression of tRNA binding protein eEF1A. Here we demonstrate a *MET22* dependent synthetic 5-FU phenotype in a *trm4 trm8* mutant lacking 5methylcytosine and 7-methylguanosine. Overexpression of eEF1A or tRNA^{Val}_{AAC} suppresses the 5-FU phenotype and tRNA^{Val}_{AAC} levels are decreased in the mutant after drug exposure in a *MET22* dependent manner. We profiled all uridine specific modifications in tRNA after drug exposure and observed reduced abundances of pseudouridine and 5-methyluridine, while other U modifications were unaffected. We assume that 5-FU triggers tRNA decay in tRNA modification mutants due to multiple modification loss.

Identification of the divergent function of the pseudokinases Scy1p and Cex1p

Sven Meinerz¹, Gabriele Fischer von Mollard²

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Presenter: Sven Meinerz

Proteinkinases are fascinating proteins involved in almost every aspect of cellular function, yet some proteinkinases, like those of the SCY1-like family, are breaking known pattern by lacking key motifs in their kinase domains, leading to a loss of catalytical activity. In mammals the pseudokinases SCYL1 and SCYL2 has been proposed to be an important part of the intracellular trafficking machinery, as their inactivation in mice leads to an early-onset progressive motor neuron disorder.

Here, we are trying to dissect the function of Scy1p and Cex1p, orthologs of the mammalian SCY1-like proteins, in yeast to find their cellular function. Cex1p has been discussed to be either a tRNA-exporter or part of the intracellular trafficking machinery facilitated by COPI or clathrin coated vesicles. Exploiting well studied trafficking pathways, we were able to show, that Cex1p is involved in the COPI-dependent Golgi-to-ER retrograde vesicle transport. Scy1p, the ortholog of the clathrin vesicle associated SCYL2, was found to be expendable for intracellular trafficking but essential for the invasion of yeast cells into agar and for the formation of flocs. Cells lacking Scy1p lost these abilities, which we explain by a reduced expression of the heavily regulated flocculin Flo11p, hinting at a possible role of Scy1p in the transduction of extracellular cues.

ID: 153; Stand: B19

TOR signaling and transcriptional regulation

Ronit Weisman

Open University of Israel, Israel

Presenter: Ronit Weisman

TOR is an atypical protein kinase that is found in two highly conserved complexes, TORC1 and TORC2. In *Schizosaccharomyces pombe*, TORC1 is essential for growth, while TORC2 is essential only under stress conditions. TORC1 regulates the phosphorylation state of transcription factors, thereby controlling the switch between growth and stress responses. Less is known about the roles of TORC2 in gene expression. We have recently demonstrated that the sensitivity of *S. pombe* TORC2 mutant cells to a variety of stress conditions is rescued by loss of positive regulators of transcription, including the SAGA complex and the BET bromodomain protein Bdf2 [1]. We also show that TORC2 mutant cells have a lower level of phosphorylation of Spt5, a subunit of the DSIF transcription elongation factor complex, together with dysregulation of both the initiating and elongating form of RNA pol II. Together, our data suggests that TORC2 may affect the RNA transcription cycle in a manner that is critical for rapid and acute upregulation of stress genes. Our data unravel a novel aspect of TORC2 signaling and point at an interesting feature of regulating transcription in response to stress.

Genetic and physical interaction of Vps13 lipid transporter with Rsp5 E3 ubiquitin ligase: coordination of lipid synthesis with lipid transport?

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Presenter: Teresa Zoladek

Several rare neurodegenerative diseases depend on mutations in *VPS13A-D* genes, including chorea-acanthocytosis and early-onset Parkinsonism. *VPS13* genes are conserved from yeast to humans and yeast with unique *VPS13* is a good model system to study function of Vps13 proteins. Recent findings show that Vps13 protein transports bulk lipids at membrane contact sites. Localization of Vps13 is dependent on interactions with specific proteins and lipids which are characteristic to different subcellular compartments. The lipids determining the localization of Vps13 are quite well defined, but the proteins are not. To investigate Vps13 protein partners we purified Vps13 from yeast cells by pull down and identified interacting proteins using mass spectrometry. Among proteins recognized, we found Rsp5 ubiquitin ligase. The Vps13-Rsp5 interaction was confirmed by immunoprecipitation and Western Blot. Rsp5 is important for the lipid homeostasis. It ubiquitinates and is involved in proteasomal maturation of the transcriptional activators, Spt23 and Mga2, which regulate the expression of genes encoding lipid biosynthetic enzymes. Increased levels of activated Spt23 or Mga2 result in the formation of gigantic lipid droplets. This prompted us to analyse genetic interaction between *VPS13* and *RSP5* genes and we found that additional copies of the *RSP5* gene introduced into *vps13* mutant cells increase their sensitivity to sodium dodecyl sulphate. We also observed the lower level of Rsp5 E3 ubiquitin ligase in *vps13* compared to the wild type cells. These results suggest that the Vps13-Rsp5 interaction may be important for coordination of the lipid synthesis with lipid transport capacity of the cell.

Is the growth rate based on glucose assimilation the best way for identify the optimal growth temperature of psychrophilic and psychrotolerant yeasts?

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Presenter: Gianmarco Mugnai

Based on the conventional definition, the optimal growth temperature is the temperature at which yeasts have the highest growth rate (GR). However, the optimal growth temperature does not always result in the highest biomass production. By common definition, psychrophilic yeasts are considered those unable to grow above 20°C and growing fastest at 15°C or below. They are often isolated from permanently cold habitats worldwide. Psychrotolerant yeasts, frequently found in habitats subjected to temperature fluctuations, grow over a wider temperature range, including low temperatures, and exhibiting a faster growth rate above 20°C.

Such classifications originate from the results of glucose growth tests; this carbohydrate was chosen over other sources as it is universally assimilated by all the species and has the highest molecular energy content. However, little is known on how the GRs of both psychrophilic and psychrotolerant yeasts may differ with variation of both, the carbon source and the incubation temperature.

The aim of this study was to elucidate the effects of the incubation temperature on the GRs of four psychrotolerant and four psychrophilic yeast species, belonging to the genera *Mrakia* and *Naganishia*. This was done by growing them on different compounds used as unique carbon and nitrogen sources. Growth tests revealed that the GR is dependent on both the temperature and the carbon source used.

The preliminary results obtained suggest that the conventional definition of optimal growth temperature based on glucose assimilation characteristics should be re-evaluated for psychrophilic and psychrotolerant yeasts according to more complex criteria.

Purine depletion promoted oxidative stress tolerance does not depend on mitochondrial functions

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¹University of Latvia, Latvia. ²KU Leuven, Belgium

Presenter: Zane Ozoliņa

Understanding the physiological and metabolic responses of auxotrophic microorganisms is object of increasing interest in microbial research. Among auxotrophic phenotypes, adenine auxotrophy exhibits unique characteristics - cells demonstrate an organized response to purine starvation, probably similar to nitrogen or carbon starvation. Moreover, our surprising findings indicate that the ability to withstand various stresses following purine starvation persists even in petite cells.

In this study, we employed purine auxotrophs with mutations in the adenine biosynthesis pathway, with and without the additional petite phenotype. We investigated the effects of short-term purine and nitrogen starvation on yeast fitness, as well as acute and chronic stress responses. Our results highlight the importance of the location of knock-out mutations within the adenine synthesis pathway, shedding light on the critical factors influencing cellular response to purine scarcity. Furthermore, we explored the involvement of mitochondrial metabolism in the intracellular response to purine starvation. Both petite and non-petite cells exhibited enhanced stress resistance and varying degrees of adaptability to challenging environments. While the precise mechanisms by which cells signal purine starvation remain elusive, our data provides significant clues regarding the underlying processes.

By employing a combination of different experiments and approaches, we uncover the intricate interplay between purine metabolism, mitochondrial activity, and stress resistance in adenine auxotrophic yeast. These findings not only expand our understanding of cellular responses to nutrient scarcity but also have implications for the evolutionarily conserved nature of purine starvation response mechanisms.

Acknowledgements

The study was supported by the Latvian Science Council's Project LZP-2021/1-0522

ID: 224; Stand: B23

Ubiquitination of CLIP-170 family protein restrains polarized growth upon DNA replication stress in fission yeast

Xi Wang, Yamei Wang, [Quan-wen Jin](#)

Xiamen University, China

Presenter: Quan-wen Jin

Microtubules play a crucial role during the establishment and maintenance of cell polarity. In fission yeast cells, the microtubule plus-end tracking proteins (+TIPs) (including the CLIP-170 homologue Tip1) regulate microtubule dynamics and also transport polarity factors to the cell cortex. In the current study, we show that the E3 ubiquitin ligase Dma1 plays an unexpected role in controlling polarized growth through ubiquitinating Tip1. Dma1 colocalizes with Tip1 to cortical sites at cell ends, and is required for ubiquitination of Tip1. Although the absence of *dma1⁺* does not cause apparent polar growth defects in vegetatively growing cells, Dma1-mediated Tip1 ubiquitination is required to restrain polar growth upon DNA replication stress. This mechanism is distinct from the previously recognized calcineurin-dependent inhibition of polarized growth. In this work, we establish a link between Dma1-mediated Tip1 ubiquitination and DNA replication or DNA damage checkpoint-dependent inhibition of polarized growth in fission yeast.

ID: 260; Stand; B24

Investigation of the *S. cerevisiae* Start checkpoint and its regulation under acute carbon starvation

Anastasia Petropoulou, Deniz Irali, Jennifer Ewald

Eberhard Karls Universität Tübingen, Germany

Presenter: Anastasia Petropoulou

G1 cells must decide whether to initiate a new division cycle, at a G1/S checkpoint termed Start in yeast and Restriction Point in mammals. Whether a cell begins a new round of division is determined in part by nutrient availability. When cells face nutrient starvation, the G1/S checkpoint prohibits transition into S phase and cells arrest at G1. In *Saccharomyces cerevisiae* the critical point for a cell to pass Start is nuclear export of the inhibitor Whi5. Once 50% of Whi5 has been exported, the cell is considered irreversibly committed to a new cycle. What happens however if a cell after Start is faced with acute starvation?

We recently showed that post-Start cells facing acute carbon starvation within 20 minutes after Start can re-import Whi5 to the nucleus. These cells return to a pre-Start state, become sensitive to mating pheromone again and re-activate CDK when nutrients are available once more. However, through which mechanism cells interrupt the G1/S transition and re-import Whi5 is still unclear.

So far, we have been able to exclude transcriptional repression of Cln1/2 through the starvation response, as well as CDK inhibition through Sic1 as plausible mechanisms. We are now further investigating other post-transcriptional mechanisms.

ID: 272; Stand: B25

Identification of spatially distinct pools of AMPK/SNF1 and their targets in yeast

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Department of Biology, University of Fribourg, Fribourg, Switzerland, Switzerland

Presenter: Marco Caligaris

The well-conserved AMPK/SNF1 kinase complex is a central cellular energy sensor in eukaryotic organisms. In *Saccharomyces cerevisiae*, SNF1 responds to both glucose levels and stress signals to ensure cell survival. SNF1 is a heterotrimeric complex formed by a catalytic α -subunit (Snf1) with Ser/Thr kinase activity, three alternative β -subunits (Gal83, Sip1, and Sip2), which regulate the localization of the complex, and a γ -subunit (Snf4) involved in the regulation of Snf1 activity. The Gal83 β -subunit is needed to localize the SNF1 complex to the nucleus during the first minutes of carbon starvation. When Snf1 is associated with Sip1, the complex is localized on the vacuolar surface, while Snf1 remains cytosolic when bound to Sip2. To separately study the activities of the three different SNF1 complexes, combinations of amino acid substitutions were introduced in the β -subunits that should, as reported, render them non-functional, without affecting their assembly into SNF1 complexes. As a result, we obtained three strains in which only one of the β -subunit, and hence one local pool of SNF1, is active and functional. Our analyses of these strains using SILAC-based phosphoproteomics should therefore allow us to identify the target profile of spatially sequestered pools of SNF1. Here, we will present our progress in this project and discuss potential new mechanisms of crosstalk between the SNF1 and other nutrient-sensing pathways.

Amino acid excretion in yeast cells is induced at the transition phase and mediated by the DHA1 transporters family

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Presenter: Luís Sousa

Amino acid excretion by yeast cells has been described many years ago [1-2] and is at the heart of several recent studies [3-4], but its biological meaning remains poorly understood. In 2004, we identified for the first time an amino acid excretion protein, Aqr1p, which belongs to the Drug H⁺-Antiporter (DHA1) family [5,6]. More recently, we have reported that two additional transporters of the same family, Qdr2p and Qdr3p, also catalyze amino acid export [7]. We have now set up conditions to exploit the permease-like sensor of external amino acids, Ssy1p, as a proxy for detecting amino acid excretion by cells growing in amino-acid free media [8,9]. This tool enabled us to show that in cells cultivated on different nitrogen media, amino acid excretion is typically induced when their growth rate begins to decrease, what we named the transition phase. This amino-acid excretion is largely dependent on the activity of a small set of the DHA1 family transporters. Additionally, it is largely dependent on autophagy, shown to be stimulated just ahead of the transition phase. Our observations suggest that limitation of a certain nutrient or growth factor during growth triggers autophagy. This in turn would provoke an increase of cytosolic amino acids which tend to be excreted via several exporters of the DHA1 family.

ID: 294; Stand: B27

Cross-talk between the Hog1 MAPK pathway and Sch9 in the regulation of neutral lipid accumulation

David Hunt, Clare Lawrence

UCLAN, United Kingdom

Presenter: David Hunt

Initiation of lipogenesis through dysregulated signal transduction is a major hallmark of cancer and contributes to sustained cell proliferation. We have previously shown that the *S. cerevisiae* MAPK Hog1p is a novel regulator of neutral lipid accumulation in yeast and that activation of downstream signalling is independent of the canonical pathway. We have also shown that Hog1p inputs to the TORC1 signalling pathway during lipogenesis. Here, we show evidence that Hog1p is involved in cross-talk with the TORC1 pathway via its substrate Sch9p during initiation of neutral lipid accumulation in *S. cerevisiae* cells. Nile red staining for relative quantification of neutral lipids in *hog1Δsch9Δ* cells suggests that Hog1p and Sch9p regulate neutral lipid accumulation by a previously uncharacterised pathway. Mutation of potential MAPK sites in Sch9p suggests that both N-terminal and C-terminal phosphorylation is required for Sch9p mediated neutral lipid accumulation. Further experiments have also demonstrated that Hog1p has a role in regulating Sch9p. We hypothesise that Hog1p regulates Sch9p and is important for its subsequent phosphorylation by TORC1. Further work will aim to further characterise and validate signalling between Hog1p and Sch9p in regulating neutral lipid accumulation.

Whi2 protein, a key player in the evolution of spatially structured yeast populations

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Presenter: Libuse Vachova

To form spatially structured colonies that differentiate into U- and L-cell subpopulations, yeast cells must divide, cooperate, and coordinate their behavior at the right time and place in the colony. To date, several regulators and signaling molecules have been identified as important for proper colony development: volatile ammonia and key nitrogen- and amino acid-responsive signaling pathways such as TORC1 and GAAC. We found that the differentiated structure of older colonies can be disrupted by groups of cheater cells that do not follow the rules of differentiation and selectively proliferate within the U-cell subpopulation and form papillae on the colony surface. We have identified Whi2p-Psr1p/Psr2p complex as a key player in papilla outgrowth (PNAS 117:15129, 2020). When the complex is not functional, papillae are not formed. In chimeric colonies, cells lacking this complex (wpp cells) have a significant fitness advantage over wt cells with the functional complex. Such an advantage begins early in colony development, long before the colony differentiates into U- and L-cells, so that the subpopulation of U-cells in chimeric colonies consists only of wpp cells with nonfunctional complex. To shed new light on the mechanism of this distinctive competitive superiority, we analyzed the proteomes of mixed colonies and colonies of single wt or wpp strains. Comparison of the proteomes along with other data suggests that an as yet unidentified extracellular compound produced by wpp cells that suppresses wt cells may be responsible for the competitive superiority of wpp cells in chimeric populations. This work was supported by GACR23-06368S.

ID: 305; Stand: B29

Roles of yeast ataxin-2 in mitochondrial homeostasis and signaling

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Presenter: Michelle Grace Acoba

The mitochondrion has diverse cellular roles, importantly in nutrient catabolism, macromolecule biosynthesis, and metabolite compartmentalization. Moreover, as a signaling hub, the mitochondrion is a key player in pathways that promote cell adaptability and survival. Efficient mitochondrial biogenesis is essential in preserving the crucial functions of this organelle; however, aside from regulation at the transcriptional level, our understanding of how this process is coordinated with extramitochondrial factors is lacking. In this work, we show that yeast ataxin-2, or Pbp1 (polyA binding protein-binding protein 1), which has been linked to RNA and stress granule biology, supports mitochondrial biogenesis and the cell's ability to transition to oxidative metabolism. This is in addition to its role in sensing the cellular redox state as influenced by mitochondrial health, placing Pbp1 at the intersection of maintaining mitochondrial functionality and signaling mitochondrial state. Dissecting the mechanisms and functional factors involved in this regulatory pathway is the focus of ongoing studies.

Mitochondrial dysfunction and glucose metabolic remodeling accelerate osmoadaptation in *S.cerevisiae*

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University of Bari "Aldo Moro", Department of Biosciences, Biotechnology and Environment (DBBA), Italy

Presenter: Maria Antonietta Di Noia

Alterations in mitochondrial function have been linked to a variety of cellular and organismal stress responses including apoptosis, aging, tumorigenesis. However, adaptation to mitochondrial dysfunction can occur through the activation of survival pathways, with the mechanisms behind poorly investigated. Metabolic adjustment is a cytoprotective strategy in stress response and it has been shown that mitochondria can support the survival of cancer cells upon hyperosmotic environment. We used *S.cerevisiae* as a model to study how mitochondrial dysfunction can affect osmoadaptation. We analyzed and compared wild type cells with cells lacking mitochondrial DNA ($\rho 0$), a mitochondrial pyrimidine nucleotide transporter (Δ RIM2) and the catalytic subunit of the transcriptional complex Hap (Δ HAP4). Our results revealed that mitochondrial dysfunction confers an advantage in the kinetics of stress response. Accordingly, wild type cells exhibit higher osmosensitivity in the presence of respiratory metabolism. All mitochondrial mutants showed higher rate of glucose consumption and increased glycerol level produced in the early phase of yeast osmoadaptation. Reactive oxygen species appeared to be a secondary product of mitochondria-mediated stress response rather than signaling molecules. In all mutants the up-regulation of CIT2, encoding the peroxisomal isoform of citrate synthase, could be observed in the presence of salt stress indicating the activation of the RTG-dependent mitochondrial retrograde signaling. Interestingly, selected TCA cycle genes, CIT1 and ACO1, showed a different regulation in $\rho 0$ cells and DRIM2, although both strains lack mitochondrial DNA. These data will allow to gain insight into the mechanisms affecting the relationships among metabolism, cell survival and mitochondria-mediated stress response.

Extracellular DNA accumulating in yeast fed-batch cultures is metabolism-specific and inhibits cell proliferation: evidence from a multidisciplinary study

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Presenter: Maria Luisa Chiusano

Extracellular DNA (exDNA) is found in biological fluids and in culture media of different cell types, deriving from either cell lysis or active release from living cells. In ecological research, a species-specific inhibitory effect of exDNA has been reported in plants and demonstrated also in other species.

By a multidisciplinary approach, including theoretical modelling, metabolomics, DNA sequencing and bioinformatics, we reveal that the growth rate decline observed in *Saccharomyces cerevisiae* fed-batch cultures and leading to a limited cell density, was determined by exDNA accumulating in the medium.

A System Dynamics model showed that the yeast growth population decline is explained by the occurrence of a self-produced inhibitor, excluding the limiting action of end-products of fermentation.

Flow cytometric analysis showed that the observed growth inhibition corresponded to an arrest in the S phase of the yeast cell cycle.

The untargeted metabolomic ¹H-NMR analysis of the culture media highlighted the statistical significance of negative correlations between DNA-related signals and the cell population growth rate, with the absence of signals indicative of other possible inhibitory metabolites.

The exDNA isolated from the culture media was sequenced and found to represent only a portion of the entire yeast genome, showing a high similarity, up to 91% overlap, with extra-chromosomal circular DNA (eccDNA) described in the literature.

Different methodologies revealed that the exDNA is mostly single-stranded (98%), and reflects the respiratory or fermentative metabolic state yeast displays during the fed-batch cultivation.

These unprecedented findings open a new scenario in the control of cell proliferation.

The mysterious proteome of *Yarrowia lipolytica*

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Presenter: Kacper Szymański

Yarrowia lipolytica is at the center of researchers' attention worldwide. One huge advantage of these yeasts is their ability to secrete large number of metabolites as well as to utilize industrial waste substances, such as raw glycerol, as a carbon source. However, among sugars, wild strains of *Y. lipolytica* are using only glucose, fructose and mannose.

Growing in a mixture of glucose and fructose, *Y. lipolytica* exhibit a similar pattern of their utilization to other organisms, including *Saccharomyces cerevisiae*. It consumes glucose first and only when glucose is completely depleted it starts to use fructose. The exact mechanism underlying this yeast behavior is still poorly explained.

RNASeq analysis of *Y. lipolytica* showed, that during yeast growth in a medium containing fructose, separately or in combination with glucose, expression of the YAL10E03300g gene is several times higher compared to a medium with glucose alone. Due to that, *Y. lipolytica* transformants with deletion and overexpression of this gene were analyzed. Drop tests, microcultures and growth kinetics were performed to assess the impact of the introduced modifications on the yeast's physiology. It was found that strains with deletion of the target gene exhibited several times better growth and fructose utilization compared to the wild-type strain. The introduced modifications had an impact on the yeast morphology as well inhibiting filamentation. Determining the function of the investigated protein can help to better understand the metabolism of fructose by *Y. lipolytica*.

Does *Komagataella phaffii* (*Pichia pastoris*) rely on respiratory complex I?

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Presenter: Özge Ata

The methylotrophic yeast *Komagataella phaffii* is one of the most preferred hosts for the production of recombinant products. However, our knowledge on its fundamental metabolic properties is limited and mostly curated from *Saccharomyces cerevisiae*. Yet, *K. phaffii* has distinct properties that differ from *S. cerevisiae*, therefore we cannot easily draw analogies. Therefore, we need basic research to fully grasp the regulation of *K. phaffii*'s carbon and energy metabolism to rewire it for biotechnological applications.

Here we aim to explore energy harvesting mechanisms of *K. phaffii*, focusing on respiratory Complex I (CI) and alternative dehydrogenases. CI, also known as NADH:ubiquinone oxidoreductase or Type I NADH dehydrogenase, is the first enzyme of the mitochondrial electron transport system and catalyzes the transfer of electrons from NADH to coenzyme Q10. Being a Crabtree negative yeast, *K.phaffii* possesses CI unlike *S.cerevisiae*, and it has two alternative dehydrogenases, *NDE1* and *NDE2*. Until now, the dependency on CI and functions of the alternative dehydrogenases are not investigated. To understand this, we generated knockout and overexpression strains of complex I, *NDE1*, and *NDE2* by CRISPR-Cas9 and screened them. We performed inhibitory experiments to reveal the dependency of *K.phaffii* on CI for the electron transfer. In the light of our results, we show that *K.phaffii* requires a functional alternative dehydrogenase in the absence of CI during growth on glucose. We also present the characteristics of knockouts and overexpression strains on methanol, which we hope to provide more insights into the energy harvesting and cytosolic NADH supply of the cells.

Does Transcription Factor SPCC320.03 Regulate Expression of Hexose Transporter Genes in Fission Yeast?

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Presenter: Merve Seda Ibisoglu

Glucose plays key role as fundamental source of carbon and energy in eukaryotes, and its uptake are therefore vital to drive various energy-consuming cellular processes. Although it is known that two pathways (cAMP/PKA and MAPK) are involved in the sensing of glucose signal in *Schizosaccharomyces pombe* fission yeast, there is limited information about the regulation of hexose transporter genes (*ght1-8*) whose expression varies in different glucose concentrations and carbon sources. We aim to investigate the possible effect of SPCC320.03 transcription factor, which is thought to be involved in the pathway, on *ght1-8* genes at different carbon sources and glucose concentration. For this purpose, we used parental and Δ SPCC320.03 strains of *S. pombe* and their transformants. Significant changes in the expression of *ght3-6* were observed in SPCC320.03 overexpressed strain, Δ SPCC320.03, and its *pSLF272- SPCC320.03* transformant grown on 2% gluconate. In SPCC320.03 over-expressed strain, *ght3-6* the expressions were suppressed. The expression of *ght3*, *ght4*, *ght6* increased in Δ SPCC320.03 compared to parental strain. In addition, the expressions of *ght3-6* were suppressed in the transformant strains containing a single copy of SPCC320.03 gene, compared to the Δ SPCC320.03 strain. On the contrary, the expression of *ght2* in SPCC320.03 overexpressed strain was regulated positively in the 2% glycerol. It has also been shown that expression of *ght1*, *ght7* and *ght8* genes may vary depending on carbon sources but is not affected by expression of SPCC320.03 gene. We suggested that DNA-binding transcription factor SPCC320.03 has a role in the regulation of *ght3*, *gh4* and *ght6* genes.

ID: 326; Stand: B35

Screening of genes involved in Whi2p-mediated regulation in yeast populations.

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Presenter: Mradul Mishra

The yeast protein Whi2p (WHiskey) together with its binding partners Psr1p/Psr2p, forms the Whi2p-Psr1p/Psr2p (WPP) complex and functions as an important factor in balancing cell population and regulating expansion of cells with fitness advantage in dense yeast populations (J. Maršíková, et al.,2020). However, the functional mechanism of the WPP complex in the development and competitive adaptation of yeast colonies is still unclear. Therefore, we focus on identifying novel biological factors (genes) involved in the function of the Whi2p-Psr1p/Psr2p complex in yeast populations. We have implemented a screening approach to examine a genome-wide deletion library of *Saccharomyces cerevisiae* to identify genes involved in the function of the Whi2p-Psr1p/Psr2p complex. The experiments involve screening for deletion strains that exhibit a whi2D phenotype, i.e. strains with functional Whi2p but with deletions of other genes that lead to a similar phenotype in colonies. These phenotypically selected strains have been further investigated using a cell competition assay to explore the potential role of selected genes in gaining a fitness advantage. Several potential genes have already been identified that regulate yeast population development and competitive adaptation similar to the (WPP) complex. The project was supported by GAUK 406122 and GACR 23-06368S.

Is growth of *Saccharomyces cerevisiae* on xylose hindered by intracellular pH homeostasis mechanisms?

Krishnan Sreenivas, Leon Eisentraut, Daniel P. Brink, Magnus Carlquist, Marie Gorwa-Grauslund, Ed van Niel

Lund University, Sweden

Presenter: Krishnan Sreenivas

Redox and energy metabolism are balanced in microbes demonstrating good anaerobic growth. *Saccharomyces cerevisiae* strains engineered for utilizing the non-native substrate D-xylose show impaired growth, and one of the suspects is believed to be an imbalanced redox and energy recovery. To elaborate on the relationship between redox, energy and growth we used a dimensionless parameter called the cofactor formation flux ratio (R_f) which is a function of growth (μ). We showed that this kinetic ratio between redox formation flux ($J_{\text{NADPH}} + J_{\text{NADH}}$) and energy formation flux (J_{ATP}) in *S. cerevisiae* engineered for xylose utilisation is high.

Previous literature reports the intracellular pH in native xylose-utilizing yeast species being lower on xylose than on glucose. In *S. cerevisiae*, ATP is the sole energy carrier involved in pH homeostasis via a cytosolic H^+ -ATPase and a vacuolar H^+ -ATPase. In this study we compared the effects of proton pumping across the vacuolar membrane or the cytosolic membrane and its physiological impact on redox and energy. We investigated whether the production of the energy carrier flux (J_{ATP}) is a limiting factor for growth on xylose in *S. cerevisiae* engineered with the oxidoreductive xylose pathway by expressing a heterologous proton-pumping pyrophosphatase (H^+ -PPase). Our hypothesis is that upon expression of the H^+ -PPase, pyrophosphate (PPi) that is normally broken down into phosphate, releasing heat as byproduct, can instead be used to pump protons across membranes. We expect this to modify the energy formation flux into $J_{\text{ATP} + \text{PPi}}$ and restore intracellular pH to levels seen on glucose.

Crosstalk between CDK and PKA signaling impacts cellular physiology in budding yeast

Prathibha Muralidhara, Elisa Kaechele, Jennifer Ewald

Universität Tübingen, Germany

Presenter: Prathibha Muralidhara

Cell metabolism and the cell cycle are bidirectionally regulated. Important metabolic signaling pathway – the Protein kinase A pathway, essential for cell growth and functioning, has been studied in detail, but always in a very specific context. Work by us and others found several pieces of evidence that there lies extensive crosstalk between the nutrient-sensing PKA pathway and the cell cycle. How exactly the cell cycle drives the activity of the PKA is still very unclear.

Our previous study showed that upstream signaling components of the Ras2-cAMP-PKA pathway are phosphorylated during cell cycle progression. Some of these phosphorylation sites resemble CDK1 motifs. One protein that has particularly caught our attention is the PKA activator Cdc25 protein. The Ras-GEF Cdc25 appears to not only have CDK sites on its uncharacterized middle region but also potential cell-cycle targeted ubiquitination sites. By combining biochemistry, yeast physiology and genetics, we are currently investigating the functional relevance of these sites on Cdc25, and how these may affect PKA activity. Preliminary experiments using Cdc25 in vivo reporters suggest that protein fragment (S336-G725) containing two predicted CDK sites and several ubiquitination sites is differentially phosphorylated in different growth conditions. Additionally, protein localization also appears to be cell-cycle regulated under specific nutrient conditions. Characterizing these sites on the Cdc25 protein will help us understand how the cell cycle and metabolic pathways integrate to influence cellular growth and physiology in budding yeast and other eukaryotes.

ID: 4; Stand: B38

Investigating the roles of Hho1p and Hmo1p during stationary phase in *Saccharomyces cerevisiae*

Reham Alnajjar, Alastair Fleming

Department of Microbiology, Moyne Institute, Ireland

Presenter: Reham Alnajjar

DNA in eukaryotic cells is packaged as chromatin, the fundamental subunit of which is the nucleosome comprised of histone proteins H2A, H2B, H3 and H4 and 146 base pairs of DNA. Higher order compaction of chromatin involves the histone H1 linker histone. Linker histone H1 is well characterized in higher eukaryotes but is less well studied in *Saccharomyces cerevisiae*. It has been proposed that Hho1p is yeast histone H1, but the structure of Hho1p is different from mammalian H1 and its function in yeast is ambiguous. Another candidate to be functioning in yeast as histone H1 is Hmo1p. In this project I am investigating the roles of Hho1p and Hmo1p in yeast to determine which is the most likely candidate to be the functional equivalent of mammalian linker histone H1. I will test the hypothesis that Hho1p and/or Hmo1p functions to repress global gene transcription in the quiescent cell population formed during yeast stationary phase. The results reveal an hho1 mutant forms fewer quiescent cells during stationary phase which lose viability over time. In the hmo1 mutant, quiescent cell formation and viability are delayed. Hmo1p plays the greatest role in gene repression in the quiescent cells. Thus, Hho1p and Hmo1p have different roles in cell development, cell survival and regulation of transcription during yeast stationary phase.

ID: 17; Stand: B39

TorC1 and Nitrogen Catabolite Repression Control of Integrated GABA Shunt and Retrograde Pathway Gene Expression

Jennifer J. Tate, Terrance G. Cooper

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Presenter: Jennifer J. Tate

Despite our detailed understanding of how the *S. cerevisiae* lower gamma-aminobutyrate (GABA) shunt and retrograde pathway genes are regulated, there is a paucity of validated information concerning control of *GAD1*, the glutamate decarboxylase gene which catalyzes the first reaction of the GABA shunt. Further, integration of glutamate degradation via the GABA shunt with other metabolic pathways is similarly lacking. To fill these gaps, we show that while *GAD1* shares a response to rapamycin-inhibition of the TorC1 kinase, it does so independently of the Gln3 and Gat1 NCR-sensitive transcriptional activators that mediate transcription of the lower GABA shunt genes. We also show that GABA shunt gene expression increases dramatically in response to nickel ions. The alpha-ketoglutarate needed for the GABA shunt to cycle, thereby producing reduced pyridine nucleotides, derives from the retrograde pathway as shown by a similar high increase in the retrograde reporter, *CIT2* when nickel is present in the medium. These observations demonstrate high integration of the GABA shunt, retrograde, peroxisomal glyoxylate cycle and beta-oxidation pathways and a more in depth understanding of *GAD1* and GABA shunt transcriptional regulation.

Investigating the molecular mechanisms regulating the yeast-to-titan switch in *Cryptococcus neoformans*

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¹University of Exeter, United Kingdom. ²University of Edinburgh, United Kingdom

Presenter: Daniel Jones

The basidiomycete fungal pathogen *Cryptococcus neoformans* is a major agent of life-threatening infections in the immunocompromised, where fungal proliferation in the lung, central nervous system and brain leads to pneumonia and meningitis. Despite this, key aspects of the *C. neoformans* cell cycle during proliferation are understudied. Notably, *C. neoformans* significantly diverges from model yeast in both the composition and regulation of conserved cell cycle events, requiring an improved understanding of the basic biology of this organism.

In the host, *C. neoformans* exist in different morphological forms, including haploid yeasts and polyploid titan cells. Although the inducing conditions for yeast-to-titan transition are established, the molecular mechanisms remain unknown. We hypothesise that changes in cell cycle regulation mediate the switch. This study investigates how the cell cycle-related transcription factors Mbs1 and Swi6 differentially regulate the proliferation of yeast and titan cells.

In *S. cerevisiae*, differential binding of the Mbs1 orthologs Mbp1 and Swi4 to Swi6 form the cell cycle regulatory complexes MBF (Mbp1/Swi6) and SBF (Swi4/Swi6), which regulate cell cycle progression. MBF responds to internal checkpoints, while SBF regulates response to stress. In *C. neoformans*, Mbs1 has both Mbp1 and Swi4 characteristics, raising questions about how this regulatory circuit has been rewired. Loss of function of either Mbs1 or Swi6 impacts morphogenesis in distinct ways. Using genetic and molecular approaches, we characterise and demonstrate how differential regulation of a conserved pathway underpins morphological differences.

ID: 168; Stand: B41

Transcriptome analysis revealed multiple targets of erythritol-related transcription factor Euf1 in unconventional yeast *Yarrowia lipolytica*

Dorota Rzechonek, Mateusz Szczepańczyk, Aleksandra Mirończuk

Wrocław University of Environmental and Life Sciences, Poland

Presenter: Dorota Rzechonek

Euf1 is a transcription factor in yeast *Yarrowia lipolytica*, whose first known targets were genes encoding erythritol utilization pathway. New transcriptomic analyses revealed that it up-regulates more than 150 genes. The most interesting examples are connected with utilization of unusual carbon sources - the degradation pathway of the branched-chain amino acids (leucine, isoleucine and valine) and the glyoxal cycle. Most of the upregulated-genes are not directly linked to erythritol metabolism, however their expression is higher when this polyol is present in the environment, that points to previously unknown connections. As erythritol is typically produced in under hyperosmotic conditions, activity of Euf1 might be a part of prolonged stress response. Discovery of connection between leucine and erythritol metabolism may be important for designing genetically modified strains of *Y. lipolytica*, as these two compounds are used as a part of popular toolkits for metabolic engineering.

This work was financially supported by the National Science Centre, Poland, project UMO-2018/31/B/NZ9/01025.

S. cerevisiae basal transcription modulation by G-quadruplex forming sequences and their impact on the transactivation potential of p53 family proteins.

Libuše Kratochvilová¹, Matúš Vojšovič², Natália Bohálová¹, Lucie Šislerová¹, Zeinab El Rashed³, Alberto Inga⁴, [Paola Monti](#)⁵, Václav Brázda¹

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Presenter: Paola Monti

G-quadruplexes (G4) in DNA are increasingly being revealed as critical players in DNA metabolism, including the accessibility and activity of promoters. Exploiting a well-defined transcriptional reporter system in yeast, we sought to systematically investigate the impact of sequences with different propensities to adopt G4 structures on both basal and induced transcription. A 22-mer G4-prone sequence (derived from the KSHV virus) and five derivatives that progressively mutate the characteristic guanine stretches were placed upstream of a minimal promoter and adjacent to a P53 response element in otherwise isogenic luciferase reporter strains. In vitro, the panel of G4 sequences was analyzed both by Thioflavin T binding test and Circular Dichroism. In yeast, the impact of the G4 sequences was measured first on the basal activity of the reporter, then on the transactivation induced by the expression of transcription factors of the P53 family. Wild-type P53, P73, and P63 wild-type isoforms, nine cancer-associated P53 missense mutants, and three ectodermal dysplasia P63 alleles were tested, exploiting inducible expression. Results show that G4-prone sequences increase basal transcriptional activity in a manner proportional to their relative propensity to adopt a G4 structure. Furthermore, G4-prone sequences can cooperate with weaker wild-type P63 and P73 proteins but not with P53. Finally, G4-prone sequences increase the transactivation ability of mutant alleles of the P53 family of partial function, indicating the importance of including an evaluation of local DNA structural motifs within promoters to predict the functional impact of disease-associated TP53 and TP63 mutations.

ID: 185; Stand: B43

Interplay between chromatin and RNAPII transcription termination

Pablo Maraver-Cárdenas, Andrés Aguilera, Ana G. Rondón

Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), University of Seville, Spain

Presenter: Pablo Maraver-Cárdenas

Transcription termination is a complex process involving the release of RNA polymerases (RNAPs) from the DNA template thus allowing completion of RNA synthesis. For RNAPII, transcription termination is coupled to mRNA 3' end processing. Upon recognition of the polyadenylation signal, the elongating RNAPII slows down, and the nascent RNA is then cleaved. Ultimately, degradation of the nascent RNA still attached to RNAPII trigger its release from DNA. Defects in transcription termination negatively affect gene expression and are linked to diseases such as cancer and viral infections.

While the role of chromatin in transcription initiation and elongation is well-established, its impact on transcription termination remains unclear. Previous results implicate the chromatin remodellers CHD1, ISW1 and ISW2 in the process of termination at specific termination regions. Furthermore, nucleosome positioning at termination regions facilitates termination by serving as a barrier to transcription progression. To identify key factors and molecular pathways connecting transcription termination with chromatin, we performed a screening of a small collection of mutants affected in chromatin metabolism using a designed plasmid with multiple termination sites. We assessed the termination efficiency of RNAPII for each mutant at specific genes and genome-wide. Preliminary findings indicate that changes in chromatin structure and accessibility play a crucial role in modulating transcription termination. With this study, we aim to contribute significantly to our understanding of regulation of gene expression and the underlying mechanisms of transcription termination.

ID: 349; Stand: B44

Bio-reduction of wine volatile acidity using yeast cells

Filipa Mendes, Joana Guedes, Tiago Cardoso, Maria João Sousa, Manuela Côrte-Real, Susana R. Chaves

CBMA— Center of Molecular and Environmental Biology, Departamento de Biologia, Universidade do Minho, Portugal

Presenter: Susana R. Chaves

Acetic acid is the main component of the volatile acidity of grape musts and wines. It can be formed as a by-product of alcoholic fermentation or as a product of the metabolism of acetic and lactic acid bacteria, which can metabolise residual sugars to increase volatile acidity. Acetic acid levels are critical for wine quality, and usually range from 0.2 to 0.6 g/l. At higher concentrations, acetic acid has a negative impact on the quality of wine, with 1.2 g/l the maximum acceptable limit for volatile acidity by OIV (2010). At higher values, there are few processing options available to winemakers for the removal of sensorial objectionable levels of volatile acidity, and they are complex and expensive. Bioreduction methods using yeasts have been known for a long time but have not been sufficiently well characterized for commercial application. In this work we explored to reduce excessive volatile acidity of wines by direct inoculation of acidic wine with a well characterized highly acetic acid resistant yeast. We also screened natural yeast diversity for strains that could possess similar deacidification properties. Our final expectation is to develop a simple, inexpensive, and efficient biological process for the deacidification of acidic wines by yeasts, which will allow correcting the volatile acidity of acidic wines.

This work was supported by the project EXPL/BAA-AGR/1096/2021 (WINE-TUNING) and by the “Contrato-Programa” UIDB/04050/2020 funded by national funds through FCT.

ID: 257; Stand: B45

Investigating the interaction landscape of ubiquitination enzymes using a NanoLuc-based protein complementation assay

Natalia Łazarewicz^{1,2}, Gaëlle le Dez¹, Robert Wysocki², Gwenael Rabut¹

¹University of Rennes, France. ²University of Wrocław, Poland

Presenter: Natalia Łazarewicz

Protein-protein interactions (PPIs) create a complex network that determines proper functioning of eukaryotic cells. Among PPIs, protein ubiquitination is one of several key enzymatic cascades in the cell, regulating many processes such as cell growth, proliferation and signaling. This process involves three classes of enzymes, among which ubiquitin ligases (E3s) are the most common and determine the variety of ubiquitination processes. As these enzymes recognize the modified substrate proteins and provide ubiquitination specificity, they became attractive targets for treatments against diseases caused by aberrations in protein ubiquitination. Therefore, understanding the molecular action of ubiquitin ligases is essential to develop processes-specific drugs.

Here, we present the studies on ubiquitin ligases, which are involved in diverse cellular processes such as DNA damage response or cell cycle control in the yeast *Saccharomyces cerevisiae*. We investigate the mechanism of action, interacting partners, and substrates' recognition mechanisms to decipher their cellular consequences. For this purpose, we use a modern protein-fragment complementation assay (the NanoBiT[®] technique).

Our preliminary results showed that among the high confidence results we found the interactors previously described in the literature as well as novel putative interactors. Using luciferase-based technique, we were able to show proteasomally degraded substrates. In addition, we optimized a high-throughput cell growth and measurements, which can be applied in research of different enzymatic pathways. This may also contribute to the development of novel therapies for various diseases in the future.

This research was funded by the National Science Centre, Poland grant number: 2021/41/N/NZ2/00551(NCN, Poland)

ID: 281; Stand: B46

The yeast endonuclease Rnt1 processes and degrades a wide range of messenger RNAs

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Presenter: Lee-Ann Notice

RNA processing and degradation are critical for maintaining cellular homeostasis. These processes regulate steady-state RNA levels in the cell and ensure the destruction of aberrant mRNAs. Key enzymes that participate in RNA processing and degradation are endoribonucleases that cleave transcripts internally, providing entry sites for the RNA-degrading exoribonucleases. However, despite their crucial involvement in maintaining normal cell physiology, endoribonucleases remain largely uncharacterized, and although a large fraction of newly transcribed RNA is degraded in the nucleus, nuclear mRNA degradation mechanisms have not been well studied. This project aims to characterize the role of a nuclear endoribonuclease, Rnt1, in eukaryotic mRNA processing and degradation. Although Rnt1 is known to process many ncRNAs by recognizing a double-stranded RNA stem loop with an AGNN sequence, its involvement in mRNA degradation is much less defined. To identify Rnt1 cleavage sites throughout the yeast transcriptome, we employed an RNA sequencing approach along with rigorous bioinformatics in a strategy called comparative parallel analysis of RNA ends (compARE). This confirmed most known Rnt1 ncRNA processing sites, and revealed many previously unknown cleavage sites in protein-coding regions. Investigation into the sequence- and structure-specific determinants of mRNA target selection by Rnt1 showed striking similarity to the requirements for Rnt1 selection of ncRNA targets. Relocalizing Rnt1 to different subcellular compartments further revealed that the nuclear localization of Rnt1 may also play a major role in its selection of mRNA targets. We anticipate that these findings will fill considerable gaps in the current understanding of eukaryotic nuclear mRNA processing and degradation.

Mitochondrial retrograde pathway plays an important role in regulation of colony development

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Presenter: Vitezslav Plocek

Although yeasts are unicellular microorganisms, in nature they form complex multicellular structures, colonies and biofilms. To analyze development of multicellular structures, we use colonies of laboratory strain *Saccharomyces cerevisiae* BY4742, which form specialized spatially localized subpopulations during their development (FEMS Yeast Res. 18(4), 2018). We show that mitochondria-nucleus communication mediated by retrograde pathway (RTG) plays an important role in colony development. RTG pathway in colonies comprises three distinct branches (Cit2-branch, Ato-branch and viability branch) that regulate the properties and fate of different cell subpopulations, and the production of specific markers (Oncotarget 7:15299, 2016). Using proteomics, we identified novel targets of RTG pathway in colonies (Int J Mol Sci 22, 2021). We found that RTG pathway plays a key role in changes in the metabolism of U cells in the upper part of colonies during their transition to the alkali developmental phase. Importantly, this regulation occurs when the TORC1 pathway is active, although TORC1 was described as a negative regulator of the RTG pathway. In U cells, RTG pathway regulates processes leading to amino acid biosynthesis (including the transfer of metabolic intermediates between compartments), represses the expression of mitochondrial ribosomal components (which may lead to a reduction in mitochondrial functions) and is involved in the adaptation of U cells to changing nutritional conditions during the transition from acidic to alkaline developmental phase. Our findings expand our understanding of the function of RTG pathway and suggest new ways in which this pathway contributes to colony development. Supported by GACR19-09381S and INTER-COST-LTC20036.

Investigating the role of 3'-untranslated region sequence in condition-dependent mRNA fate

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Presenter: Weronika Danecka

3'-untranslated regions (3'-UTRs) are non-coding sequences of mRNA that are involved in mRNA translation, localization and stability. In some 3'-UTRs these functions are mediated by interactions with RNA-binding proteins or changes in mRNA secondary structure. Moreover, 3'-UTR isoforms, which result from alternative polyadenylation, might contain different motifs important for the mRNA fate. However, the exact motifs and mechanisms involved in determining mRNA fate remain largely unknown. The goal of this project is to understand the role of 3'-UTR isoforms and motifs in adaptation to growth environment and stress conditions using *Saccharomyces cerevisiae* as model organism.

Using fluorescent reporter assays, we test how native and modified 3'-UTRs and terminator sequences control reporter gene expression in physiological and stress conditions. Our library design focuses on studying sequences that are conserved across fungi or relevant to adaptation to environmental conditions. We measured the reporter protein and mRNA abundance as well as changes in polyadenylation site usage using flow cytometry and RNA-Seq. Our results indicate that inserting cis-regulatory elements in the 3'-UTR changes the fluorescent reporter expression and poly(A) site distributions, and that the impact of these modifications depends on the terminator context. To study the role of non-conserved 3'-UTR sequences, we have tested native and modified terminator sequences from yeast species adapted to different temperatures within the reporter system and in their native genomic context. We have identified sequences that might play a role in temperature adaptation within *Saccharomyces* genus by increasing mRNA stability.

ID: 337; Stand: B49

Molecular mechanisms of habituation to pheromone signal in *Saccharomyces cerevisiae*

Aliaksandr Damenikan, Yves Barral

ETHZ, Switzerland

Presenter: Aliaksandr Damenikan

The ability to adapt is one of the most fundamental properties of life. Genetic adaptations, recorded as sequences of DNA, allow populations to change according to their environment. Individual organisms can adapt too, by changing their phenotype based on their own experience. Such adaptations are often called learning and require storing and maintenance of historic information (i.e. memory). Budding yeast show ability to learn during sexual behaviour. A haploid yeast cell senses the presence of potential sexual partners through signal molecule called pheromone. When presented with a deceiving pheromone signal (purified compound without a partner), cells become insensitive to it with time, or, in other words, they habituate. This manifests mainly as a reversal of a pheromone-induced cell cycle arrest in G1. Previous work in our lab has established that several cell cycle regulators, particularly those that control G1/S transition, are important for the adaptation. We have shown that Cyclin 3 (Cln3), an upstream regulator of the cell cycle entry, is being upregulated during pheromone response and its levels remain high after habituation. Here we explore the molecular mechanisms of this memory-reminiscent dynamics of Cln3.

ID: 339; Stand: B50

Function driven reconstruction of yeast cell cycle transcriptome

Maria Chiara Langella, Dario Borrelli, Francesco Monticolo, Edoardo Pasolli, Maria Luisa Chiusano

Department of Agricultural Sciences, University of Naples "Federico II", via Università 100, 80055 Portici (NA), Italy

Presenter: Maria Luisa Chiusano

The expression of genes playing known roles in the cell cycle of the budding yeast *Saccharomyces cerevisiae* (e.g. genes coding for cyclins) has been extensively described (eg. Hartwell 1974, Evans et al. 1983, Stern et al. 1984, Baum et al. 1988, Fitch et al. 1992, Booher et al. 1993 Harrington et al. 1996, Koch et al. 1993, Schowb and Nasmyth 1993, Kock and Nasmyth 1994, Pines 1995, Nasmyth 1996, Sia et al. 1996, Yamamoto et al. 1996, Mcierny et al. 1997, Wolfe and Shields 1997, Mendehnull and Hodge 1998 ...).

In addition, microarray analyses considering the progression through yeast cell cycle revealed periodic fluctuations of mRNA levels associated to hundred of genes that have been therefore classified as cell cycle-associated (eg. Cho et al. 1998, Spellman et al. 1998, de Lichtenberg et al. 2005, Pramila et al. 2006) and organised in integrated data collections (Santos et al. 2015) to provide references for the scientific community.

This study aims to reconcile cell cycle associated genes from previous classifications with results from deep transcriptome analyses based on RNA-sequencing (Teufel et al. 2019). Computational analyses driven by functional annotation permitted to reconstruct the entire atlas of yeast cell cycle expression profile. This effort confirmed wellknown evidences while highlighting discrepancies in previous classification efforts and depicting useful motifs that may better figure out the expression patterns associated to yeast cell cycle phases.

**Poster session: “DNA replication &
recombination; DNA damage & repair”,
“Synthetic and Systems Biology of Yeasts”,
“Yeast biotechnology”**

Wednesday 23rd August, 13.30-15.15

ID: 28; Stand: C01

Recruitment of Rad51 recombinase to the repair foci depends on SUMOylation

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Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland

Presenter: Tuguldur Enkhbaatar

Homologous recombination (HR) is dedicated to repairing DNA double-strand breaks (DSBs) and preventing chromosomal rearrangements and cell death. A Rad51 recombinase, crucial for this process, binds ssDNA, arising during the resection step of the HR, to create a presynaptic filament. This filament searches homology and invades the duplex DNA, allowing the homologous sequence to be used as a template during DNA replication in the following repair steps.

Posttranslational modifications modulating Rad51 activity, e.g., phosphorylation impacts its DNA binding affinity, ATPase activity, and stability. We showed that episomally expressed three E3 ligases affected Rad51's SUMOylation and ubiquitination profiles. Mms21 overproduction resulted in mono-SUMOylation, Rsp5 overproduction in both poly-SUMOylation and ubiquitination, whereas Slx8 overproduction in SUMO-targeted poly-ubiquitination.

To investigate how the differences in ubiquitination and SUMOylation patterns influence the Rad51 role in DNA repair, we followed the YFP-Rad51 foci formation and their intensity after DSB-induction with zeocin. The fluorescence microscopy results showed that Mms21-dependent mono-SUMOylation of Rad51 increased the number of Rad51 repair foci in both control conditions and after zeocin treatment, while Slx8 overproduction decreased the number of Rad51 foci formed after zeocin treatment. Rsp5 overproduction slightly increased the number of Rad51 repair foci in zeocin-treated cells, simultaneously decreasing the intensity of these foci.

Our findings suggest that Mms21-dependent mono-SUMOylation of Rad51 stimulates Rad51 recruitment to DNA lesions, while Slx8 promotes the degradation of Rad51 SUMOylated forms, likely contributing to the release of recombinase from DNA. These results provide new insights into the complexity of HR regulation.

ID: 76; Stand: C02

Investigating the yeast Swi-Snf chromatin remodelling complex

Hadel Aljaeed, Alastair Fleming

Microbiology department, Ireland

Presenter: Hadel Aljaeed

Genomic DNA in eukaryotic cells is packaged as chromatin. The fundamental subunit of chromatin is the nucleosome which is composed of 147 bp of DNA wrapped around an octamer of two each of the core histone proteins, H2A, H2B, H3, and H4. The structure of chromatin is generally repressive to any process that requires access to the DNA, including transcription, replication, recombination, and DNA damage repair. However, the structure of chromatin can be altered or 'remodeled' via ATP-dependent chromatin remodeling complexes. The yeast Swi-Snf complex was the first ATP-dependent chromatin remodelling complex that could activate global gene transcription discovered. I have examined the role of each Swi-Snf subunit in cell function and transcription by examining deletion mutants of the Swi-Snf complex. The RNA Seq data revealed that Swi3p was the most unique subunit in the complex. Furthermore, Cyc8p protein levels in the swi3 mutant were abolished. Our findings indicate Swi3p as being important for Swi-Snf function and for mediating a possible direct interaction with the Tup1-Cyc8 co-repressor complex.

Identification of cohesin interactome in budding yeast

Ireneusz Litwin¹, Malgorzata Nowicka², Katarzyna Markowska¹, Ewa Maciaszczyk-Dziubińska², Paulina Tomaszewska^{2,3}, Robert Wysocki², Karol Kramarz¹

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Presenter: Malgorzata Nowicka

Genomic instability is the main cause of developmental disorders and tumorigenesis. In eukaryotic cells there are several factors that preserve genome integrity and are responsible for equal division of genetic material. One of the most important is the cohesin complex. Cohesin is essential, ring-shaped multiprotein complex that holds sister chromatid together until anaphase, ensuring biorientation of chromosomes and equal genome division. Moreover, cohesin is crucial for chromosomal metabolism, including ribosomal DNA (rDNA) region organization, DNA repair and gene transcription, thus regulation of cohesin activity is crucial for cell viability.

In order to identify cohesin interactome, we performed proteomic screen, analysis of obtained data allowed us to identify over 300 potential cohesin interactors. Most of identified proteins are involved in transcription and RNA metabolism, chromatin organization and DNA repair.

Hence, mass spectrometry analyses are prone to contamination and false positive results, we decided to confirm cohesin physical interaction with previously identified potential interactors. We successfully confirmed over 10 physical interaction cohesin-protein with proximity ligation assay and coimmunoprecipitation, next we performed experiments in order to determine if interaction cohesin-protein affects cohesin functions.

Here, I will present our results that will shed a light on cohesin complex regulation and role of this interactions in maintaining genome stability in *Saccharomyces cerevisiae*.

ID: 182; Stand: C04

Systematic investigation of disease-related short tandem DNA repeat instability

Daniele Novarina, Sandra Ollivaud, Liesbeth Veenhoff, Michael Chang

European Research Institute for the Biology of Ageing, Netherlands

Presenter: Daniele Novarina

Short tandem DNA repeats, consisting of stretches of repeating tracts of 2-12 bp DNA units, are intrinsically unstable due to the formation of non-B DNA structures, which can interfere with DNA replication and repair. To date, the expansion of 13 such repeats has been linked to over 50 neurodegenerative diseases in humans, including Huntington's disease, Friedrich ataxia, fragile X syndrome and several spinocerebellar ataxias. These diseases are collectively referred to as "repeat expansion diseases" (REDs). Even though the pathogenetic mechanisms might be different among these diseases (from gene inactivation to toxic gain of function at the mRNA or at the protein level), in each case the initial event is the expansion of the corresponding repeated sequence at the DNA level. Therefore, elucidating the mechanisms leading to DNA repeat instability and the cellular pathways counteracting it is essential for understanding (and ultimately curing) these diseases.

We take advantage of the *Saccharomyces cerevisiae* genetic model to systematically investigate the instability of the 13 RED-linked human short tandem repeats by inserting repeat tracts of varying length into the yeast genome. We measure their length- and orientation-dependent fragility with the gross chromosomal rearrangement (GCR) assay, and we assess their potential localization at the nuclear pore complex (NPC) during S-phase via live-cell microscopy, since CAG relocation to the NPC has been shown to be important for their stability. Furthermore, we perform genome-wide screens using the GCR assay and our recently-developed high-throughput replica-pinning technique to identify genes and pathways that affect the fragility of these sequences.

Who is what and what is who: how does *Saccharomyces cerevisiae* interact with exogenous DNA in the nucleus?

Rachel Mellon, Hassan Mustapha, Yves Barral

ETHZ, Switzerland

Presenter: Rachel Mellon

How eukaryotic cells differentiate chromosomal DNA from exogenous DNA in the nucleus is poorly understood. Particularly in unicellular eukaryotes: are they able to protect their genome from potential pathogens; how and why do they accept exogenous DNA? Using the budding yeast *Saccharomyces cerevisiae*, we sought to understand how these cells interact with exogenous DNA in the nucleus.

To investigate this, we established a method using the Cre/loxP system to assay the frequency of which plasmid DNA transiently enters the nucleus. We observe that plasmid DNA which enters the nucleus and is expressed is only heritably retained in ~50% of the cells. These results not only indicate that transient expression of exogenous DNA is not sufficient for heritable retention in *S. cerevisiae*, but also support the existence of mechanisms governing chromosomal-exogenous DNA discrimination in the nucleus. Which begs the question, what mechanisms are at play?

In order to identify players involved in this process, a genetic screen using transposon-based mutagenesis was carried out to identify mutations affecting transformation efficiency. The screen revealed multiple processes restrict transformation of exogenous DNA, such as the endocytic, degradation and nuclear import machinery, as well as chromatin remodelling factors and DNA transcription, replication silencing and repair.

Next steps involve using the developed Cre/loxP system along with mutations affecting transformation efficiency such as those involved in DNA repair. This will allow us to gain a mechanistic understanding of chromosomal-exogenous DNA discrimination within the nucleus of *S. cerevisiae*.

Development of a high-throughput genome-wide method to assess Ty1 retrotransposon insertion upstream of tRNA genes in *Saccharomyces cerevisiae*

Rutuja Pattanshetti, Marjan Barazandeh, Corey Nislow, Vivien Measday

University of British Columbia, Canada

Presenter: Rutuja Pattanshetti

Transposable elements are DNA elements comprising repeated sequences that can change their location within a genome. Retrotransposons mobilize via RNA intermediates and this phenomenon, in humans, can cause diseases. In the S288c genome of *Saccharomyces cerevisiae*, Ty1 is the most abundant retrotransposon, present at ~32 copies. Ty1 typically integrates within a 1kb window upstream of genes that are transcribed by RNA Polymerase III, such as transfer RNA (tRNA) genes. Because the lifecycle and structure of retrotransposons resembles that of retroviruses, studying Ty1 in yeast will help better understand human retrotransposons and retroviruses.

Our aim is to develop and validate a high-throughput, genome-wide method to assess Ty1 insertion upstream of tRNA genes in *S. cerevisiae*. In a proof-of-principle study, a wildtype (BY4741) and a condensin mutant (*brn1-9*) strain were transformed with a Ty1-donor plasmid containing a Ty1 element under galactose-induced expression. PCR using primers that recognize a barcoded Ty1 sequence and a tRNA gene upstream sequence were used to identify and amplify Ty1 insertions. Using amplicon sequencing, we mapped the insertion patterns and compared frequencies of Ty1 insertion between the wildtype and the mutant.

Our sequencing results confirmed that the barcoded Ty1 inserted upstream of the targeted tRNA genes with higher insertion frequencies in the wildtype in comparison to the mutant. We are now attempting to multiplex our PCR reactions to interrogate multiple tRNA insertion events concurrently and testing different analytical methods to measure any qualitative and quantitative differences in Ty1 insertion frequency and position between wildtype and mutant strains.

Condensin is required for accurate targeting of the Ty1 retrotransposon in *Saccharomyces cerevisiae*

Mariah Lumpa, Hazel Cui, Stephanie Cheung, Vivien Measday

University of British Columbia, Canada

Presenter: Mariah Lumpa

Long terminal repeat (LTR) retrotransposons, such as Ty1 in *Saccharomyces cerevisiae*, are mobile elements that replicate via an RNA intermediate and integrate their complementary DNA (cDNA) into the genome via a retrotransposon encoded integrase (IN). Ty1 cDNA is targeted upstream of genes transcribed by RNA Polymerase III (RNA Pol III), such as tRNA genes, by Ty1-IN interacting with RNA Pol III. The condensin complex, that condenses chromatin into chromosomes, is enriched at tRNA genes and interacts with the RNA Pol III transcriptional machinery. The condensin subunits Smc2 and Smc4 form a hinge joint while Ycs4 and Ycg1 interact with Brn1 to bind DNA. Given that both Ty1-IN and condensin localize to tRNA genes and interact with RNA Pol III, we evaluated the effect of condensin on Ty1 targeting and mobility. Ty1 insertion patterns are altered in condensin mutants, most distinctly in *brn1-9* and *smc2-8* mutant strains. In the *brn1-9* mutant, no Ty1 insertion is observed upstream of *tGLY* genes. However, Ty1 mobility is 3.4-fold higher in the *brn1-9* mutant than in wild type (WT), indicating that Ty1 may be targeted to other locations in the genome in *brn1-9* strains. Additionally, Ty1 mobility in *smc2-8* is 1.9-fold higher than WT. Ty1 mRNA and cDNA levels are similar to WT in the *brn1-9* and *smc2-8* mutant strains indicating that defects in Ty1 insertion are not due to reduced Ty1 expression or reverse transcription. Next, we will test if condensin subunits interact with Ty1-IN to determine if condensin directly aids in Ty1 targeting.

ID: 302; Stand: C08

Understanding the regulation of Mms21 in genome stability

Cheung Li, Aleksandra Avramenko, Nkechinye Baadi, Yee Mon Thu

Colby College, USA

Presenter: Yee Mon Thu

Organisms have evolved multiple mechanisms to respond to DNA damage or chromosome instability. Protein sumoylation orchestrates many of these genome maintenance mechanisms. Sumoylation is mediated by E1, E2 and E3 ligases, which collectively facilitate the covalent linkage of small ubiquitin-like modifiers (SUMO) peptide to target proteins. Since sumoylation is functionally conserved from yeast to human, we use *Saccharomyces cerevisiae* as a model for genetic studies to elucidate molecular mechanisms of SUMO regulation. Specifically, we investigate how an E3 ligase Mms21 is regulated in response to DNA damage. Defects in Mms21's function or deviation from its normal expression have been reported in genetic diseases. We generated mms21D22 mutation, which lacks the last 22 amino acids of the protein but does not affect the residues in the E3 ligase domain. mms21D mutants exhibit negative genetic interaction with rad5 mutants but do not show any genetic interaction with slx4 or mms4 mutants. These data imply that the C-terminus of Mms21 regulates processes that act in parallel with the template switching pathway but act in tandem with a subset of homologous recombination pathway that utilizes structure specific endonucleases. Mutational analyses reveal that the effect of the C-terminus is not due to the SUMO interacting motif or the serine residues targeted by the check point. We are currently performing genetic interaction studies to gain insights into the functional significance of the C-terminus of Mms21.

Multifaceted mechanism of caffeine-mediated DNA damage responses in *Saccharomyces cerevisiae*

Ji Eun Choi^{1,2}, Woo-Hyun Chung^{1,2}

¹College of Pharmacy, Duksung Women's University, Korea, Republic of. ²Innovative Drug Center, Duksung Women's University, Korea, Republic of

Presenter: Ji Eun Choi

Caffeine is known to affect various physiological conditions such as energy metabolism, cell growth and proliferation. A genome-wide screening of genes required for caffeine tolerance in *Schizosaccharomyces pombe* revealed several candidates, including AP-1-like transcription factor, Pap1 and its downstream target genes particularly involved in caffeine efflux. I observed that a budding yeast AP-1 homolog, Yap1 required for oxidative stress resistance, has a caffeine tolerance function. Even though the *yap1* mutant cell is not sensitive to caffeine, overexpression of Yap1 makes cells resistant to high concentration of caffeine. Caffeine sensitivity shown in mutants lacking the two multidrug transporters, Sng2 or Pdr5, is completely recovered by overexpression of Yap1, suggesting that neither *SNQ2* nor *PDR5* are important downstream target genes of Yap1 activation in the presence of caffeine. Among the Yap1-dependent target genes, a fluconazole resistant gene, *FLR1*, is necessary but not sufficient for caffeine resistance. Low concentrations of hydrogen peroxide restore cell viability against caffeine toxicity. On the other hand, caffeine is involved in reduction of intracellular ROS level, mutation rate and Rad52 foci formation but not physically induced DSBs. Altogether, we identified a novel crosstalk between ROS signaling and caffeine resistance.

ID: 317; Stand: C10

Interplays between checkpoint kinases, nucleases and helicases in the DNA damage response

Michela Galli, Chiara Frigerio, Michela Clerici

Università degli Studi di Milano - Bicocca, Dipartimento di Biotecnologie e Bioscienze, Italy

Presenter: Michela Galli

DNA double strand breaks (DSBs) are the most cytotoxic lesions that threaten our genome and could lead to genome instability if they are not properly repaired. They are sensed by the protein kinase ATM (Tel1 in yeast), which orchestrates a complex genetic network that arrests the cell cycle through the activation of a checkpoint pathway and repairs the damage. Tel1/ATM represents a relevant target for cancer therapy because either germline or sporadic ATM mutations were identified in different tumors. To provide novel targetable proteins in anticancer therapy in combination with ATM mutations/inhibition, we searched for novel synthetic cytotoxic interactions with Tel1/ATM mutations in the budding yeast *Saccharomyces cerevisiae*. We performed a genomic screening searching on one hand for mutants that exacerbate the sensitivity to DNA damaging agents of cells lacking Tel1, and on the other hand for mutants that can suppress this sensitivity. Among the identified genes, we found nucleases and helicases involved in different DNA repair pathways, thus suggesting that the activity of these enzymes becomes crucial for the cells to survive to DNA damage in the absence of Tel1. We then investigated the molecular mechanisms underlying the identified genetic interactions by evaluating the capability of cells lacking Tel1 and the identified mutants to activate the DNA damage checkpoint pathway and the DNA repair mechanisms. Understanding the molecular mechanism underlying these genetic interactions could contribute to unravel novel Tel1/ATM interplays in the DNA damage response and to discover new therapeutic targets for anticancer treatments.

ID: 318; Stand: C11

Exploring the interconnections between DNA-RNA hybrids and the DNA damage response

Chiara Frigerio, Michela Galli, Michela Clerici, Diego Bonetti

Università degli Studi di Milano - Bicocca, Dipartimento di Biotecnologie e Bioscienze, Italy

Presenter: Chiara Frigerio

DNA-RNA hybrids are normally generated at highly transcribed regions, centromeres and telomeres and participate in a number of physiological processes such as transcription, immunoglobulin class switching and epigenetic modifications. When their homeostasis is perturbed, they can become source of DNA damage and genomic instability, a hallmark of cancer and other genetic diseases. How these structures affect genome stability is not completely understood. The aim of this project is to investigate how DNA-RNA hybrids can activate the cellular response to DNA damage and to study the interplays between DNA-RNA hybrids metabolism and the DNA damage response. We combine mutations in genes involved in DNA-RNA hybrids processing, as those encoding the Sen1/Senataxin helicase or the RNase H ribonucleases, with mutations in genes involved in the DNA damage response in *Saccharomyces cerevisiae*. This approach allowed us to identify both negative and positive genetic interactions between Sen1, RNase H and nucleases that operate in the DNA damage response. Since Senataxin and the DNA damage response genes are mutated in tumours and genetic diseases, understanding the molecular mechanisms at the basis of these genetic interactions could be useful for the identification of pathogenesis mechanisms and of new targets and strategies for therapies.

ID: 335; Stand: C12

The effects of near identical H2A and H2B genes on Ty1 retrotransposon insertion.

Jonah Lu, LeAnn Howe, Vivien Measday

University of British Columbia, Canada

Presenter: Jonah Lu

Nucleosomes are the preferred target for retroviral and retrotransposon insertion into a host genome, however the histone requirements are not well understood. The Ty1 retrotransposon in *Saccharomyces cerevisiae*, which targets upstream of RNA Polymerase III-transcribed genes, has been proposed to insert itself at the H2A/H2B interface of the nucleosome. In this study we examined the effects of the near identical genes coding for H2A and H2B on Ty1 insertion. In *S. cerevisiae*, H2A and H2B are encoded from bidirectional promoters at both the *HTB1-HTA1* or *HTA2-HTB2* loci. Despite their different regulatory elements, their gene products differ by only a few amino acids. We found that when *HTB1-HTA1* or *HTA2-HTB2* were deleted, Ty1 insertion was altered with the *HTA2-HTB2* deletion strains having a more pronounced effect on insertion site position. We hypothesize that the *HTB1-HTA1* and *HTA2-HTB2* loci have different roles for targeting Ty1 insertion. To test this, we created strains where all the H2A and H2B loci were deleted and inserted a plasmid carrying either *HTB1-HTA1* or *HTA2-HTB2* with and without the 3' untranslated region (UTRs). Using these strains, we will determine whether chromatin structure has been altered by measuring histone protein levels with mass spectrometry and observing any changes in nucleosome positioning using MNase-seq. We will also examine Ty1 retromobility in these strains using a GFP reporter assay and analyzing Ty1 insertion patterns upstream of tGLY genes.

ID: 8; Stand: C13

Mapping environmental suppression of gene essentiality

Nuria Bosch-Guiteras, Jessica Burnier, Elise Eray, Amandine Batté, Jolanda van Leeuwen

University of Lausanne, Switzerland

Presenter: Nuria Bosch-Guiteras

Genes are typically classified as essential based on the lack of viability of cells deleted for the gene under standard laboratory conditions. However, mutant phenotypes are often modified by environmental factors, suggesting that a proportion of the genes catalogued as essential could possibly be dispensable under different environmental conditions.

To systematically assess the fraction of environment-dependent essential genes, we screened deletion strains of 846 yeast genes (~80% of all essential genes in *Saccharomyces cerevisiae*) for viability in 21 environments targeting a variety of fundamental biological pathways and processes. Our results suggest that 1 to 10% of essential genes are not required for viability in a specific environment.

In general, more essential genes could be suppressed by non-drug environments, such as changes in osmolarity or temperature, than by addition of a drug, possibly because the former category can exert more general mechanisms of suppression than a drug that specifically targets a particular gene or pathway. For example, we found that various cytokinetic proteins were dispensable in the presence of salt, and that several proteins involved in RNA processing and translation initiation were not required for viability at elevated temperatures.

These results contribute to our understanding of how gene essentiality can vary between contexts as well as the underlying causes. This knowledge provides insight on the effect of environmental conditions on genetic traits and may benefit, for example, the design of synthetic minimal genomes and the development of specific treatments against pathogenic fungi that have little effect on closely related benign species.

ID: 13; Stand: C14

Characterizing yeast colony growth dynamics

Tünde Gaizer, János Juhász, Bíborka Pillér, Helga Szakadáti, Csaba I Pongor, [Attila Csikász-Nagy](#)

Pazmany Peter Catholic University, Hungary

Presenter: Attila Csikász-Nagy

Colonies built by natural isolate strains of the budding yeast, *Saccharomyces cerevisiae* show various morphologies. To understand the differences in the growth behaviour of these strains we study the quantitative features of yeast colonies in isolation or in mixed cultures. We investigate the limiting factors, which slow down colony growth and study how various strains interact with each other. We quantify the number of the cells, the density, the size, the shape and internal structure of the colonies, initiated from various inoculation scenarios to reveal the key factors affecting the proliferative capacity of yeast strains. This quantitative data is used to train an agent-based mathematical model that is capable of capturing growth differences of various yeast strains. We use the fitted models to design experiments which can distinguish between alternative scenarios explaining why colony growth slows down in time. Imaging of the internal structure of mixed colonies of two strains also help us to test how our models can capture local interactions inside colonies.

ID: 27; Stand: C15

Metabolic engineering of *Rhodotorula toruloides* for astaxanthin production using Golden Gate Assembly Platform

Inna Lipova, Srdjan Gavrilovic, Nemailla Bonturi, Petri-Jaan Lahtvee

Tallinn University of Technology, Estonia

Presenter: Inna Lipova

Astaxanthin is a red-colored keto-carotenoid widely used as a feed additive for aquaculture. Currently, majority of commercially available astaxanthin is produced using chemical synthesis. For development of circular economy, efforts have been directed to establish production of astaxanthin using microbial cell factories and upcycled substrates, such as biomass hydrolysates. *Rhodotorula toruloides* is a non-conventional yeast that naturally produces lipids and carotenoids growing on a wide range of carbon sources including lignocellulosic hydrolysates and pre-treated food waste. Diverse substrate consumption and tolerance to inhibitors make *R. toruloides* a suitable candidate for the metabolic engineering to obtain astaxanthin. Astaxanthin is produced from β -carotene in two oxidation steps. To obtain desired final product a biosynthetic pathway was designed which contains β -carotene ketolase (*crtW*) and β -carotene hydroxylase (*crtZ*) from *Haematococcus pluvialis*, *Pantoea ananatis* and, *Brevundimonas* sp. SD212. The Golden Gate Assembly Platform (RtGGA) with modifications and additional steps was used to construct cassettes that were randomly integrated in six different combinations (*bCrtW+bCrtZ*; *bCrtW+hpCrtZ*; *bCrtW+paCrtZ*; *hpCrtW+bCrtZ*; *hpCrtW+hpCrtZ*; *hpCrtW+paCrtZ*). All six constructs were successfully assembled in pGGA plasmid which was verified by sequencing and then randomly integrated in *R. toruloides* genome. Successful integration of astaxanthin pathway in *R. toruloides* was confirmed by PCR of extracted genomic DNA of transformants. The transformants were characterized in terms of growth, total carotenoids and astaxanthin detection. Obtained knowledge is currently used as an input for the design of strategies to transform *R. toruloides* into future potential astaxanthin producer.

Global analysis of genetic suppression of partial loss-of-function alleles

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Presenter: Sabine N.S. van Schie

For most genetic variants identified in genomes, their functional significance remains unclear. Predicting a variant's impact is complex, because it depends not only on the molecular consequences to the affected protein, but also on the genetic context. In some cases, the deleterious phenotype of a particular mutation can be rescued by concomitant mutations in suppressor genes. Previous studies of suppression predominantly mapped suppressors of deletion alleles. Here, we used a collection of temperature sensitive (TS) alleles of nearly all essential yeast genes to uncover principles of suppression of partial loss-of-function alleles. We could isolate suppressor strains for 89% of the essential genes in our collection, suggesting that nearly all deleterious point mutations can be suppressed. Whole-genome sequencing of the ~2500 isolated suppressor strains identified the mutations underlying the TS phenotype as well as candidate suppressor mutations. We find mostly (>90%) novel suppression interactions, highlighting new connections between genes. In addition to interactions among functionally related genes, 9% of the suppressors involved general suppression mechanisms that affect the expression or stability of the TS mutant mRNA or protein, and 21% of the suppressor mutations occurred in the TS allele itself, restoring protein function. Finally, we explored the genomic positions of the TS mutations to pinpoint suppression interactions that are specific to the mutation of a particular protein domain. This work is generating the most extensive global suppression network for any organism, identifying novel functional connections between genes, and improving our understanding of how mutations can interact to produce unexpected phenotypes.

Genome scale metabolic model of the hybrid lager yeast *S. pastorianus*

Soukaina Timouma¹, Laura-Natalia Balarezo-Cisneros¹, Jean-Marc Schwartz², Daniela Delneri¹

¹Manchester Institute of Biotechnology, United Kingdom. ²Division of Evolution and Genomic Sciences, United Kingdom

Presenter: Soukaina Timouma

Budding yeast species and their related hybrids are widely used in food and beverage fermentations, as well as in many biotechnological applications. Among these species, *S. pastorianus* is a natural hybrid that evolved from different hybridization events between *S. cerevisiae* and the cold-tolerant *S. eubayanus*. *S. pastorianus* strains are used in the lager-beer fermentation. The development of new hybrids with improved phenotypic traits is one of the priority of the beverage industry. Genome-scale metabolic models (GSMMs) have shown to be powerful tools to guide metabolic engineering experiments in different microorganisms. In this framework, we constructed a GSMM (*iSP_1513*) for the hybrid *S. pastorianus* CBS 1513. *iSP_1513* model takes into account the functional redundancy caused by the presence of orthologous parental alleles in the Gene-Protein-Reaction rules. It comprises 4062 reactions, 1808 alleles and 2747 metabolites. Essentiality datasets, metabolite secretion, as well as growth data on various carbohydrates, were used to validate this model. Flux Balance Analysis and Flux Variability Analysis have been used to predict growth in different environments. Reactions to favour/repress have been predicted to optimize the production of isoamyl acetate, a flavour compound that gives a banana/pear aroma to the beer. Other conditions, such as the effect of high and low temperatures on metabolite production, have been investigated by mapping transcriptome data. This GSMM represents an important step towards understanding the metabolic capabilities of natural yeast hybrids and will enable a predictive top-down approach to strain development processes alongside with the established bottom-up selection methods.

Exploring the effect of genetic background on suppression interactions in natural yeast isolates

Claire Paltenghi, Jolanda Van Leeuwen

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Presenter: Claire Paltenghi

Genetic suppressors are mutations that confer a fitness gain to a cell carrying a deleterious, or even lethal, mutation in another gene. Suppression interactions often identify previously unknown connections between functionally related genes, making suppressor mutations a powerful tool to study gene and pathway function. However, for *Saccharomyces cerevisiae*, most suppressor mutations have been described in the lab reference strain S288C, which could potentially limit the diversity of discovered suppressors since these could be specific to the genetic background in which they occur. To gain an understanding of the effect of the genetic background on the identified suppressors, spontaneous suppressor mutations in genetically diverse *S. cerevisiae* natural strains were isolated. To do this, temperature-sensitive alleles of the essential genes *TAO3*, *SEC17* and *GLN1* were introduced into three natural yeast isolates and suppressor colonies able to grow at the restrictive temperature were selected. We identified the genetic variants responsible for the suppression using whole-genome sequencing, validated their effect on fitness, and introduced the suppressors from the natural strains in the lab strain S288C to assess the specificity of the suppressor to its natural genetic background. All 10 tested suppression interactions were conserved across yeast strains, which suggests that the level of conservation of suppressor mechanisms across genetic backgrounds is high. As there are only a few fundamental ways of rewiring biological processes or pathways through genome alteration such that mutation of an essential gene can be suppressed, this may constrain the number of ways through which compensatory evolution can occur.

Endeavouring reconstitution of the human cGAS-STING pathway in yeast

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Presenter: Sara López-Montesino

The GMP-AMP cyclic synthase (cGAS) is a pathogen recognition receptor that senses cytosolic DNA associated with viral infection or cell damage. When DNA is detected, cGAS generates a cyclic dinucleotide second messenger, cGAMP, which stimulates the STING effector to activate the TBK1 protein kinase, necessary for downstream events leading to the eventual secretion of interferon and other inflammatory mediators. This pathway is essential for the control of infection, autoimmunity, and tumor immunity.

In this work we studied the effects of the heterologous expression of components of this pathway in *Saccharomyces cerevisiae* under the control of the inducible *GAL1* promoter. We developed fusions to fluorescent proteins to assess their subcellular localization. The expression and phosphorylation of TBK1 and STING proteins was tested by immunoblotting with specific antibodies, and non-phosphorylatable STING (S366A), TBK1 (S172A), as well as kinase-dead TBK1 (K38A) were constructed.

In yeast, cGAS-GFP was localized to cytosolic spots close to the endoplasmic reticulum and mitochondria, colocalizing with Mdm34, a component of the ERMES complex that serves as a molecular tether between these two organelles. By studying truncated versions, we observed that its C-terminal domain is necessary to form cytosolic aggregates whereas the N-terminal inhibit the nuclear cGAS localization. TBK1, but not its kinase-dead versions, was toxic for the yeast cell. STING co-localizes with TBK1 at the endoplasmic reticulum and is phosphorylated by this kinase independently of the presence of cGAS. These results suggest that the yeast model is useful to perform molecular studies on this important cell signaling pathway.

ID: 125; Stand: C20

Understanding the genetic networks and functional profile of ncRNAs in yeast

Tanda Qi, Laura Balarezo Cisneros, Soukaina Timouma, Marcin Fraczek, Raymond O'keefe, Daniela Delneri

The University of Manchester, United Kingdom

Presenter: Tanda Qi

Non-coding RNAs (ncRNAs) are being increasingly recognised as important regulators of cellular activity, prompting greater attention to be directed towards these pervasive transcripts. However, the majority of the studies were limited to only a few specific candidates, which highlighting the need for a comprehensive ncRNA profiling that could provide a systematic understanding of these “genomic dark matter”. By exploiting our in-house ncRNA deletion collection (400 mutants, encompassing SUTs, CUTs, snoRNAs and tRNAs), we aim to understand the connectivity of the ncRNA networks, the function of ncRNA in various conditions and the impact of environments on ncRNA regulation in a large-scale. A synthetic genetic array (SGA) study was performed to investigate ncRNA epistasis interactions by generating ncRNA-ncRNA double mutants between 5 query strains, previously shown to affect the transcription, and the ncRNA deletion collection. The double mutants were screened in five different conditions, namely YPD, YP+7% glycerol, YPD+ 4mM H₂O₂, YPD+1M NaCl, and SD in 30°C, as well as YPD in 37°C, to identify environmental specific genetic interactions. Moreover, we found that the deletion of one stable unannotated transcript, SUT480, has an impact on the mitochondria stability. This genetic screening coupled with transcriptome data sheds light on the potential regulatory function and plasticity of ncRNAs.

Expression of the human NLRP1 inflammasome sensor in the model yeast *Saccharomyces cerevisiae*.

Óscar Barbero-Úriz, Marta Valenti, Elba del Val, María Molina, Víctor Cid, Teresa Fernández-Acero

Universidad Complutense de Madrid, Spain

Presenter: Óscar Barbero-Úriz

In higher eukaryotes, inflammasomes are signaling complexes that activate innate immunity and regulate cell death pathways in response to aggressions. The nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein 1 (NLRP1) is an inflammasome-forming cytosolic sensor that initiates an inflammatory response in presence of certain pathogens. Its activation is caused by proteolytic cleavage exerted by bacterial or viral proteases (3Cpro and 3CLpro). Proteolysis results in the removal of the auto-inhibitory N-terminal PYD domain, which causes an N-degron-mediated degradation of the NACHT and LRR domains. Eventually, the C-terminal domains (FIIND-CARD) are self-proteolyzed (forming the UPA-CARD fragment), thus preventing their proteasome-mediated degradation and leading to oligomerization into the NLRP1 inflammasome.

In this work, we study different versions of NLRP1 using *Saccharomyces cerevisiae* as a platform. Yeast lacks a homologous pathway but confers a cellular environment to assess diverse aspects of NLRP1 function. We have individually overexpressed diverse domains of the NLRP1 protein. By expressing the two domains at the C-terminus fused to fluorescent proteins in yeast, we have observed that FIIND-CARD assembles thick aggregates whereas UPA-CARD displays a more diffuse pattern, partially coincident with yeast lipid droplets. Interestingly, both can recruit the co-expressed inflammasome adaptor, human ASC. We are co-expressing the NLRP1 PYD-NACHT domains with the SARS-CoV-2 proteases 3CLpro and PLpro and their catalytically inactive mutants (C146A and C114A respectively) to test whether these proteases can cleave NLRP1 in the yeast model. Our approach aims to provide novel humanized yeast-based tools to gain knowledge into the molecular aspects of inflammasome assembly.

The genomic landscape of autophagy nutrient response dynamics in yeast

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Presenter: Nathalia Chica

Autophagy is tightly regulated in response to environmental cues, leading to fluctuations that impact the balance between component synthesis and degradation, and maintaining cellular homeostasis. A systems-level approach can provide insights for understanding autophagy dynamics by considering the complex interplay between autophagy and other cellular processes, as well as the impact of changing environments. In this study, we used high-content imaging combined with deep learning to quantify influences on autophagy response kinetics in the yeast genome-wide KO library subjected to changing nutrient conditions. Using this repository of genomic profiles, we established a connection between the gene network architecture and the genome-wide tuning of autophagy. Our findings reveal how autophagy behaves under different combinations of genetic backgrounds and nutritional states, which may further our understanding autophagy regulation in other cellular systems and disorders.

ID: 156; Stand: C23

Combinatorial optimisation of biosynthetic pathway performance in yeast

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Presenter: Alisa Nira

We are developing broadly applicable tools designed to facilitate optimisation of biosynthetic pathway productivity in *Saccharomyces cerevisiae*. One test case under investigation is the bikaverin biosynthetic pathway. As is typically the case for polyketides, the natural host strain that normally produces bikaverin is less easily cultivated than yeast and generates a low yield of the product. This project combines two main strategies: first, the use of computational tools to optimise gene expression, pathway kinetics and metabolic burden; second, a novel combinatorial gene cluster assembly strategy that generates a landscape of alternative combinations of transcriptional and posttranscriptional expression control elements. Different pathway control scenarios are analysed using ordinary differential equation model of *S. cerevisiae* metabolism, gene expression and growth. Robotics facilitate exploration of broad landscapes of gene expression rates and ratios. Multi-objective optimisation routines are applied to identify trade-offs between growth and different bikaverin production metrics. Sensitivity analysis will reveal how differential use of transcription and translation resources can be expected to impact pathway performance and growth. Designs that are predicted to show optimum performance will be identified for further exploitation.

ID: 165; Stand: C24

Evaluating GPD1 gene regulation in yeast through synthetic biology tools and bioinformatic approaches

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Presenter: Camila Baeza

Alcoholic fermentation or 'glucose fermentation pathway' is a yeast metabolic process, by which glucose is converted into ethanol, carbon dioxide, and other by-products such as acetate and glycerol. The industrial importance of the glucose fermentation pathway has led to important efforts toward transcriptional control of genes responsible of its products yield. In this work, we combined optogenetics and CRISPRi (interference) technologies to achieve the transcriptional repression of the GPD1 gene. We used the FUN-LOVSP (single plasmid) optogenetic switch for light-activated expression of the catalytically inactive version of the Cas9 protein (dCas9), which was fused to the Ume6 transcriptional repressor domain (RD) from yeast. By using different guide RNAs (gRNAs), we directed dCas9-Ume6-RD to the GPD1 promoter, assessing its transcriptional repression through the luciferase reporter gene. The results showed that under constant blue light, the transcriptional activity of the GPD1 promoter was highly repressed by gRNA2. Afterwards, we analysed the GPD1 promoter looking for SNPs into a collection of 1011 *Saccharomyces cerevisiae* strains, identifying SNPs that correlate with Gpd1 protein expression levels in Synthetic Complete and Minimal media. Based on this information, five GPD1 promoter variants coming from the Malaysian, African beer, French Guiana, French Dairy, and Sake yeast clades were selected for transcriptional validation. In conclusion, synthetic biology and bioinformatics approaches enabled us to uncover the complex regulation controlling GPD1 gene expression.

ID: 170; Stand: C25

Easy Modular Integrative fuSion-ready Expression (Easy-MISE) Toolkit for Fast Engineering of Heterologous Productions in *Saccharomyces cerevisiae*

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Presenter: Letizia Maestroni

Nowadays, the yeast *Saccharomyces cerevisiae* is the platform of choice for demonstrating the proof of concept of the production of metabolites with a complex structure. However, introducing heterologous genes and rewiring the endogenous metabolism is still not standardized enough, affecting negatively the readiness-to-market of such metabolites. We developed the Easy Modular Integrative fuSion-ready Expression (Easy-MISE) toolkit, which is a novel combination of synthetic biology tools based on a single Golden Gate multiplasmid assembly meant to further ameliorate the rational predictability and flexibility of the process of yeast engineering. Thanks to an improved cloning screening strategy, double and independent transcription units are easily assembled and subsequently integrated into previously characterized loci. Moreover, the devices can be tagged for localization. This design allows for a higher degree of modularity and increases the flexibility of the engineering strategy. We show with a case study how the developed toolkit accelerates the construction and the analysis of the intermediate and the final engineered yeast strains, leaving space to better characterize the heterologous biosynthetic pathway in the final host and, overall, to improve the fermentation performances. Different *S. cerevisiae* strains were built harboring different versions of the biochemical pathway toward glucobrassicin (GLB) production, an indolyl-methyl glucosinolate. In the end, we could demonstrate that in the tested conditions the best-producing strain leads to a final concentration of GLB of 9.80 ± 0.267 mg/L, a titer 10-fold higher than the best result previously reported in the literature.

Unraveling the biology of BRAFV600E isoforms by a genome-wide screening in yeast

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Presenter: Tiziana Cervelli

BRAF is a Ser-Thr protein kinase belonging to the highly oncogenic RAS/RAF/MEK/ERK signaling pathway. About 50% of melanomas have the BRAFV600E mutation conferring constitutive monomeric activation of BRAF kinase activity, so that an uncontrolled increase in cell proliferation is observed. Two BRAFV600E protein isoforms, ref and X1, coexist in melanoma cells, but their specificities are unknown. Through the expression of the two isoforms, one by one, in yeast we are deciphering their specificity.

We expressed BRAFV600E-ref and X1 separately in a collection of yeast strains deleted for non-essential genes, evaluated the fitness alteration of each deleted yeast strain and compared the results with appropriate controls to assess differences. An interaction network and gene enrichment analysis of the isoform-specific functional interactors highlighted BRAFV600E-ref as specifically involved in autophagy, while BRAFV600E-X1 in *de novo* fatty acid (FA) synthesis. Further investigations in yeast highlighted that the expression of BRAFV600E-X1 determines a stronger accumulation of LDs than BRAFV600E-ref. Besides, we demonstrated that such an accumulation is prevented when yeast cells are treated with cerulenin (inhibitor of FA synthetase), or are deleted for Elo1/2 elongases of FA, suggesting that it is caused by increased *de novo* FA synthesis. On the other hand, the expression of BRAFV600E-ref determines a stronger increase in autophagy than the expression of BRAFV600E-X1. Interestingly, the overexpression of BRAFV600E-ref/X1 in HeLa cells confirmed their specificity of action on autophagy and *de novo* FA synthesis, respectively.

ID: 188; Stand: C27

pCEC-red: an easy-to-use vector for CRISPR/Cas9 genome editing in *Saccharomyces cerevisiae*

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Presenter: Pietro Butti

CRISPR/Cas9 is currently the gold standard for genome editing in *Saccharomyces cerevisiae*. Regrettably, plasmid-based systems that allow the easy insertion of user-defined gRNAs are still lacking. The standard techniques are usually based on restriction and ligation or on PCR amplification of the whole plasmid backbone. The latter method frequently results in an unpractical two-plasmids system, in order to avoid the amplification of the long Cas9 cassette. Additionally, many traditional systems rely on auxotrophic markers, which cannot be exploited in industry-ready prototrophic strains.

We present pCEC-red, an easy-to-use single-plasmid CRISPR/Cas9 system in which the gRNA is inserted by Golden Gate Assembly, with an immediate red/white screening and assembly efficiencies of about 100%. The plasmid bears a dominant marker, to overcome strain background limitations, and allows precision editing with high efficiency. The total turnover time from parts assembly to the engineered strain is about two weeks.

The system was tested using the standard *ADE2* deletion assay, which showed an efficiency of about 90% for both precision editing and large DNA region replacement. Additionally, a proof of concept of how the pCEC system can be applied to microbial cell factories development will be presented.

Pathway design for mixotrophic production of biochemicals from CO₂ and methanol in yeasts

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Presenter: Golnaz Memari

Autotrophy and mixotrophy in industrial strains have the potential to contribute to the mitigation of climate change, and they are therefore of a great interest towards a more sustainable production of biochemicals.

In previous studies, functional autotrophy was engineered in yeasts by integrating the Calvin-Benson-Bessham (CBB) cycle in *Pichia pastoris* (*Komagataella phaffii*) (Gassler et al. 2020), and similarly in *Escherichia coli* (Gleizer et al. 2019), allowing growth on CO₂ as the sole carbon source. The autotrophic *P. pastoris* strain uses methanol for energy supply. In this project, we aim to design alternative pathways for assimilation of both CO₂ and methanol for the conversion of CO₂ into value-added compounds. Using methanol in both assimilatory and dissimilatory pathways lowers the overall methanol demand. Blueprints for such a mixotrophic pathway are the bacterial serine or serine-threonine cycles. Implementation of such a complex pathway in the metabolism of *K. phaffii*, requires a substantial rerouting of central metabolic fluxes to maintain growth.

By using methods for synthetic biology such as Golden Gate Assembly and CRISPR-Cas9, we are aiming to design thermodynamically feasible and energetically favorable pathways for the production of organic acids from a mixed feed of methanol and CO₂. For this purpose, additional enzymatic loops are added to the serine cycle by either introducing bacterial genes or activating native yeast pathways. Functionality of the novel cyclic pathways is assessed by the capacity to produce the target molecules and intermediates from methanol and CO₂.

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ID: 254; Stand: C31

Development of a yeast platform for expression and optimization of PET plastic degrading enzymes

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Presenter: Raphael Loll-Krippleber

Over the past 70 years since the introduction of plastic into everyday items, plastic waste has become an increasing problem. With over 400 million tonnes of plastics produced every year, solutions for plastic recycling and plastic waste reduction are sorely needed. Recently, multiple enzymes capable of degrading PET (Poly Ethylene Terephthalate) plastic have been identified and engineered. In particular, the enzymes PETase and MHETase from *Ideonella sakaiensis*, have been shown to allow depolymerization of PET into the two precursors used for its synthesis, ethylene glycol (EG) and terephthalic acid (TPA). Importantly, EG and TPA can be re-used for PET synthesis allowing complete and sustainable PET recycling. We have recently established a platform using *Saccharomyces cerevisiae* to develop a whole-cell catalyst expressing the MHETase enzyme, which converts MHET (monohydroxyethyl terephthalate) into TPA and EG, on the cell surface (PMID: 36587193). Using a similar strategy, we have now assessed multiple construct architectures for efficient expression of PETase which acts upstream of MHETase and convert PET into MHET. Using a newly developed high-throughput plate assay that allows quantitative measurement of enzyme activity, we demonstrate that despite differences between construct architectures, all PETase constructs are active against a model substrate. Importantly, expression of a published improved variant of PETase (FAST-PETase) increases activity of all PETase constructs as well, demonstrating that our yeast platform can be used to optimize PET-degrading enzyme expression and to test enzyme variants with improved activity.

Sequence and chromatin features guide resection initiation at DNA double-strand breaks

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Speaker: Robert Gnügge

DNA double-strand breaks (DSBs) are common genomic lesions that must be accurately and efficiently repaired to ensure genome stability and organism survival. One major DSB repair pathway, called homologous recombination (HR), relies on the nucleolytic degradation of DSB ends in a process termed end resection. The Mre11-Rad50-Xrs2 (MRX) complex initiates end resection by nicking the 5'-terminated DNA strands internal to DSBs. Previous studies established how cofactors and protein blocks at DSBs stimulate MRX nicking *in vitro*. However, it is unknown how MRX cleavage is influenced by the sequence and chromatin landscape surrounding a DSB *in vivo*. Here we show that both DNA sequence and chromatin features impact on MRX nicking. We mapped MRX nicks quantitatively and at single-nucleotide resolution next to multiple defined DSBs in the yeast genome. Consistent with previous *in vitro* assays, we observed that the Ku70-Ku80 complex directed DSB-proximal nicks. Repetitive MRX nicking then extended the resection tracts. MRX cleavage was enhanced in linker DNA between nucleosomes and reduced in highly transcribed regions. Consistently, MRX nicking was influenced by chromatin remodelers that are involved in nucleosome dynamics and promoter regulation. Additionally, we observed that MRX preferentially cleaved DNA containing a specific sequence motif and a related DNA meltability and bending profile, and these findings are consistent with recent structural studies. Our results provide fundamental mechanistic insights into the initial step of DSB repair by HR.

Grafting the Ribulose Monophosphate Pathway into *Komagataella phaffii* for Increasing Energy Efficiency on Methanol

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Presenter: Miriam Kuzman

Industrial biotechnology offers great potential to mitigate CO₂ when based on sustainable and renewable resources. Here, one-carbon (C₁) substrates offer a sustainable opportunity for a carbon-neutral and circular bioeconomy. Methanol can be directly produced from greenhouse gases such as methane and carbon dioxide. Due to this, the ability of microorganisms to utilize C₁ substrates is gaining increasing attention.

Komagataella phaffii is an industrial production host mainly used for heterologous protein production, and more recently, also for non-protein products. The aerobic methylotrophic yeast can utilize methanol as sole carbon and energy source. This constitutes a great chance for its establishment within a methanol-based bioeconomy. However, the natural methanol assimilation pathway of the yeast, the xylulose monophosphate (XuMP) cycle, has rather poor energy efficiency. Optimization towards a better biomass yield on methanol is desirable to enable efficient and cheap production processes.

In this study we aim to metabolically engineer *K. phaffii* towards a more energy efficient methanol assimilation, utilizing the ribulose monophosphate (RuMP) cycle from *Bacillus methanolicus*. We were able to re-engineer the yeast with a peroxisomal heterologous RuMP cycle. The resulting strain was able to grow on methanol as sole carbon and energy source. Although the strain's biomass yield on methanol was still inferior compared to the wild type, this proof of principle study sets the first-generation *K. phaffii* RuMPi strain. By further metabolic engineering and adaptive lab evolution, the resulting next generation strain can give new momentum for the use of methanol as alternative, carbon-neutral feedstock in biotechnological production processes.

Yeast as a model to study biological noise in autophagy

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Presenter: Karolina Spustova

Biological noise is defined as a cell-to-cell variation that is observed in populations of genetically identical cells. Cellular processes such as transcription and translation are intrinsically stochastic. Furthermore, changes in the availability of nutrients, as glucose or amino acids, can lead to fluctuations in cellular metabolism and gene expression. These fluctuations can impact both cell commitment and fate, ultimately affecting cellular fitness.

Autophagy is a critical process for maintaining cellular homeostasis regulated in response to changing nutrient conditions, suggesting that autophagy may play a role in minimizing the effects of biological noise within cells and in reducing variability in cell populations. However, noise can also affect autophagy commitment by causing fluctuations in gene expression or protein levels, resulting in unpredictable and suboptimal autophagic response and cellular dysfunction. Therefore, understanding the interplay between biological noise and autophagy is essential for gaining insights into the mechanisms that underlie autophagy response and cellular homeostasis.

This study aims to explore the impact of biological noise on autophagy and identify the regulatory pathways that govern autophagy commitment and noise suppression in response to changing nutrient conditions in budding yeast. To investigate the relationship between gene functions and biological noise more comprehensively, we are constructing a toolbox of quantitative fluorescent reporters to measure noise at various regulatory levels of autophagy control. By studying the impact of noise on autophagy, we hope to gain systems-level understanding of mechanisms that mitigate the effects of biological noise in autophagy commitment and regulate optimal levels of activity in cells.

ID: 341; Stand: C35

Development of a yeast cell factory aiming the conversion of agro-industrial residues into malic acid

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Presenter: Davi de Castro Gomes

There is a worldwide consensus on the need to use alternative sources of energy to replace those of fossil origin. Organic acids, like lactic and malic acids (MA), are molecules with great commercial potential, particularly with an industrial application as precursors of biopolymers and other biocompounds. Microorganisms can have their metabolisms redesigned using synthetic biology tools to become high-producers platforms. In this context, the project aims to develop an industrial strain of *Saccharomyces cerevisiae* capable of producing high titers of malic acid, using C5 and C6 sugars from lignocellulosic biomass. The malate synthetic pathway relies on fine-tuned expression of PYC2, a mutant version of MDH3 Δ SKL, and heterologous expression of SpMAE1 transporter. The expression cassettes with the metabolic pathway were stably integrated into the genome of an industrial xylose-fermenting strain using a CRISPR-Cas9 system. Two variants of the malate transporter were tested and the deubiquitinated version of SpMAE1 transporter was shown to have a higher stability at yeast cell membrane after 16h of fermentation, which will promote higher yields of malate export. To increase the titer levels of MA, we are developing a biosensor based on the 2-component regulatory system of *Bacillus licheniformis*. Yeast synthetic promoters with insertion of binding sites for the transcription factor MalR are being evaluated. The biosensor will be associated with a promoter expression library carrying the MA pathway to screen for high-producer cells. The data obtained by the approaches would result in an innovative and efficient system for production and sensing of malic acid in yeast.

Optimizing expression of the therapeutic protein Irisin from probiotic yeast *Saccharomyces boulardii*

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Presenter: Saumya Mathur

Inflammatory bowel diseases, chronic disorders which cause inflammation in the intestine, have few effective treatment options, since current options mostly depend on indirect administration of the drug. Because the intestine is a natural environment for a large and diverse group of microbes, we can use genetic engineering tools derived from synthetic biology to genetically engineer microbes into a probiotic that can deliver a therapeutic peptide directly at the site of inflammation. Irisin, a hormone-like peptide derived from a cleaved product of the FNDC5 gene in mammals, is a leading candidate for such therapeutic purposes. Through optimization of protein expression, I have genetically engineered a probiotic yeast strain – *Saccharomyces boulardii*, a sub-strain of *Saccharomyces cerevisiae* – to express irisin using an oxidative stress-inducible promoter, as well as a strong constitutive promoter. Reactive oxygen species, or ROS, can be used to induce this synthetic promoter at non-cytotoxic concentrations, as this is a common yet interestingly underrated biomarker of inflammation. Current results show that using constitutive promoters, in combination with a C-terminal 12X-His tag for protein purification and an N-terminal TRX2 solubility tag, can improve recombinant protein expression in *S. boulardii*. Upscaling this production and purification produces sufficient quantities of fusion protein to perform an ROS assay and elucidate the anti-inflammatory and anti-oxidant properties of irisin. The results of this work are presented here, along with implications for future goals stemming from this project.

Development of a High throughput robust yeast-based assays towards the identification of human ChaC1 inhibitors

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Presenter: Shradha Suyal

Human ChaC1 enzyme degrades cytosolic glutathione and its inactivation in adult cells enhances cellular GSH. As it is highly upregulated in several cancers, inhibiting ChaC1 becomes a novel means for elevating glutathione levels during stressed conditions. We are therefore interested in identifying potential inhibitors of the hChaC1 enzyme. Toward this goal, we created a “humanized yeast” by expressing the hChaC1 protein in yeast strains and developed two different assays for identifying ChaC1 inhibitors. In one assay, inhibition of ChaC1 prevents growth, and in another assay, inhibition allows the growth of yeast cells. The expression of ChaC1 was optimized under promoters of different strengths and the yeast strains were sensitized against drug efflux by deletion of the pleiotropic drug efflux pump, Pdr5. The assays developed were robust, with Z' scores of >0.9, which were scaled down to a 96-well format for High throughput screening. A small molecule compound library was screened using an automated liquid handling system. Parallely, in an in silico approach, the 3D structure of hChaC1 was predicted using homology modeling. Its active site map was predicted through substrate docking & MD simulations, which were further validated using mutagenesis studies in yeast. The major binding residues include 38'YGSL'41, D68, R72, E115, and Y143. Subsequently, a systemic pharmacophore-based virtual screening was performed. The hits were further narrowed down based on their interactions with the catalytic site residues. Experimental evaluation of the top hits from both approaches is underway for the development of novel inhibitor-based therapeutics against the ChaC1 enzyme.

Surface displayed BRD of SARS-CoV-2 in the yeasts as efficient inexpensive peroral vaccine against COVID-19

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Presenter: Kostyantyn Dmytruk

The emergence of the coronavirus disease 2019 (COVID-19) as a global pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has underscored the critical need to prioritize the development of safe and effective vaccines. Numerous vaccine candidates are currently being developed, with many already in use. Even though the pandemic has essentially ended, there is still an urgent need to develop affordable and efficient platforms to effectively address potential future challenges.

We generated *Komagataella phaffii* and *Saccharomyces boulardii*-based SARS-CoV-2 vaccine. The full-length receptor binding domain (RBD) of the spike protein of SARS-CoV-2 was expressed on the surface of both yeast species. Expression of RBD in *K. phaffii* was carried out under control of the homologous MOX promoter. Strong constitutive *Saccharomyces cerevisiae* TEF1 promoter was used to facilitate the expression of RBD in *Saccharomyces boulardii*. The Sag1-anchoring domain was used to construct surface-engineered yeasts. Western blotting and immunofluorescence assays were performed to confirm the location of RBD expression. Mice vaccinated orally with both types yeast-based vaccines produce significant humoral immune responses. The serum of immunized animals was assessed by a neutralization test (SARS-CoV-2 Neutralizing Ab ELISA Kit #BMS2326). The level of neutralizing antibodies reached 93% for animals vaccinated by *K. phaffii* surface displayed BRD cells.

Thus it was demonstrated that the surface display systems of *K. phaffii* and *S. boulardii* have the potential to function as a universal technology platform and can be utilized to create other types of oral viral vaccines.

Towards measuring the function of all possible missense variants in human porphyria genes using yeast

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Presenter: Warren van Loggerenberg

The fact that every possible single nucleotide change in the human genome is already carried by someone alive today supports the idea that the functionality of variants should be tested proactively, for all variants. Yeast offers a tractable and faithful platform for multiplexed variant function assays, yielding comprehensive 'variant effect maps' for target genes. Here we describe variant effect maps for the human acute hepatic porphyria genes HMBS and CPOX genes and, for CPOX, demonstrate use of yeast as a model of the impact of human heavy metal exposure on variant effects. We also describe resulting sequence-structure-function insights and the impact of HMBS and CPOX variant effect maps on clinical variant interpretation.

Identifying and assessing causative variants at single-gene resolution for salt tolerance in the yeast *Saccharomyces cerevisiae*

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Presenter: Gašper Žun

For genetic dissection of quantitative traits, sporulation of heterozygous cells of the yeast *Saccharomyces cerevisiae* is a powerful tool as it enables to generate numerous genotypically and phenotypically different haploid segregants. To determine quantitative trait loci (QTLs) of NaCl tolerance, two parental haploid strains were crossed. A portion of formed segregants was robotically replica plated onto agar plates with increasing NaCl concentration. High-throughput phenotyping provided not only the quantitative data of salt tolerance of individual segregants, but also the population distribution of the trait.

To decrease the size of QTLs, firstly, numerous segregants were analysed. Secondly, the causative loci were reduced by inbreeding to increase meiotic events within the loci intervals. Namely, the most salt tolerant segregant of each progeny was iteratively back-crossed with each of the parental strains, creating two lineages, in which genetic elements of the opposite parental strain were diluted. Whole genome sequencing of the individual winning segregants from each generation and lineage as well as the pools of segregants under extreme salt conditions allowed us to perform the QTL mapping and extract the causative variants.

Finally, alleles of the potential causative genes (QTGs) within the QTLs were swapped in otherwise isogenic strain backgrounds using the CRISPR-Cas9 approach. The putative QTGs were evaluated by phenotyping the allele-swapped strains. As will be presented, by generation-dependent reduction of the causative loci and allele swaps we dissected the complex trait down to the QTGs, assessed the contribution to the variation and associated their function to the molecular process.

Spontaneously established syntrophic yeast communities improve bioproduction

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Presenter: Simran Kaur Aulakh

Nutritional co-dependence (syntrophy) is observed in natural microbial communities and has underexplored potential to improve biotechnological processes by using cooperating cell types. So far, design of yeast syntrophic communities has required extensive genetic manipulation, as the co-inoculation of most eukaryotic microbial auxotrophs does not result in cooperative growth. Here we employ high throughput phenotypic screening to systematically test pairwise combinations of auxotrophic *S. cerevisiae* deletion mutants from a genome-scale knock-out library. While most auxotrophic co-culture pairs indeed do not enter syntrophic growth, we identify 49 auxotroph pairs which defy the general rule and spontaneously form syntrophic, synergistic communities. We then characterise the stability of nine selected co-cultures and show that initial inoculation ratios of co-cultured populations significantly influence growth dynamics. We demonstrate that a pair of tryptophan auxotrophs grow by exchanging a pathway intermediate rather than the end product indicating that the metabolic space for metabolic interactions is larger than currently assumed. Exploiting this observation, we introduced a malonic semialdehyde biosynthesis pathway split between different pairs of auxotrophs, including the tryptophan auxotrophs, and obtained a 6-fold increase in relative production titer of a biotechnologically relevant compound. Our results report the spontaneous formation of stable syntrophy in a specific subset of *S. cerevisiae* auxotrophs and illustrate the biotechnological potential of dividing labour between a naturally cooperating intraspecies community.

ID: 202; Stand: C43

A modular cloning (MoClo) toolkit expansion for optimization of heterologous protein expression and secretion in *Saccharomyces cerevisiae*.

Nicola O'Riordan, Paul Young

University College Cork, Ireland

Presenter: Nicola O'Riordan

Saccharomyces cerevisiae is an attractive host for heterologous expression of certain categories of secreted proteins due to its GRAS ("generally recognized as safe") status and widespread use in the food, beverage, and industrial biotechnology sectors. Unfortunately, many heterologous proteins fail to enter, or efficiently progress through, the secretory pathway, resulting in poor yields. Secretion signal peptides (SSPs) and translational fusion partners (TFPs) can be used to direct foreign proteins towards the secretory pathways in *S. cerevisiae*, using either post- or co-translational translocation to the endoplasmic reticulum. Modular cloning (MoClo) utilises Type IIS restriction enzymes to facilitate the efficient and seamless incorporation of designated 'part' types such as promoters, protein coding sequences, and terminators into expression vectors. We have expanded the yeast MoClo toolkit to enable the efficient incorporation of a panel of fourteen well-characterised SSPs and TFPs into *S. cerevisiae* expression cassettes. The system was validated using three different difficult to express and/or secrete proteins of interest. The intracellular and secreted protein levels were compared and the optimal SSPs and TFPs for each individual protein was determined. Large, protein of interest specific variations in overall expression levels, as well as secretion efficiency were observed. These observations highlight the potential value of the panel of MoClo compatible parts described to allow facile screening of SSPs and TFPs for optimal secretion of a given protein in *S. cerevisiae*. This offers promise for the industrial production of multiple heterologous proteins with potential applications in the food, and biopharmaceutical industries.

EasyGuide expanded: using yeast in vivo cloning for CRISPR/Cas9 applications in *Saccharomyces cerevisiae* and *Escherichia coli*

Joneclei Barreto¹, Maria Cecília Lelis¹, Lucas de Bem^{2,1}, Ana Paula Jacobus^{1,2,3}, Jonas Contiero¹, Jeferson Gross¹

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Presenter: Joneclei Barreto

Saccharomyces cerevisiae has a great capacity to assemble DNA fragments having homology arms as short as 20 bp. Recently, we have described the EasyGuide approach that uses the power of yeast in vivo recombination to support CRISPR/Cas9 applications in *S. cerevisiae* (ACS Synth. Biol. 2022, 11, 11, 3886–3891). Now we present a new streamlined version of the EasyGuide plasmids (pEasyG4) as an efficient system not only for yeast genome editing, but also as a versatile platform for in vivo multipart-DNA assembly. Based on the minimized pEasyG4 vector backbone, we have developed a strategy to link DNA fragments in any desired conformation to assemble “donors” used for genomic modifications by CRISPR/Cas9 in both *E. coli* and *S. cerevisiae*. These assembled fragments can be easily amplified by PCR from yeast DNA preps and promptly transformed into *S. cerevisiae*, or purified and used in *E. coli* CRISPR/Cas9 experiments. For *E. coli*, we have been making insertions, deletions and modifications of individual base pairs in the genome; in addition to this, metabolic engineering for the production of polyhydroxybutyrate by *E. coli* using this strategy is being developed with the help of the EasyGuide yeast cloning platform. By applying the EasyGuide principle to *E. coli*, we have established a system for in vivo cloning of gRNAs into the bacterial cell. The EasyGuide system is now being adapted to support the assembly of gRNAs and donors from simple oligonucleotides, greatly facilitating CRISPR/Cas9 genome editing procedures for *S. cerevisiae* and *E. coli*.

Debugging S288C through ReMaSSing: mapping defective alleles and selecting high-fitness recombinants in different environments

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Presenter: Lucas de Bem

The *Saccharomyces cerevisiae* S288C is a widely used lab-model that often provides a weak parental strain for QTL mapping. In addition to its inherent stress sensitivity, the lab strain can accumulate mutations through bottlenecks of colony propagations. To map these variations, we crossed the S288C with the stress-tolerant and fast-growing PE-2_H4 (Brazilian bioethanol). During germination, a pool of haploids was selected using an antibiotic resistance construct (*pSTE3-Marker*) expressed only in *MAT α* cells. Millions of haploid recombinants were selected over 12 generations for fitness growth in optimal medium, followed by backcrossing with S288C, sporulation, germination, and haploidy selection preceding a new propagation. After five cycles of Reiterated Mass Selection and Backcrossing (ReMaSSing), we identified defective S288C alleles related to *HTA1*, *HAP1*, *PHO84*, and *MKT1*. Interestingly, when following selection in lignocellulosic hydrolysate (LCH), we found the same pool of QTL, and newly mapped *VPS70*^{P199L} and an unresolved QTL on chromosome XV. Using CRISPR/Cas9, we replaced the S288C alleles with those from the PE-2_H4 and confirmed the increased fitness in YPD 2% conferred by alleles *MKT1*^{D30G}, *PHO84*^{L259P}, and the *Ty* deletion from the *HAP1* 3' region. Remarkably, most of these QTLs were identified in previous studies, but *HTA1* has a defect exclusive to our laboratory S288C strain. Additionally, we confirmed that *VPS70*^{P199L} is LCH-tolerant. This demonstrates that ReMaSSing allows the identification of defective SNPs related to specific conditions. Accordingly, a S288C strain "debugged" by reverse engineering all five identified QTL, outperforms the strong parental strain PE-2_H4 in both YPD 2% and 30% LCH.

ID: 266; Stand: C46

Yeast strain optimisation by randomised combinatorial overexpression of auxiliary genes

Sebastian Ro Toft Hansen, Asser Sloth Andersen

Novo Nordisk A/S, Denmark

Presenter: Sebastian Ro Toft Hansen

The yeast *Saccharomyces cerevisiae* is very well characterised and is often used as a host for recombinant production of secreted proteins. In any production process, a bottleneck will inevitably occur that limits the hosts ability to produce the product of interest. For recombinant production of secreted proteins, the bottleneck may appear in any of the steps from transcription through exocytosis of the folded protein. Increasing the concentration of proteins involved in the limiting step by overexpression of the encoding genes is a proven strategy to alleviate bottlenecks. However, it is often unknown which cellular process is limiting, and thus, a rational approach often requires a large amount of research to identify the bottleneck.

Here, a screening strategy is presented that uses randomised combinatorial overexpression of auxiliary genes encoding proteins involved in relevant cellular processes, with the goal of improving recombinant secreted protein production. In a single transformation reaction, DNA fragment libraries of promoters and gene ORFs are recombined into expression cassettes by in vivo recombination (IVR) - these cassettes are themselves recombined to create a randomised DNA substrate to repair a DNA double-stranded break created using a CRISPR/MAD7 nuclease. IVR-based assembly of the integration substrate is facilitated by a set of universal homology arms that enables randomised assembly. When a diverse pool of randomised yeast strains has been generated, overproducers of recombinant proteins can be isolated using single-cell analysis of candidate cells, either using microfluidics or yeast surface display methodologies, the latter of which is utilised here.

Natural nanoparticles from yeast: the promise for facilitated drug delivery

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Presenter: Saulius Serva

Viruses are inherently organized supramolecular structures, designed by nature for viral genome packaging and delivery. Instances of virion application in biomedicine have been substantiated, however safe and multipurpose delivery systems are particularly missing. The yeast is highly relevant for human research, further enabled by granting of *Saccharomyces cerevisiae* a GRAS (Generally Regarded As Safe) status. The intrinsic benign yeast dsRNA viruses are uninjective and exclusively intracellular. Deep integration into the cellular metabolism presumes virus significance for host survival in diverse ecosystems, including the human body environment. *S. cerevisiae* L-A virions are genuine packing system of viral genome: the sole capsid protein associates into 60 asymmetric dimers, forming a relatively loosely packed yet firmly structured 39 nm diameter particle. The expression of capsid protein in virus-naive host leads to spontaneous formation of genome-free virus-like particles (VLPs), ready for complexation of cargo molecules. We cloned and sequenced all to date known *S. cerevisiae* - and many alike - dsRNA viruses from natural yeast. Gene engineering of both outer and inner shells of capsid has been demonstrated to be compatible with virion structure, conferring the loading and targeting options for a particle. The proof-of-principle of cargo transfer into mammalian cells was demonstrated by assembling of VLPs carrying external nucleic acids and peptides, confirming the perspectives of the different payload. The bioengineering of capsids is performed to prepare versatile nanoscaffolds with precise addressability option.

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Engineered *Saccharomyces cerevisiae* for *de novo* biosynthesis of curcumin

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¹Centre of Biological Engineering, Portugal. ²LABBELS –Associate Laboratory, Portugal

Presenter: João Rainha

Extensive research has been conducted on curcumin, a polyphenolic compound found in turmeric (*Curcuma longa*), due to its potential as a natural cancer-fighting drug. However, curcumin exhibits limited accumulation levels in the plant over an extended period, and its extraction is costly and ineffective. Curcumin biosynthesis involves the phenylpropanoid pathway, which converts aromatic amino acids into ferulic acid, its precursor. Subsequently, 4-coumarate-CoA ligase and type III polyketide synthases (PKSs) convert ferulic acid into curcumin using malonyl-CoA as an extender substrate. Here, we present the pioneering development of a genetically modified *Saccharomyces cerevisiae* strain able to produce curcumin from glucose. We used CRISPR-Cas9 to genome integrate the curcumin biosynthetic pathway. Initially, curcumin synthesis from supplemented ferulic acid was evaluated. We tested different enzyme combinations and the highest curcumin levels were obtained using a bacterial feruloyl-CoA synthetase and type III PKSs from *C. longa*. Subsequently, we employed *p*-coumaric acid-overproducing *S. cerevisiae* strain, enabling the expression of the enzymes responsible for the conversion of *p*-coumaric into ferulic acid. These enzymes included caffeic acid O-methyltransferase from *Arabidopsis thaliana* and two components of the bacterial enzyme 4-hydroxyphenylacetate 3-hydroxylase (HpaB/C). After confirming the successful biosynthesis of ferulic acid, the remaining part of the pathway required for curcumin synthesis was expressed in this strain. The resulting strain was able of produce curcumin from glucose. Next, to reduce *p*-coumaric acid accumulation, an additional copy of HpaB/C was integrated. As a result, the final strain exhibited a significant improvement, yielding a curcumin titer of 2.1 mg/L.

An integrated system approach to redesign industrial yeasts as microbial cell factories

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Presenter: Leandro Vieira dos Santos

The increasing climate concerns guided the development of environmentally friendly technologies towards a more sustainable development in the world. In this context, microbial cellular metabolisms can be redesigned, creating efficient platforms to produce biofuels and biochemicals from lignocellulosic biomass. However, several limitations still need to be addressed to produce a robust microbial platform for future biorefineries. The goal of this project was to design an integrated system approach to: i. rewiring microbial metabolism and investigate the signaling network that regulates xylose/arabinose (C5) fermentation in *Saccharomyces cerevisiae*; ii. engineering membrane transporter proteins for improved xylose uptake and alleviated catabolic repression for C5/C6 (glucose) co-fermentation; iii. investigate cellular effects against multiple lignocellulosic inhibitors and engineer more tolerant cells; iv. design synthetic biology tools to improve production of biochemicals. As major results, a genomic atlas was produced with a set of 98 mutations connected to xylose and arabinose fermentation and will be used to elucidate the genetic basis of C5 metabolism in yeast. Epistatic combinations which boost C5 fermentation were integrated using a CRISPR/Cas system to design an efficient industrial strain. Mutant transporters with improved activity were obtained by different approaches and molecular mechanisms associated with improved tolerance against inhibitors were identified. Based on a robust high-performing engineered yeast cell, metabolic pathways to produce biochemicals as malic acid and MEG were integrated into yeast genome. Biosensors coupled with synthetic promoter libraries have been used to improve strain performance and create promising microbial cell factories to be used in the bio-based industry.

Poster session: “Yeast biotechnology”

Thursday 24th August, 12.30-14.00

METABOLIC ENGINEERING OF THE YEAST *CANDIDA FAMATA* FOR IMPROVEMENT OF RIBOFLAVIN PRODUCTION FROM XYLOSE.

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¹Institute of Cell Biology, NAS of Ukraine, Ukraine. ²University of Rzeszow, Poland

Presenter: Ljubov Dzanaeva

Flavinogenic yeast *Candida famata* are characterized by the ability to grow on unconventional substrates, in particular on xylose. It is important to expand the spectrum of substrates and to increase the yield of riboflavin in particular from xylose-containing lignocellulosic renewable raw materials.

The metabolism of xylose in yeast starts with its reduction by xylose reductase (EC 1.1.1.21) to xylitol, which is further oxidized to xylulose, this reaction is catalyzed by xylitol dehydrogenase (EC 1.1.1.9). Xylulose, at the next stage, is phosphorylated by xylulokinase (EC2.7.1.17) to xylulose-5-phosphate. This sugar in the non-oxidizing phase of PPP is converted by phosphopentose epimerase to ribulose-5-phosphate which is used as the riboflavin precursor. Strains with constitutively overexpressed *XYL1*, *XYL2* and *XYL3* genes could be characterized with elevated riboflavin production from xylose through the better supplying the non-oxidizing stage of PPP with riboflavin precursor ribulose-5-phosphate.

To achieve this goal *C. famata XYL1* gene under control of strong constitutive *CfTEF1* promoter was cloned into pUC57_CfTEF1pr_DhTEF1tr_pGAL1_Cf_mazF_Ec_tAOX_Pp_NTC (6967 bp) vector. Obtained expression cassette was introduced into *C. famata* VKM Y-9 and BRP#6 strains, and the selection of *NTC* transformants was provided. The ability of resulted strains to biomass accumulation and riboflavin production in xylose medium was studied. *XYL1* overexpression in recombinant BRP#6 strain improved growth kinetics up to 1.3-1.5 times, while the production of riboflavin was 1.3-2 times higher as compared to the initial strain. Correlation between riboflavin production in recombinant strains with overexpressed *XYL1* gene and activity of its enzyme will be studied.

ID: 16; Stand: D02

General stress response: a mechanism to save energy and express heterologous proteins in slow-growing yeast cultures

Nuran Temelli, Simon van den Akker, Gwénolé Paquet, Mugesh Kumararajan, Ruud A. Weusthuis, Markus M.M. Bisschops

Bioprocess Engineering, Wageningen University, Netherlands

Presenter: Nuran Temelli

Slow growing yeast cells in fed-batch or continuous cultures are highly relevant for industrial biotechnology as they allow uncoupling of growth from product formation to increase yields. To effectively apply slow-growing cultures, most of the metabolic energy and substrate should be directed to product formation, e.g., heterologous protein expression. When its specific growth rate decreases, yeast gradually activates the conserved General Stress Response (GSR). The GSR confers resistance against multiple stresses, but we hypothesize that it also contributes to energy saving. For example, the GSR results in increased levels of heat shock proteins that act as chaperons that can reduce expensive protein turnover. To test this hypothesis, we manipulated the main transcription factors that activate the expression of stress response genes in *Saccharomyces cerevisiae*. This resulted in only minor changes of the maximum specific growth rate. Stress resistance was however strongly affected, which correlated well with observed changes in stress-responsive promoter (pHSP12) driven protein expression. To study the impact on cellular energetics, the cellular maintenance energy requirements of mutant and parental strains were estimated using chemostat and fed-batch cultures. A reduction of the GSR strongly increased the maintenance energy requirement, which amongst others translated into reduced biomass yields and protein titers in fed-batch cultures. These findings strongly suggest that GSR not only plays a role in actual stress resistance, but also in energy saving in yeast. The GSR is hence a relevant target to further improve yeasts as microbial cell factories.

ID: 33; Stand: D03

Post-translational lipidations as minimal plasma membrane targeting motifs in *Saccharomyces cerevisiae*

Liv Teresa Muth, Michelle Patricia, Siele Jochems, Inge Van Bogaert

BioPort Research Group, Centre for Synthetic Biology, Faculty of Bioscience Engineering, Ghent University, Belgium

Presenter: Liv Teresa Muth

Prenylation, a post-translational modification, increases the binding capacity of a protein to endomembranes through C-terminal lipidation. Using minimal prenylation motifs provides a versatile and robust tool to direct proteins to distinguished subcellular locations. In this comparative study, prenylation motifs from various organisms, and their combination with secondary signals like polybasic sequences, palmitoylation, and additional prenylation were tested for their abilities to target specific endomembranes in *Saccharomyces cerevisiae*.

Qualitative and quantitative confocal microscopic image analysis were used to investigate the localization of prenylated mCherry as a reporter protein in both exponential and stationary phase. Adding the native upstream polybasic sequence or adjacent palmitoylation recognition site(s) enhanced targeting the reporter to the plasma membrane. Furthermore, expanding the polybasic sequence with additional lysines gradually shifted the localization of the reporter from endomembranes toward the plasma membrane.

This research provides a combinatorial toolbox of minimal C-terminal modifications that can be integrated into multiple layers of localization information for basic and applied research whenever membrane binding is required, e.g., for ectopic protein expression studies or anchoring sensors to specific sites within the cell. As a proof-of-concept, the best-performing motifs to target the plasma membrane were used to create a so-called 'transport metabolon' in a *S. cerevisiae* cell factory. 'Transport metabolons' describe a close connection between the enzymatic assembly line of the product of interest and cross-membrane transport. Increased proximity of subsequent enzymes and corresponding importers enhances product formation, reduces the accumulation of toxic or inhibitory intermediates, and prevents feeding alternative metabolic pathways.

Discovery of monoterpene indole alkaloids and bioactivity screening in *S. cerevisiae*

Samuel A. Bradley¹, Beata J. Lehka¹, Frederik G. Hansson¹, Daria Volkova¹, Khem B. Adhikari¹, Daniella Rago¹, Paulina Rubaszka¹, Ahmad K. Haidar¹, Lea Hansen¹, Olga Gudich¹, Konstantina Giannakou¹, Bettina Lengger¹, Pedro Pinho¹, Maxence Holtz¹, Yoko Nakamura², Anja A. Petersen³, Thomas Frimurer³, Sebastien Beasseau⁴, Sandeep Kumar⁴, Nicolas Gautron⁴, Celine Melin⁴, Jillian Marc⁴, Remi Jeanneau⁵, Sarah E. O'Connor⁶, Nicolas Gautron⁴, Yijun Qiao⁷, Davide R. Suarez¹, Vincent Courdavault⁴, Jay Keasling^{1,8,9,10,11}, Jie Zhang¹, Michael Jensen¹

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Presenter: Frederik G. Hansson

Monoterpene indole alkaloids (MIAs) are a large class of plant secondary metabolites derived from tryptamine and secologanin. Their structural diversity is reflected in their impressive array of pharmaceutical applications, being used to treat ailments ranging from hypertension to cancer. However, this complexity, precludes their efficient chemical synthesis at scale, limiting pharmaceutical companies to extracting MIAs from source plants. Microbial synthesis, in which the plant biosynthetic pathways are refactored in microbial chassis, is emerging as a viable alternative. The advanced molecular toolboxes for these chassis, relative to plants, also presents new opportunities for engineering biosynthetic pathways to produce synthetic derivatives of bioactive alkaloids, thereby introducing even more chemical diversity. Once produced, these potential drugs will need to undergo a long screening process for potential uses in medicine, where human G-protein coupled receptors (GPCRs), are important therapeutic targets. Human GPCRs can be expressed in yeast and activation can be coupled to a visible signal, making yeast a robust host for drug screening of potential human therapeutics. In this work, we first demonstrate production of both natural and new to nature compounds derived from MIAs. Then, optimization of the cell factories by addressing bottlenecks and promiscuity of enzymes lead us to meaningful titers of the compounds. Finally, we test these compounds against a panel of human GPCRs expressed in yeast and demonstrate bioactivities. In total, this work exemplifies yeast as a viable chassis for studying biosynthesis of novel synthetic alkaloid of relevance for drug discovery pipelines of otherwise difficult-to-source pharmacophores.

Genetic inactivation of the Carnitine/Acetyl-carnitine mitochondrial carrier of *Yarrowia lipolytica* leads to enhanced odd-chain fatty acid production

Eugenia Messina¹, Camilla Pires de Souza², Claudia Cappella¹, Pasquale Scarcia¹, Isabella Pisano¹, Simona Nicole Barile¹, Luigi Palmieri¹, Jean-Marc Nicaud³, Gennaro Agrimi¹

¹University of Bari Aldo Moro, Italy. ²Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, France. ³Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Italy

Presenter: Eugenia Messina

The non-conventional yeast *Yarrowia lipolytica* can utilize various substrates producing acetyl-CoA in the cytosol or peroxisome, including fatty acids and acetate. Acetyl-CoA is transported to the mitochondrial matrix for complete oxidation to CO₂ using the Carnitine/Acetyl-Carnitine shuttle. Recently, the mitochondrial carrier YlCrc1 involved in this shuttle was identified in *Y. lipolytica*. The YlCrc1 knock-out strain failed to grow on ethanol, acetate and oleic acid, demonstrating how in *Y. lipolytica*, this shuttle is the only pathway for transporting peroxisomal or cytosolic acetyl-CoA to mitochondria. In contrast, *Saccharomyces cerevisiae* has two pathways for acetyl-CoA to reach the mitochondrial matrix, the glyoxylate cycle and the Carnitine/Acetyl-Carnitine shuttle.

YlCrc1 can represent a key point for the regulation of fatty acid metabolism since it is induced by oleate much more than carnitine acyltransferases and its overexpression significantly reduced the cellular lipid content.

As oleaginous yeast, *Y. lipolytica* directs most of the acetyl-CoA generated towards the synthesis of lipids, which occurs in the cytosol. A metabolic engineering approach involving the deletion of YlCrc1, and the recombinant expression of propionyl-CoA transferase from *Ralstonia eutropha* (RePCT), allowed the simplification of the lipogenic medium for odd-chain fatty acids (OCFAs) synthesis and improved their accumulation in *Y. lipolytica*. OCFAs are promising microbial-based compounds with several applications in the medical, cosmetic, chemical and agricultural industries. These findings further highlight that the manipulation of the expression levels of YlCrc1 mitochondrial carrier can be a promising target for metabolic engineering approaches involving the use of cytosolic acetyl-CoA.

ID: 104; Stand: D06

Buffalo milk whey as a matrix for the isolation of potential probiotic strains

Andrea Bonfanti¹, Romano Silvestri¹, Ettore Novellino², Maria Maisto², Giancarlo Tenore², Cristina Mazzoni¹

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Presenter: Andrea Bonfanti

Milk whey is the main by-product of dairy industry and if disposed into the environment it can be very polluting; nevertheless, whey has a high nutritional value, being rich in proteins and vitamins. The fermentation of food matrices can bring new benefits and acquire new nutraceutical properties. In this work, buffalo whey was characterized both at the metagenomic level and with culture isolation methods in order to use autochthonous strains for whey fermentation for the production of a new nutraceutical.

The metagenomic analysis of DOP Campania Buffalo milk whey revealed the presence of yeasts, which has been confirmed by a culturomic approach.

In recent years, beside to the already well-known and tested activity of some bacteria as probiotics, it has emerged that some species of yeasts, including *Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces*, *Debaryomyces*, *Candida*, *Pichia*, *Hanseniaspora*, and *Metschnikowia* can also perform healthy benefit on humans¹.

Some important advantage of yeast probiotics, compared to bacteria, is that they are highly resistant against gastrointestinal enzymes, pH, bile salts, organic acids, and variations in temperature. Moreover, yeasts, as eukaryotic cells, are resistant to bacteriophages and most antibiotics, and cannot transfer antibiotic-resistance genes to bacteria. Finally, yeasts have shown to have immunostimulatory effects in different animal taxa^{2,3}.

Data on the evaluation of probiotic properties of some isolated yeast strains from Buffalo milk whey will be presented.

Conversion of methanol into value-added organic acids using engineered *Komagataella phaffii*

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Presenter: Simone Bachleitner

Organic acids are used as chemical building blocks for a variety of materials used in chemical, food, and pharmaceutical industry. Currently microbial fermentation for organic acid production uses first-generation feedstocks such as glucose and starch. However, these carbon sources are derived from agricultural cultivation and compete with food, feed, and plant fiber material production. Methanol, a single carbon source offers a more sustainable possibility to produce value-added organic acids, as technology allows electrochemical reduction of CO₂ to methanol. Thus, using CO₂-derived methanol as a carbon source potentially reduces carbon emissions and promotes sustainable bioprocesses.

Here we use the natural methylotrophic yeast *Komagataella phaffii* as a production platform, and with genetic tools such as CRISPR-Cas9 and Golden Gate Cloning, we integrated the heterologous pathways for itaconic and lactic acid synthesis. First screenings enabled the production of 2 g/L itaconic and 0.6 g/L lactic acid, respectively. While further genetic engineering and process optimization in bioreactor cultivations enhanced itaconic acid titers to 50 g/L, upscaling of lactic acid turned out to be more challenging. A glimpse in gene expression indicated that the presence of lactic acid itself might negatively affect methanol utilizing genes, thus hampering methanol utilization and lactic acid synthesis.

Uncovering the molecular evolutionary mechanisms for simultaneous xylose/glucose consumption in industrial *Saccharomyces cerevisiae* strain

João Bueno¹, Jefferson Gross², Leandro Santos^{1,3}

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Presenter: João Bueno

The world's dependence on fossil fuels for energy supplies can be reduced by using bio-based chemicals produced by microbial platforms capable of converting biomass sugars into biorenewable compounds. However, the uptake of the five-carbon sugar xylose derived from biomass is impaired by the presence of glucose. This inhibition is called glucose-catabolic repression and increases the total fermentation time in industrial hydrolysates. The aim of this study is to identify molecular evolutionary mechanisms that can alleviate catabolite repression and design a C5 industrial *Saccharomyces cerevisiae* yeast that can co-ferment xylose-glucose. We used an industrial-derived strain engineered with the C5 metabolic pathway and with glucose metabolism disabled by the knock-out of all hexokinase genes. Evolutionary pressures were imposed on different cell populations to fix mutations associated with catabolic repression. High-coverage whole-genome sequencing of seven evolved populations revealed promising genetic modifications: duplications on chromosomes I, III, and VI, and Single Nucleotide Polymorphisms (SNPs) on *HXT2*, *HXT6*, and *ZWF1*. Using a CRISPR-Cas system, the SNP on *ZWF1* was confirmed to substantially improve xylose consumption in yeast cells. In a transporter-free platform with the xylose consumption pathway, two mutants on *HXT6* lost its ability to transport glucose and became specific xylose transporters. We re-introduced glucose metabolism into our best evolved isolate and its growth performance in glucose-xylose was superior compared to the non-evolved strain. These findings demonstrate different evolutionary responses to alleviate glucose repression in industrial C5 strains and identify promising genetic targets for engineering efficient microbial platforms to consume glucose-xylose simultaneously in lignocellulose-based biorefineries.

ID: 133; Stand: D09

Exploring the potential of neglected biomass, new enzymes, and non-conventional yeast for fuel production

Fellipe de Mello, Beatriz Vargas, Lara Sousa, Ana Clara David, Giovanna Maklouf, Jade dos Santos, Marcelo Carazzolle, Gonçalo Pereira

University of Campinas, Brazil

Presenter: Fellipe de Mello

The expansion of climate emergency scenarios worldwide has raised awareness of the need to advance the full exploration of biomass for fuel production. While bioethanol - a leading alternative to fossil fuels - is mainly produced from the fermentation of sugarcane and corn-derived hexoses with *Saccharomyces cerevisiae*, advances in yeast genetic engineering empower the efficient metabolization of pentoses from lignocellulosic residues or other complex sugars available in different biomass. In this context, the drought-resistant and highly productive agave (*Agave sisalana*) is a promising option for ethanol production in dry and degraded areas, that could guarantee the surplus of energy demand in the circumstances of a paradigm shift in fuel production. Therefore, yeast cell factories that efficiently ferment pentoses, from sugarcane bagasse; starch, from corn; or inulin, from agave are paramount for the development of this industry in the near future. In this work, new xylose isomerase genes, uncovered from ruminants' metatranscriptomic data, enabled growth performance in xylose similar to the gold standard *Orpinomyces*, and ethanol production in *S. cerevisiae*. Also, unprecedented alfa- and glucoamylases expressed in *S. cerevisiae* showed elevated starch degrading activity, helping to move towards feasible consolidated bioprocessing of corn. Inulinases prospected from *A. sisalana*'s phytopathogens have shown the ability to ferment the plant's fructans, envisioning the use of this feedstock for chemical commodity production. In parallel, a library of non-conventional yeasts isolated from the Brazilian biodiversity is being screened as industrial platforms with robust phenotypes, where endogenous xylose metabolism and resistance to extreme stresses have been uncovered.

Deciphering phenotypic and genomic features in *Saccharomyces cerevisiae* strains with high dominance potential and formic acid resistance

Rebecca My¹, Paolo Antoniali², Stefano Campanaro³, Laura Treu³, Lorenzo Favaro¹

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Presenter: Rebecca My

Lignocellulosic biomass is one of the most promising substrates for the production of bioethanol. However, the fermentation of this feedstock is still non-profitable. In fact, lignocellulosic substrates require a pretreatment step, which releases inhibitors detrimental to the growth of *Saccharomyces cerevisiae* and to the fermentation itself. Nowadays, in sugarcane-to-ethanol industrial plants, there is usually a rapid succession of yeast strains, and the dominant one(s) can overcome the starter. Therefore, both dominant potential and inhibitors tolerance are crucial traits for the selection of superior yeast strains.

Thanks to a hybrid approach combining biotechnology and bioinformatics, this study investigated a wide collection of *S. cerevisiae* strains composed of laboratory, industrial and oenological strains using a comparative genomic analysis. A cluster of 20 strains was then selected on the basis of their promising robustness and tested for their ability to survive when mixed together. During the selection process, different stresses typical of lignocellulosic ethanol production were applied, including: i) low readily assimilable nitrogen (RAN) (30 mg/L); ii) high concentrations of acetic acid (3 g/L); iii) formic acid (1.0 and 1.2 g/L). Few strains showed outstanding fitness and were selected for genomic insights. Gene copy numbers and SNPs were analysed in order to prioritize variants and better assess their linkage with phenotypes. A novel cluster of variants impacting key genes involved in both formic acid and yeast dominance was found and deeply investigated. These findings can support the development of superior *S. cerevisiae* strains for lignocellulosic bioethanol production.

Enhancement of poly(ethylene terephthalate) degradation *via* pre-treatment methods by the engineered *Yarrowia lipolytica* strain

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Presenter: Katarzyna E. Kosiorowska

Increasing amounts of plastic waste in the environment have been witnessed in the past few decades. Among the most popular plastics, whose global production constitutes 8.1% is poly(ethylene terephthalate) (PET). This threat posed by the accumulation of synthetic polymers in the environment requires a comprehensive approach to prevent further devastation of ecosystems. One of the most promising methods enabling the reduction of the negative impact of plastic waste on the environment is the use of biotechnological methods allowing the enzymatic degradation of PET. One enzyme that exhibits high efficiency in hydrolyzing ester bonds present in this polymer is PETase from *Ideonella sakaiensis*. In our study, we employed an unconventional yeast *Yarrowia lipolytica*, as a platform for extracellular production of bacterial PETase. The codon-optimized gene was cloned into the yeast strain using a system based on rDNA integration. The resulting strain was tested for its ability to degrade various types of PET plastic. Different pretreatment methods of plastic material, to verify their effect on its degradation level were applied. The results show that the application of the artificial aging process in artificial seawater increase the amount of TPA released to the media. The yield is 4-fold higher compared to the untreated PET plastic. This study proves that during prolonged yeast culture under applied conditions, enzyme activity is not impaired. The conducted studies also demonstrate the feasibility of degrading plastics directly in yeast culture carried out at low temperature.

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ID: 197; Stand: D12

Analysis of putative erythrose reductases in yeast *Yarrowia lipolytica*

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Presenter: Mateusz Szczepańczyk

The unconventional yeast *Yarrowia lipolytica* produces erythritol as an osmoprotectant to adapt to osmotic stress. The analysis of the array of putative erythrose reductases, responsible for the conversion of D-erythrose to erythritol, was conducted to reveal their influence on polyol synthesis profile. Strains with single and multiple knockouts were tested for their ability to produce polyols in osmotic stress conditions. Results indicate that lack of six of the reductase genes does not affect erythritol production significantly, as the content of this polyol in the medium is comparable to the control strain. Deletion of eight of the homologous erythrose reductase genes resulted in a 91% decrease in erythritol synthesis, a 53% increase in mannitol synthesis, and an almost 8-fold increase in arabitol synthesis as compared to the control strain. Additionally, glycerol utilization was impaired in the media with induced higher osmotic pressure. Those results may shed new light on the production of arabitol and mannitol from glycerol by *Y. lipolytica* and help to develop strategies for further modification in polyol pathways in these microorganisms.

This work was financially supported by the National Science Centre, Poland, project UMO-2018/31/B/NZ9/01025.

ID: 206; Stand: D13

Optimal trade-off between boosted tolerance and growth fitness during yeast adaptation to ethanol shocks

Ana Paula Jacobus^{1,2}, Stella Diogo Cavassana¹, Isabelle Inácio Oliveira¹, Joneclei Alves Barreto¹, Thalita Peixoto Basso³, Luiz Carlos Basso³, Jeferson Gross¹

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Presenter: Jeferson Gross

Ethanol tolerance is a key trait for biofuel producing yeasts. We subjected four populations (P1-P4), derived from the Brazilian Bioethanol strain *Saccharomyces cerevisiae* PE-2, to an adaptive evolution protocol for survival to acute ethanol treatments. About 70-80 reiterated cycles of a 2-hour ethanol shock, followed by a recovery growth (YPD 2%), raised yeast alcoholic tolerance from initial 19% up to 30% (v/v) at the end. Clones P1c-P4c, isolated from final populations, showed much higher survival rates after acute alcoholic exposure, and displayed marked fitness improvements for ethanol shocks in competition assays with the parental strain. Whole-genome sequencing of evolved clones revealed 46 mutated alleles, which are enriched in cAMP/PKA pathway components. Consistent with a low PKA phenotype, *HSP12-GFP* biosensor measurements showed that evolved clones have constitutive induction of Msn2/4-mediated stress responses. Key mutations were reverse engineered into the parental strain. Competition assays under 20% (v/v) ethanol treatments highlighted several adaptive alleles, with the best outcome for combining *cyr1*^{A1434T} and *ath1:MX*. Despite adaptation to acute treatments, most reverse-engineered strains showed decreased fitness for growth under 8% ethanol, or without alcohol. We found an optimal trade-off between alcoholic tolerance and fitness growth for the strain *cyr1*^{A1434T}/*usv1*Δ, which outcompetes its parent both in 20% ethanol shocks and during growth under 8% (v/v) ethanol, while having a minor fitness loss when propagated in YPD 2% without ethanol. Fermentation assays showed that selected strains have a robust performance under stress conditions mimicking the industrial ethanol production in Brazil.

ID: 215; Stand: D14

Phenotypic characterisation and expression analysis of the low pH tolerant yeast *Kazachstania bulderi*.

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¹The University of Manchester, United Kingdom. ²BP Biosciences Centre, USA

Presenter: Alistair Hanak

Yeasts are a key class of microorganism, widely used in commercial food and drink production and industrial biotechnology. Non-conventional yeast species have huge phenotypic and genetic diversity and represent an untapped reservoir of traits with potential for use in industrial settings. *Kazachstania bulderi* is a non-conventional yeast species that is able to grow efficiently under low pH conditions with glucose and δ -gluconolactone as a substrate. These traits make *K. bulderi* an interesting candidate for use in sustainable biotechnology processes including low pH fermentation and the production of green chemicals such as organic acids. Here, we present extensive phenotypic characterisation of three strains of *K. bulderi*, including the type strain, under a range of low pH and other stress conditions. Specifically, their tolerance to several industrially produced organic acids and antimicrobial compounds was assessed. To accelerate the understanding of the genetic underpinning of the low pH trait, RNA-seq based transcriptomic analysis has been carried out in fed-batch under constant pH of 5.5. and 2.5. Relevant differential expression (DE) genes at low pH will be discussed, including *K. bulderi* species-specific genes which are over-represented in the pool of DE genes. This data combined with the high-quality annotated genome sequence of *K. bulderi* will pave the way for strain development in this non-conventional yeast.

ID: 217; Stand: D15

Development and genetic characterization of new hybrid yeast chassis for sustainable bioproduction.

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Presenter: William Royle

Members of the *Saccharomyces* genus present as a genetically diverse set of microorganisms capable of tolerating a range of environmental stressors and demonstrate a high fermentation capacity. This makes them ideal candidates as chassis strains for bioproduction of industrially relevant chemicals. Hybrids between yeast species can combine beneficial traits from each parent and may exhibit hybrid vigor, more readily adapting to harsher environments. However, interspecies hybrids are often sterile, and as such, an evolutionary dead end. Fertility can be restored by tetraploidisation, allowing full exploitation of genetic diversity through the resulting hybrid progeny. Here, the generation of multigenerational interspecies hybrids is exploited to develop bespoke chassis strains for industrial biotechnology. We demonstrate the large fitness range across a collection of F12 progeny of *Saccharomyces cerevisiae* x *S. jurei* and *S. cerevisiae* x *S. kudriavzevii* hybrids, in response to growth in permissive conditions relevant to both chemical and pharmaceutical industries. We identified hybrid spores that show either very good or poor fitness at low pH, at high concentrations of iso-butanol, and in media containing antifungal drugs. To further elucidate the genetic basis for specific traits in the hybrids, we carried out pooled-segregant genomic sequencing to identify interspecies specific QTL regions involved in tolerance to fluconazole, flucytosine and micafungin. Specific targets were validated via reciprocal hemizygosity analysis. Overall, this study highlights the potential to exploit the natural genetic variation in developing ideal hosts for specific biotechnological purposes.

IMPAIRED SERINE CATABOLISM IN A YEAST MODEL OF CONGENITAL SIDEROBLASTIC ANEMIA

Carlo Marya Thomas Marobbio¹, Silvia De Santis¹, Antonella Santoro¹, Roberta Seccia¹, Angelo Vozza¹, Federica Nettis¹, Daria Di Molfetta¹, Alessandra Castegna¹, Loredana Capobianco², Luigi Palmieri¹, Eleonora Paradies³

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Presenter: Carlo Marya Thomas Marobbio

The biosynthesis of heme is critical during the development of erythroblasts and involves the uptake of glycine into mitochondria, which is catalysed by the SLC25A38 carrier protein. Disruption or loss-of-function of the SLC25A38 gene has been shown to be responsible of congenital sideroblastic anemia, a severe form of anemia characterized by the presence of ring sideroblasts in the bone marrow. Hem25p, the yeast orthologue of SLC25A38, plays a key role in the heme synthesis by catalyzing the transport of glycine into the mitochondrial matrix. Although Hem25p significance in heme biosynthesis is well-established, its involvement in amino acid metabolism is not yet fully unravelled. In this study, we have shown that a yeast strain lacking the HEM25 gene exhibits slow growth when serine is the sole nitrogen source. This phenomenon is most likely due to impaired mitochondrial glutamate production, which is a byproduct of serine catabolism. In fact, by complementing the yeast mutant strain with the human mitochondrial glutamate carriers, we effectively rescued this growth defect. This finding sheds new light on the metabolic function of Hem25p and underscores its significance beyond the involvement in heme biosynthesis.

Cp-thionin II production using *Komagataella phaffii*

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Presenter: Kamila Oliveira

Komagataella phaffii, also known as *Pichia pastoris*, it is one of the main expression systems for recombinant proteins production due to its high yield and potential bioprocessing benefits. On the other hand, Cp-thionin II antimicrobial peptide is a defensin originally isolated from cowpea (*Vigna unguiculata*) seeds that shows activity against both Gram-positive and -negative bacteria. Therefore we aim to produce a recombinant Cp-thionin II using the *K. phaffii* as a heterologous expression system. The Cp-thionin II was fused to the His₆ tag and the construction was cloned into the expression vector pPICZαA using the pAOX1 promoter. Based on SDS-PAGE and Western blot analysis, qRT-PCR and MALDI-TOF techniques, the recombinant Cp-thionin II was expressed as a single major secreted protein with a 7335,21 Da when a high recombinant *K. phaffii* cell density bioprocess was performed. After purification by IMAC and RP-HPLC, a 744.02 µg.L⁻¹ concentration of the purified peptide was obtained. These results indicate that the Cp-thionin II defensin could be successfully produced for the first time by *K. phaffii* under the methanol-inducible pAOX1 promoter.

Characterising an orthogonal thiamine-responsive riboswitch for expression control in the yeast *Komagataella phaffii*

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Presenter: Paweł Małecki

Aside from the ability to grow on methanol as a sole carbon source, a wide array of traits sets the yeast *Komagataella phaffii* apart from the baker's yeast, occasionally leading to labelling the species as non-conventional. Due to its unique characteristics, including a favourable heterologous protein secretion profile and the ability to grow to high cell density in liquid culture, *K. phaffii* is attracting growing attention as a protein production host, as well as in basic research. An important obstacle to the cell engineering of *K. phaffii* is the narrow selection of known inducible expression systems. The vast majority of them respond to the availability of specific carbon sources, forcing the coupling of the induction and biomass generation. Establishing a regulable expression system with an inducer not being a carbon source is necessary to overcome this problem. A riboswitch repressed by thiamine pyrophosphate (TPP) within an intron of *Ogataea polymorpha* DUR31 gene has been previously characterised in its native host. As the principle of action of a riboswitch depends entirely on the RNA structure and, therefore, on the RNA sequence itself, riboswitches are expected to function in a host-independent manner across different organisms. In the presented work, we have successfully expressed two variants of GFP of different stability under the control of *O. polymorpha* DUR31 gene riboswitch in *K. phaffii*, demonstrating the first riboswitch known to work in this host. While a notable change between expression levels in the presence and absence of thiamine in the medium was observed, the system was not tightly repressed in the off-state, limiting its practical use.

Increasing global translation activity and its impact on *Komagataella phaffii*

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Presenter: Jennifer Staudacher

Yeasts, especially *Komagataella phaffii*, are widely used organisms for commercial, heterologous protein production. *K. phaffii* is known for its high secretory efficiency and biomass yield, however, specific productivities are low and tightly coupled to biomass formation. Evaluation of *K. phaffii* transcriptomics datasets revealed the yeast translation machinery as a major target for cellular regulation of protein production.

By overexpression of proteins, involved in most steps of the translation process, we could identify translation initiation factors as the main limit. Specifically, factors associated with the closed-loop conformation, a structure that increases stability and rates of translation initiation before start codon scanning is initiated, had the strongest effects. Overexpression of these factors alone or in combination increased titers of different heterologous proteins by up to 3-fold in fed-batch processes. Global translational activity, as measured by OPP-labelling assays, correlated nicely to the enhanced secreted recombinant protein levels. Furthermore, selected transcript levels and total protein content were higher in the engineered cells. Additional RNA-Seq data revealed the immense impact this overexpression has on the transcriptome. Our data shows the great flexibility of yeast for adaption to such huge intracellular changes. Further, it displays the interconnection as well as, partially, regulation of different protein synthesis steps.

ID: 261; Stand: D20

Construction of an engineered *Saccharomyces cerevisiae* industrial strains expressing cellulases efficiently

Gabriel de Jong, Andre Santos, [Taisa Dinamarco](#)

University of São Paulo, Brazil

Presenter: Taisa Dinamarco

Brazil is the world's second largest ethanol producer. However, growing demand for this biofuel requires investing in new technologies to produce ethanol from lignocellulosic biomass, as second-generation ethanol. We have characterized different cellulases from *A. fumigatus*, which we expressed in industrial *S. cerevisiae* isolated from ethanol plants in Brazil as the strains PE-02 (Usina da Pedra), SA (Usina Santa Adélia), and the thermotolerant LBGA-01. The enzyme was expressed by using CRISPR/Cas9-mediated yeast engineering techniques and the EasyClone-MarkerFree kit. The plasmids were used for cloning cellobiohydrolase and endo-1,4- β -glucanase their respective gRNA for integration into chromosomal loci. After transformation, PE-2 tested positive for cellobiohydrolase. The SA and LBGA-01 strains showed positive fragments for cellobiohydrolase and endo-1,4- β -glucanase genes. The enzymatic activity was tested in solid medium containing CM-Cellulose as substrate. The presence of larger degradation halos around the colonies as compared to the negative control indicated that the enzymes were actively expressed. Also, the enzymatic activity in liquid YP medium for up to 72 hours was determined by the DNS reducing sugar method. The reactions were performed according to Bernardi et al, 2021. The enzymatic activity only increased after cultivation for 72 hours. The most pronounced result was observed for the LBGA-01 strain: compared to the negative control, the enzymatic activity increased by more than 14-fold over time after cultivation for 72 hours. These results showed that the LBGA-01 strain has active endo-1,4- β -glucanase and cellobiohydrolase.

ID: 262; Stand: D21

Constitutive expression of bovine chymosin in *Komagataella phaffii*.

Roberta Ferreira Barros, Fernando Araripe Gonçalves Torres

UnB, Brazil

Presenter: Roberta Ferreira Barros

K. phaffii (*Pichia pastoris*) is considered one of the most important expression platforms in the context of industrial biotechnology due to its ability to secrete recombinant proteins at high levels. However, this is commonly achieved when using the induced AOX1 promoter which relies on the presence of methanol, a toxic and flammable compound.

Bovine chymosin is a protease of commercial interest in the food industry being used in milk coagulation for cheese making. The present work aimed to develop an expression system for the industrial production of bovine chymosin in *K. phaffii* using the constitutive PGK1 promoter, eliminating the need for methanol.

The prochymosin B gene was cloned into a vector which contains a fragment of the PGK1 promoter, alpha secretion factor and kanR resistance gene. Clones secreting chymosin were selected by the formation of transparent halos in LB milk medium. Chymosin production was confirmed in polyacrylamide gel by the presence of a 40 kDa band identified as prochymosin which was processed to its mature (36 kDa) form after activation in acidic pH. After selecting the best producing clone, it was possible to obtain on culture supernatant 121.8 IMCU.ml⁻¹ of enzyme according to the relative milk-clotting activity test method (IDF Standard 157:2007/ISO 11815) after 72h cultivation in complete medium containing glycerol (YPG). The supernatant was concentrated with a 30 KDa membrane, lyophilized and the protein was purified by molecular exclusion chromatography.

Exploring the biocontrol activity of *Starmerella bacillaris* against *Botrytis cinerea* during grape drying

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Presenter: Jacopo Sica

Environmental and health concerns associated with chemical pesticides have prompted the need to reduce their usage in the agri-food sector. Post-harvest fruit management is a crucial phase for developing strategies that minimize pesticide exposure for consumers. One potential solution lies in utilizing microorganisms as biocontrol agents. This study focuses on exploring the biocontrol activity of the yeast *Starmerella bacillaris* against the spoilage fungus *Botrytis cinerea* during the drying of grapes. Grapes belonging to two different cultivars, Garganega and Raboso, were subjected to an initial inoculation of *S. bacillaris*. To observe changes in the populations of *S. bacillaris* and the growth dynamics of *B. cinerea*, real-time quantitative PCR (qPCR) was employed throughout the withering process. Furthermore, DNA metabarcoding was used to compare the microbial biodiversity on the grape surface and assess the potential influence of the treatment on the microbial community structure. The development of a successful qPCR-based method for the identification and quantification of the selected *S. bacillaris* strain provided evidence of its persistence on the grape surface until the end of the withering process. DNA metabarcoding revealed that the application of *S. bacillaris* led to an increase in fungal community biodiversity on the surface of Raboso grape bunches. Moreover, this treatment resulted in a decrease in the proportions of filamentous fungi, including *B. cinerea*. These findings were further corroborated by the qPCR method, demonstrating that the application of *S. bacillaris* effectively inhibited the growth of *B. cinerea* on Raboso grape bunches.

ID: 276; Stand: D23

Fatty acid decarboxylase candidates to expand olefin production in yeast

Mateus Vicente Eugênio de Paiva, Isabelle Taira Simões, Talita Gabriela Salles Ramos, Gabriela Felix Persinoti, Leticia Maria Zanphorlin, Wesley Cardoso Generoso

Brazilian Biorenewables National Laboratory (LNBR) - Brazilian Center for Research in Energy and Materials (CNPEM), Brazil

Presenter: Wesley Cardoso Generoso

Current technologies to produce renewable hydrocarbons are mostly based on chemical processes that demand high temperature and pressure, high loads of hydrogen and heavy metals, which make them industrial and environmentally hostile. Olefins (alkenes) are example of hydrocarbons that can be produced by biotechnological routes and are key molecules to generate other chemicals, due to the carbon-carbon double bonds. Furthermore, applying small load of hydrogenation, these alkenes can be incorporated directly into the traditional fuel production as paraffins, which represents a meaningful reduction of fossil resource usage. The discovery of a fatty acid decarboxylase (OleT_{JE}), which converts free fatty acids and H₂O₂ into olefins as a product, is a promising alternative for sustainable production of these compounds. However, OleT_{JE} succumbs to the presence of oleate, the most abundant substrate available. Therefore, based on the recent discovery of an oleate consuming decarboxylase (OleTP_{RN}), we built a Sequence Similarity Network (SSN) analysis with members of CYP152 family to identify new putative oleate consuming enzymes. Five genes from an OleTP_{RN} containing cluster were screened for the ability to convert oleate efficiently. The putative decarboxylases (and OleTP_{RN}) were cloned into p426 vectors with PTS1 tag and used to transform a *Saccharomyces cerevisiae* strain with deletion of FAA2, FAA4 and CTA1, to accumulate H₂O₂ for olefin production. The recombinant strains were cultivated in media using oleate as sole carbon source and, for OleTP_{RN} and a candidate (OleTP_{CD}), olefins were positively produced. Although still preliminary, these results expand the possibility of hydrocarbon production through sustainable directives.

ID: 278; Stand: D24

***Saccharomyces cerevisiae* primary metabolism in winemaking context: natural diversity and modification strategies**

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Presenter: Jessica Noble

In winemaking, *Saccharomyces cerevisiae* is able to perform the conversion of sugars to ethanol and CO₂ with an outstanding yield of 92% on average. Other primary metabolites, such as glycerol and organic acids, are also produced in significant quantities. Yields of production of metabolites of the Central Carbon Metabolism (CCM) can vary among yeast strains even if ethanol yield is known to be strongly constrained. The first part of our study consists in a wide screening strategy to assess the diversity among *Saccharomyces cerevisiae*, measuring five metabolites from the CCM after complete fermentation in wine-like laboratory conditions using 51 strains from diverse genetic origins. Our results highlight a low, but still measurable, ethanol production diversity among strains and a link between other minor metabolites and genetic origin. Aiming at modifying the ethanol yield and being aware of legal and societal brakes limiting the use of GMO's, we then implemented two strategies: adaptive laboratory evolution (ALE) and random mutagenesis. We designed an ALE strategy using 2-deoxyglucose, a glucose analogue which acts as a glycolysis inhibitor, to impact ethanol production of three wine strains and we generated in parallel hundreds of mutants using UV-mutagenesis. A large phenotyping analysis of the evolved and mutant strains revealed strong variations in metabolites, notably organic acids. Further work is currently ongoing, based on genomic and transcriptomic approaches, to identify the genetic basis of those modifications.

ID: 280; Stand: D25

Improvement of *Saccharomyces cerevisiae* for biosynthesis of folates

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Presenter: Valeria Mapelli

Folate vitamers are essential micronutrients for humans, as they function as cofactors in one-carbon transfer reactions. Folic acid (vitamin B9) is the commercially available form of folate vitamers and is chemically synthesized. The production of natural folates by microbial fermentation represents a more sustainable and preferable alternative due to the possibility to synthesize an array of folate vitamers.

In this work, we focused on maximizing folate production by engineering the production of the two main building blocks concurring to the synthesis of folate; 4-aminobenzoic acid and dihydropteridine. A classical genetic engineering strategy has been compared to a strategy based on the use of a genetic engineering toolkit allowing the integration of the genes of interest into highly expressed genetic loci. The best *Saccharomyces cerevisiae* strain generated and studied in this work resulted in the production of 620.0 ± 12.30 $\mu\text{g/L}$ of folate, which is one of the highest titres ever reported using *S. cerevisiae* as cell factory.

ID: 282; Stand: D26

Development of a yeast-based system to remove and recover heavy metals

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Presenter: Sara Granuzzo

High exposure to heavy metals (HM) is extremely toxic to living organisms, and it becomes important to reduce their environmental levels, but also their recovery from waste material, to support recycling. Yeast *Saccharomyces cerevisiae* has been so far proposed as valid alternative since its bioadsorption abilities, and promising results have been further obtained by the use of genetic engineering.

Here, for the first time, we modified natural yeast strains to arm their cell surface by expressing the metal-binding protein γ Cup1, able to selectively associate copper ions. We then characterized the binding properties of the strains by performing multiple functional and biochemical assays.

Collectively, our data demonstrated that natural strains are able to express the surface-localize γ Cup1 protein, further supporting the significative improvement of the armed yeast cells in capturing and recovering copper from contaminated solutions. Technological perspectives in the use of our modified strains will also be discussed.

Xylitol production by cells of *Pichia fermentans* immobilized in alginate-beads and *Aspergillus oryzae* pellets

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Presenter: Alberto Amaretti

Xylitol is a pentose-polyol largely applied in food and pharmaceutical industry, that can be produced from lignocellulosic biomass valorizing the second-generation feedstock. Its biotechnological production requires the adoption of scalable solution applicable at industrial scale process. In this context, immobilized-cell system offers numerous advantages; fungal pellet carriers are increased in attention but their utilize in xylitol production is already not investigated. In this study the yeast strain *Pichia fermentans* WC 1507 was applied to xylitol production. The best performance conditions were observed at pH higher than 3.5, low oxygenation, in a medium with (NH₄)₂SO₄ and yeast extract as nitrogen source. Yeast cells of were immobilized in *Aspergillus oryzae* 76-2 pellet biocapsules (BC) and alginate beads (AB), that were tested in three consecutive production-runs. The effect of a 0.2 % w/v alginate coating was also examined for both the carriers. At the third run all the carriers performed better than a free cell control and the uncoated BC and AB were similar in xylose consumption, xylitol production, and conversion yield, with uncoated AB that reached the highest values of 98 g/L, 67 g/L, and 68 % respectively. The AB carriers was finally tested in a laboratory-scale bioreactor, achieving 95 g/L xylose consumed, 49 g/L xylitol produced, and 52 % conversion. These results demonstrated that *P. fermentans* is a promising strain for xylitol production in cells-entrapped system and fungal BC is a valuable support for xylitol bioproduction by immobilized technique.

Exploring the genetics governing quantitative phenotypes in the yeast *Komagataella phaffii*

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Presenter: Marina Jecmenica

Komagataella phaffii is a methylotrophic yeast widely used in industry for recombinant protein production. Particularly derivatives of the natural isolate CBS2612 have been used for manufacturing a wide variety of products and were studied extensively. While lots of efforts have been made for the thorough investigation of these industrially-relevant strains, the majority of natural isolates within this species remains unexplored. Yet, as demonstrated in other yeast species, particularly isolates of *Saccharomyces ssp.*, exploiting intra-species variation can benefit strain development for industrially-relevant phenotypic traits. Using quantitative trait loci (QTL) mapping, we aimed at resolving the recombinant protein production potential, as well as temperature tolerance between a cross of a natural isolate and a close descendant of CBS2612. Both parental strains show highly contrasting phenotypes with regard to product yields in small-scale cultivations and also growth at non-optimum temperatures of 39°C. We generated F14 recombinant inbred lines, of which single hybrids were screened and ranked for their ability to secrete a fluorescence protein and for their growth performance at high (39°C) and at low temperatures (12°C). Hybrids exhibiting extreme values of respective phenotypes were pooled, genomic DNA was extracted for whole-genome re-sequencing and finally, allele frequencies were compared among contrasting pools. Single nucleotide polymorphisms (SNP) within QTL regions were analysed for their effect and indeed, meaningful candidate alleles were identified for each phenotype. While final allele swaps are currently still being conducted, this study already highlights the great potential of QTL mapping for the identification of beneficial gene variants.

ID: 296; Stand: D29

CRISPR-assisted hybrids generation enables faster evaluation of fruitful breeding in winery yeast strains

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Presenter: Francesca Righetto

Yeast breeding is widely applied in food and industrial biotechnology to obtain new hybrid strains endowed with better performance. However, standard procedures are expensive and time-consuming, and moreover the final hybrid may not fit with the desired properties.

Here, we provide a novel method to markedly decrease the time required for hybrids generation from several years to a few months, allowing a faster evaluation of their technological features.

By the use of the CRISPR/Cas9 system to modify the HO locus, we performed the conversion from homo- to hetero-thallic natural strains, and then we used the haploid *Saccharomyces cerevisiae* cells to obtain the hybrids by crossing two spores of opposite mating type.

As a proof of concept, we applied the strategy to yeast strains used in oenology, replicating a cross already performed by standard methods.

The hybrid was quickly generated and its properties were compared with the reference hybrid, by the characterization of both fermentative performance and levels of relevant metabolites produced by the yeast cells during fermentation.

Overall, data supported that our strategy allows to rapidly create, from two parental strains, new hybrids, featured by specific properties, which are extremely useful to evaluate the cross as fruitful. Importantly, possible uses of the method are not limited to the wine industry, but may be interesting also for other fermentative-based applications (as bioethanol production).

ID: 298; Stand: D30

Harnessing the oxidative stress in yeast for the production of carotenoids

Zimo Jin, Asia Vighi, Yueming Dong, Codruta Ignea

McGill University, Canada

Presenter: Codruta Ignea

Reactive oxygen species (ROS), normally produced during aerobic respiration, are the major forces of oxidative stress in most organisms. ROS have also been implicated to many serious human pathologies, including diabetes, Alzheimer's disease, and aging. With recent advances of synthetic biology and metabolic engineering, several important antioxidant compounds have been produced in microbial cell factories. However, the yields of the compounds of interest are not sufficient for industry scale production. In this study, we coupled heterologous production of antioxidant compounds with the inherent yeast defense machinery under hydrogen peroxide induced stress. We show that the viability of engineered yeast cells could be restored by shifting metabolic flux into antioxidant production under oxidative stress. We proved that natural selection could be used to enhance and optimize the production of desired products under stress conditions at cell factory level. Using β -carotene as a case study, we showed that increasing oxidative stress correlated with increased production of β -carotene. Our results have shown changes in the yeast sterol profile in response for the external oxidative stress induced by hydrogen peroxide. This strategy could be used to enhance the production of other antioxidant compounds in *S. cerevisiae* or other organisms.

In vivo monitoring of reactive oxygen species in the yeast *Komagataella phaffii* using a genetically encoded fluorescent H₂O₂ biosensor

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Presenter: Victor Mendes Honorato

The interest in the methylotrophic yeast *Komagataella phaffii* (syn *Pichia pastoris*) has been increasing significantly over the years. Due to its unique features, such as high secretory capabilities and ability to utilise methanol as carbon source, *K. phaffii* presents itself as a very interesting non-conventional yeast to be used in the contexts of recombinant protein production and single carbon technology.

Alongside recombinant protein production and utilisation of methanol, hydrogen peroxide is one of the recurrently observed by-products. Generated either in the course of oxidative protein folding in the endoplasmic reticulum or during the initial steps of methanol assimilation, H₂O₂ can be significantly toxic to the cells, and at high levels cause not only the disruption of different cellular activities, but also damage membranes and organelles. Therefore, a better understanding and monitoring of this non-radical reactive oxygen species (ROS) is essential.

Here, we present the first application of the pH-independent genetically encoded fluorescent H₂O₂ responsive biosensor, named HyPer7 (Pak et al., 2020), in *K. phaffii*. By cultivating cells in microbioreactor (Biolector - M2P Labs), we were able to have on-line monitoring of the biosensor oxidation and reduction. HyPer7 responsiveness was first tested by adding exogenous stressors (H₂O₂ and DTT). Then H₂O₂ dynamics were measured in intact cells during growth on glucose or methanol. Cultivation of cells using methanol revealed a general increase in biosensor oxidation, with significant oxidation peaks shortly after the administration of methanol. Increased sensor oxidation indicating ER-derived H₂O₂ generation was also observed in strains secreting recombinant proteins.

Exploring the potential of *Yarrowia lipolytica* for microbial oil production from short-chain fatty acids through differential gene expression analysis

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Presenter: Mia Žganjar

Converting organic waste into commercially viable microbial oils is challenging, yet it simultaneously uncovers opportunities for economically sustainable oleochemical production. A potentially useful strategy involves a two-step conversion process: bacterial anaerobic fermentation of organic waste into short-chain fatty acids (SCFAs), followed by yeast fermentation into microbial oils.

To optimize this process, over 1400 yeast strains were evaluated for efficient growth and their ability to accumulate neutral lipids in relatively high quantities on media with SCFAs as the sole carbon and energy source. These strains were cultured on solid media with different compositions, each incorporating different concentrations of individual SCFAs: acetic acid (C2), propionic acid (C3), or butyric acid (C4). Their growth parameters were compared against those of the control oleaginous strain, *Yarrowia lipolytica* CECT1240. Strains growing on SCFAs were further examined for neutral lipid accumulation using a high-throughput Nile red dye staining approach.

Y. lipolytica strain EXF-17398 emerged as the most promising candidate strain, outperforming the control strain in both growth and lipid accumulation. To gain further insight into the biological mechanisms driving this performance, mRNA-Seq and multi-condition differential gene expression analysis (DEA) were conducted. The results of both the screening and DEA, along with a functional analysis of expression patterns, will be presented.

Our findings bring us closer to understanding the molecular pathways driving efficient SCFA conversion in *Y. lipolytica*, potentially enhancing microbial oil production processes and offering a more sustainable, economically viable alternative to fossil fuels.

ID: 307; Stand: D33

Lipid synthesis from algal mannitol by modified *Yarrowia lipolytica* strain

Mateusz Szczepańczyk,, Dorota Rzechonek, Adam Dobrowolski, Aleksandra Mirończuk

Wroclaw University of Environmental and Life Sciences, Poland

Presenter: Adam Dobrowolski

Yarrowia lipolytica is an oleaginous yeast that possesses an ability to utilize a wide variety of carbon sources, from glucose and glycerol to lignocellulosic biomass and by-products of large industrial processes. One of the carbon sources not easily utilized by yeast *Y. lipolytica* is mannitol, which does not have a well-described utilization pathway. This polyol is one of the largest components of algal biomass, which is referred to as third-generation biomass. Brown algae contain up to 30% of mannitol in the dry biomass. The unquestionable advantages of algae is their quick growth and no need for fertilization or fresh water. Additionally, mannitol is easily obtained from algal biomass in a process that does not require enzymatic catalysis. Genetic modifications of *Y. lipolytica* lead to increased growth on pure mannitol as a sole carbon source, as well as an algal extract containing high concentrations of mannitol. While the mechanism behind that is still unknown the improved parameters of mannitol utilization coupled with an increase in fatty acids synthesis by overexpression of diacylglycerol acyltransferase gene (DGA1) can result in the production of high amounts of lipids from waste algal biomass. Genetic engineering of the strain towards increased mannitol utilization can also provide a better understanding of the pathways responsible for polyol metabolism. The results of this study suggest the possible use of *Y. lipolytica* to convert algal mannitol to microbial oils.

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ID: 310; Stand: D34

Utilization of Volatile Fatty Acids for Lycopene Production by Modified *Yarrowia lipolytica* Strains

Paweł Moroz, Aleksandra Bartusiak, Zbigniew Lazar

Wrocław University of Environmental and Life Sciences, Poland

Presenter: Paweł Moroz

In the current trends of industrial biotechnology, focused on the biosynthesis of valuable compounds of microbial origin, a growing interest was placed on designing processes according to the principles of circular economy. One of the research directions are focused on the utilization of volatile fatty acids as a raw material for the production of lycopene using modified strains of *Yarrowia lipolytica*.

Volatile fatty acids are a byproduct of the fermentation of organic waste from agriculture and industry. Due to their low cost and the ability to convert them into acetyl-CoA (propionyl-CoA) using appropriate enzymatic pathways, they are an interesting substrate for intracellular lipid production by oleaginous yeasts. Acetyl-CoA plays a crucial role as a precursor in lipid synthesis and is an important intermediate in the carotenoid biosynthesis pathway.

Microbial synthesis of carotenoids, represents a promising alternative process compared to traditional methods of obtaining these valuable compounds. *Y. lipolytica*, due to its oleaginous nature, produce significant amount of this crucial intermediates. In this context, the research aimed to investigate the potential of volatile fatty acids as an alternative carbon source for lycopene biosynthesis by engineered *Y. lipolytica* strains. Acetate, propionate and butyrate were used. All three fatty acids have proven to be good alternative carbon sources for lycopene production and their use increases the attractiveness of this process due to the positive impact on environmental protection and sustainable waste management.

Founding: National Science Centre, Poland, OPUS19 (No 2020/37/B/NZ9/02985) "Utilization of volatile fatty acids for waxes biosynthesis by the yeast *Yarrowia lipolytica*".

***Yarrowia lipolytica* as a factory for biosynthesis of ranaspumin-2, a biosurfactant with foaming properties**

Aleksandra Bartusiak¹, Anna Kancelista¹, Bartosz Wąsik¹, Julia Zwolińska¹, Mikołaj Augustyniak¹, Przemysław B. Kowalczyk², Zbigniew Lazar¹

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Presenter: Anna Kancelista

Any substance able to lower the surface or interfacial tension in both liquid-liquid and liquid-solid systems are called surfactants. They can be used as wetting agents, emulsifiers, foaming agents or dispersants. Surfactants used in households, automotive, mining and cosmetics are usually produced by chemical synthesis, however, nowadays great emphasis is placed on the search for biodegradable compounds that, despite a higher price, will meet the expectations of a sustainable, environmentally friendly economy. One of such compounds may be proteins, which due to their amphiphilic nature (hydrophilic and hydrophobic regions), act as surfactants lowering surface tension and stabilizing foams. Secretion of proteins, including heterologous ones, may be performed by *Yarrowia lipolytica* yeast, especially using renewable sources, such as agricultural waste or by-products from various industries.

In the presented work, the production of extracellular proteins was performed using *Y. lipolytica* transformants able to biosynthesize ranaspumin-2 using various waste substrates (crude glycerol and volatile fatty acids) as carbon sources. Ranaspumin-2, a component of the nest foam of the túngara frog (*Engystomops pustulosus*), has the remarkable ability to significantly reduce the surface tension of aqueous solutions. Its secretion showed a synergistic effect to proteins naturally produced by *Y. lipolytica* in the context of stabilizing foams. Understanding and exploiting foaming properties contributes to the development of innovative and sustainable protein-based biosurfactants that can be used in flotation.

Founding: NCN, Poland, OPUS19 (No 2020/37/B/NZ9/02985) "Utilization of volatile fatty acids for waxes biosynthesis by the yeast *Yarrowia lipolytica*".

Glycerol and freeze-dried pumpkin cake hydrolysates as renewable carbon sources for resveratrol biosynthesis by *Yarrowia lipolytica*

Paweł Moroz¹, Patrycja Szczepańska¹, Tomasz Janek¹, Wojciech Łaba¹, Rodrigo Ledesma-Amaro², Zbigniew Lazar¹

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Presenter: Zbigniew Lazar

Resveratrol, a natural polyphenolic compound present in grapes, berries, peanuts and their products has beneficial effects for human health. It shows anti-cancer and anti-inflammatory properties, what makes it an attractive compound for pharmaceutical, food and cosmetic industries. Currently, plant extracts from Japanese knotweed and grapes are the primary commercial sources of resveratrol. As an alternative way to obtain this compound, its microbiological production by genetically engineered *Yarrowia lipolytica* strains using waste substrates as carbon sources is proposed. Raw glycerol supplemented with aromatic amino acids - tyrosine and phenylalanine was used. Furthermore, freeze-dried pumpkin cake hydrolysates, containing large amount of the required amino acids (0.24 mM Tyrosine and 0.18 mM Phenylalanine), were used as a renewable raw material – separately as well as together with raw glycerol. In flask cultures using glycerol based medium, supplemented by amino acids, the best *Y. lipolytica* transformant, T2P2+ARO1/2, reached 280 mg/L of resveratrol, which was twice as much as for its parental T2P2 (104 mg/L) strain. No amino acids supplementation resulted in lower resveratrol production. Lowering the pH of the culture to 3.5 did not result in lower resveratrol titer, due to that this pH was used during cultures with glycerol supplemented by freeze-dried pumpkin cake hydrolysates as cheap and renewable substrate. Surprisingly, the best resveratrol producing strain, reached approximately 270 mg/L of resveratrol in medium with freeze-dried pumpkin cake hydrolysates alone at pH 3.5. Further optimization of the process parameters will help to increase productivity of the culture and develop a cost-effective process.

ID: 322; Stand: D37

Metabolic engineering of *Yarrowia lipolytica* for sustainable wax production using volatile fatty acids as carbon source

Patryk Kupaj, Marta Węclaś, Anna Kancelista, Tomasz Janek, Zbigniew Lazar

Wroclaw University of Environmental and Life Sciences, Poland

Presenter: Patryk Kupaj

Waxes are esters of higher fatty acids and fatty alcohols. Natural waxes were obtained from sperm whales (*Physeter microcephalus*) and were characterized by physical properties important for automotive industry. However, the conventional methods of obtaining waxes involve environmental interference and ethical concerns, thus alternative approaches for their production may be processes using microorganisms.

Yarrowia lipolytica, due to its oleaginous nature, offers a promising alternative for wax production. It can use a wide range of waste substrates, including volatile fatty acids (VFAs), naturally produced during the anaerobic decomposition of organic waste, which appear to be interesting substrates for wax production. The three main VFAs generated in this process are acetic acid, propionic acid, and butyric acid.

This study focuses on metabolic engineering of *Y. lipolytica* to produce waxes. Six wax synthases from: *Tyta alba*, *Xenopus tropicalis*, *Homo sapiens*, *Phytophthora nicotianae*, *Marinobacter hydrocarbonoclasticus* and *Acinetobacter baylyi* were investigated. Furthermore, fatty acid reductase from *Marinobacter aquaeolei* was introduced into the genome. Cultures using glycerol and VFAs were conducted to analyze wax biosynthesis. The results demonstrate that *X. tropicalis*, *H. sapiens* and *M. hydrocarbonoclasticus* were the most efficient wax synthases. It was also observed that among the VFAs, the most efficient carbon source for synthesizing waxes was butyric acid. Bioreactor cultures for the best wax producing strain yielded around 2.5 g/L of waxes.

Founding: NCN, Poland, OPUS19 (No 2020/37/B/NZ9/02985) "Utilization of volatile fatty acids for waxes biosynthesis by the yeast *Yarrowia lipolytica*".

ID: 325; Stand: D38

Genetic Optimization of Bio-based Hemoprotein Production by Yeast Cell Factories

August Frost, Irene Jacobsen, Tomas Strucko, José Martinez

Technical University of Denmark, Denmark

Presenter: August Frost

In the event of acute blood loss, it is crucial to re-establish adequate oxygen transport to tissues in order to survive. Accordingly, blood loss is conventionally treated with blood transfusion, which is a common procedure, as about one in seven hospital patients needs blood. Nevertheless, even in the 21st century, the inherent nature of this lifesaving intervention is associated with a multitude of side effects, limitations, and complications. To address these issues, ongoing research is focused on developing hemoglobin-based oxygen carriers (HBOCs) as an alternative to traditional blood transfusions. Compared to donated blood, HBOCs have many advantages, such as eliminating the risk of blood-borne diseases, extending the shelf life, ensuring universal compatibility between blood types, simplifying the storage requirements, and being independent of donors.

However, the current state of HBOCs involves bio-based production challenges of recombinant human hemoglobin in yeast cell factories, which is the obstacle that this work attempts to solve. As part of the solution, improvements to multiple phases of the bio-manufacturing process from the genetic modification of yeast to the purification of human hemoglobin have been achieved. This, for example, includes (I) optimization of analytical techniques for heme/hemoglobin measurements, (II) reducing degradation of intermediates and hemoglobin, (III) understanding of oxygen regulation by -omics for increased heme formation, (IV) high-throughput screening of large heme-optimized strain library to identify best combinations of genetic modifications, (V) assessing the compatibility of these best combinations with other hemoproteins, and finally (VI) exploring the potential of other non-conventional yeasts.

Assessing the effect of ALE mutations on the sugar sensing and signaling of industrial xylose-engineered strains

Gisele de Lima Palermo^{1,2}, Viktor Persson², Marie Gorwa-Grauslund², Leandro Vieira dos Santos^{1,3}

¹State University of Campinas (Unicamp), Brazil. ²Lund University, Sweden. ³University of Manchester, United Kingdom

Presenter: Gisele de Lima Palermo

The need of sustainable sources has unveiled the potential of lignocellulosic biomass as the main carbon supply for biochemical production by *Saccharomyces cerevisiae*. Although this yeast is an established cell factory in industry, it does not natively consume the five-carbon sugar xylose—the second most abundant sugar in lignocellulosic biomass. Over the last decades, genetic engineered strains have been developed for xylose fermentation, but they still display lower consumption rates compared to glucose. Xylose sensing and signaling are suspected to be, alongside transport, a major bottleneck for xylose utilisation. It has been shown that *S. cerevisiae* does not recognize xylose as a fermentable carbon source which leads to inefficient metabolism. Through ALE and reverse genetic engineering, we identified several mutations in non-obvious targets that improved xylose fermentation rate. In this project, we investigate whether and how these mutations affect xylose sensing using biosensors to monitor the three main sugar signaling pathways (Snf3p/Rgt2p, cAMP/PKA and SNF1/Mig1p pathways) through flow cytometry. The biosensors were integrated for the first time in an industrial strain of *S. cerevisiae* using CRISPR/Cas9 and were validated in different carbon sources. Currently, we are expanding the analysis to monitor the changes promoted on sugar signaling due to mutant variants of genes identified by ALE. The results are expected to expand our understanding of the role of sugar sensing on C5 fermentation and how engineered evolved strains may change their sensing to fine-tune xylose metabolism, aiming the design of efficient xylose-fermenting strains to convert biomass-derived sugars into bioproducts.

ID: 343; Stand: D40

Comparison of yeast cells immobilization procedures for biosensor applications

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Presenter: Ohiemi Benjamin Ocheja

A biosensor is a device that consists of a transducer in conjunction with a biorecognition element which converts a biochemical signal into a quantifiable signal. Immobilization is an important step in biosensor development as it brings the biorecognition element and the transducer in close contact and increase the stability of the biorecognition element thus enhancing the operational and storage stability of the biosensor. Here we carried out and compared two immobilization procedures for biosensor applications. In both cases, yeast cells in exponential phase of growth were used. For the first protocol, immobilization was performed in sodium alginate and cellular metabolic activity was assayed by HPLC. For the second protocol, polydopamine (PDA) was used as a matrix for immobilization of the cells on a glass carbon electrode and electron transfer was assessed by cyclic voltammetry and chronoamperometry. In terms of time, immobilization in sodium alginate beads took 1.5 h, while that of PDA was 3 h. PDA mimics the adhesive mussel byssus that allows adhesion in underwater conditions, thus PDA immobilization could be applied in electrochemical biosensors development. Conversely, sodium alginate has been used in optical and amperometric biosensors. Equipment requirement for PDA immobilization experiments includes electrodes, electrochemical cell and potentiostat connected to a computer while metabolism of yeast in alginate beads was assayed using a HPLC machine. Equipment cost is less in the PDA than in the Na alginate immobilization experiment. Finally, the choice of immobilization procedure will depend on the application of the biosensor.

ID: 345; Stand: D41

Exploiting the capacity of *Debaryomyces hansenii* for the production of recombinant proteins using industrial salt-rich by-products as feedstock

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¹Technical University of Denmark, Denmark. ²Novo Nordisk, Denmark. ³Arla Foods, Denmark

Presenter: Monica Estrada

Debaryomyces hansenii (*D. hansenii*) is a halotolerant non-conventional yeast that can grow in high salinity environments (up to 4 M NaCl), metabolize several carbon sources (e.g., lactose, glycerol, arabinose, and xylose), withstand a wide range of temperatures and pH, and tolerate some fermentation inhibitors. These inherent characteristics allow *D. hansenii* to grow in harsh environments and use alternative feedstock, like industrial by-products. Recently, efficient molecular tools have been developed to engineer this yeast, facilitating the generation of strains with knockouts and knockins.

The dairy industry produces vast amounts of food and water waste daily, which is still rich in nutrients but contains high concentrations of salts. Moreover, the biopharmaceutical industry also generates several salty by-products derived from their downstream processes. These effluents must be treated before being dumped into the waterways, which is usually tedious and expensive. Therefore, finding biological alternatives to revalue these by-products is a crucial need.

The aim of this project is to demonstrate *D. hansenii*'s ability to grow and produce recombinant proteins (e.g., Yellow Fluorescent Protein (YFP)) from several salty industrial by-products. Cultivations at different laboratory scales (1.5, 100, and 500 mL) are performed without either sterilizing the medium or using pure water. Our results conclude that *D. hansenii* can grow and produce YFP without altering its performance from all by-products studied. Interestingly, it has priority to grow over other inherent microorganisms already present in the dairy by-products.

Laboratory evolutionary mutagenesis as a tool to increase riboflavin production by yeast on lignocellulose hydrolysates

Dominik Wojdyła¹, Justyna Ruchała¹, Andriy Sybirnyy^{1,2}

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²Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine, Ukraine

Presenter: Justyna Ruchała

Riboflavin, also known as vitamin B₂ is a precursor of FMN and FAD, which play a key role as cofactors in energy metabolism and in numerous oxidation reactions in all aerobic forms of life. Thanks to many genetic modifications and the use of modern methods of metabolic engineering, *Candida famata* yeast can still compete with other species used in industry for the riboflavin production. Taking into account the fact that one day natural energy sources will run out, the search for alternative energy sources has already begun and measures have been taken to reuse industrial waste, that can be reused to produce useful compounds such as vitamin B₂. As we have shown, the use of lignocellulosic hydrolysate (HL) from wheat straw, can be used as an alternative for the production of riboflavin. The most efficient strains in minimal medium with 20% HL were able to achieve a productivity of 320 mg RF/g biomass. Inhibitors created during lignocellulose hydrolysis have a negative effect on cells, which is why we decided to use evolutionary mutagenesis to select cells better adapted to difficult conditions. The strains were exposed to UV radiation, which further increased the mutation rate. The obtained mutants were characterized by better growth and/or better production of riboflavin. The best of them achieved a productivity of 350 mg RF/g of biomass. The maximum concentration of riboflavin in the medium during fermentation for the mutant was about 660 mg/L, when initial strain value was 600 mg/L.

Lactic acid synthesis by recombinant strains of thermotolerant yeast *Ogataea polymorpha*

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Presenter: Aksyniia Tsaruk

The global market for lactic acid is rapidly growing due to its wide range of utilization in industries producing food, chemicals, pharmaceuticals. This compound is mainly obtained from microbial fermentations. Yeast appears as one of the most promising lactate producers compared to other organisms, considering their ability to utilize cheap and renewable substrates along with tolerance to environmental factors that would allow a decrease in production cost. Usually, yeasts do not have genes required for lactate biosynthesis, however, metabolic engineering enables introducing heterologous lactate dehydrogenase genes for lactate production.

Lactate dehydrogenase genes from *Rhizopus oryzae* and *Bos taurus* were expressed under control of the strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase gene and introduced into the wild type strain of the yeast *Ogataea polymorpha*.

Lactate production by selected *O. polymorpha* transformants was studied in several fermentation conditions including different carbon sources, agitation rates, and addition of calcium carbonate as a neutralizing agent. The maximum amount of lactate produced by obtained transformants with bovine lactate dehydrogenase reached 18 g/L on medium with 10% xylose, high agitation and calcium carbonate supplementation, whereas with lower agitation and without neutralizing agent it was 6-fold lower. The maximum amount of lactate produced by the same transformants was 4.5 g/L on medium with 10% glucose high agitation and addition of calcium carbonate. Meanwhile, transformants with fungal lactate dehydrogenase produced maximum of up to 20 g/L and 12 g/L lactate on medium with 10% glucose and 10% xylose respectively, with lower agitation and medium neutralization.

**Poster session: “Yeast ecology and fermentations:
the intimate relationship of *S. cerevisiae* and
fermented foods”, “Yeast Commensalism &
Pathogenesis”**

Friday 25th August, 13.30-15.30

ID: 169; Stand: E01

Metabolic and fermentative screening of *Saccharomyces cerevisiae* hybridizable strains to optimize beer strains

Roberto Pérez-Torrado, David Roldán-López

Instituto de Agroquímica y Tecnología de los Alimentos, IATA-CSIC, Paterna., Spain

Presenter: Roberto Pérez-Torrado

A new way to produce low alcohol beers is the use of unconventional yeasts and their hybrids. For this, it is necessary to carry out metabolic studies in search of new strains that present a lower production of ethanol, but that, in turn, have a good fermentative and aromatic profile. For this reason, our group has carried out a metabolic study on more than 300 yeast strains that represent different subpopulations within the species of the genus *Saccharomyces* and within other genera of the family *Saccharomycetaceae*. In summary, yeasts genetically close to *S. cerevisiae* that can potentially hybridize with it. The fermentative profile of the yeasts under these conditions has been studied, through the production of CO₂ and the production of the main metabolites and aromas. This study has revealed the variability that exists between the different populations, which offers a wide variety of yeasts that could be candidates to be industrially exploited or used for the generation of hybrids with the aim of reducing the alcoholic content, generating new aromatic profiles or to solve other problems in the brewing industry.

Biodiversity of fermenting yeasts from marula fruit (*Sclerocarya Birrea*) in Botswana: A relook into the marula and elephant inebriation myth.

Tawanda Makopa, Nerve Zhou

Botswana International University of Science and technology, Botswana

Presenter: Tawanda Makopa

The inebriation of wild African elephants from eating the ripened and rotting fruit of the marula tree is a persistent myth in Southern Africa. The myth is in conjunction with several anecdotal reports of animals getting drunk of naturally occurring alcohol. However, the yeasts responsible for alcoholic fermentation to intoxicate the elephants, as a first step to debunk or prove the myth, remain poorly documented. In this study, we considered Botswana, a country with the world's largest population of wild elephants and where the marula tree is indigenous, abundant and protected to assess the occurrence and biodiversity of yeasts with a potential to ferment and subsequently inebriate the wild elephants. We collected a total of 74 marula fruit samples from 21 different locations over a stretch of 800 km in Botswana. A total of 106 yeast strains representing 24 yeast species, including known ethanol producers belonging to the following genera; *Saccharomyces*, *Pichia*, *Brettanomyces*, and *Wickerhamomyces* among others. Assessment of the fermentative capacity of selected isolates revealed convincing credentials to warrant spontaneous fermentation with a potential to suggest that inebriation of elephants in nature could be possible. Evaluation of volatile organic compounds produced, as another probable factor influencing elephant foraging behaviour on the fermenting fruits, revealed complex aroma profiles for the selected yeast isolates. Therefore, considering prevalence of the fruits, substantial quantities of ethanol generated from isolated fermentation process in this investigation, and the potential for greater quantities when mixed consortia undergo spontaneous fermentation, it can be inferred that there is insufficient evidence to refute the aforementioned myth.

Influence of cocoa bean fermentation methods on fungal communities as revealed by metagenomics approaches

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Presenter: Edoardo Puglisi

The process of cocoa fermentation is spontaneous and influenced by numerous variables: dissimilarities in the final product are thus often substantial and unpredictable. A greater resolution and detection sensitivity of the main fermenting microbial groups can be achieved by high-throughput sequencing (HTS) of phylogenetic amplicons. However, this approach may be influenced by DNA extraction methods and the specificity of primers, which may affect the range and variety of microbial populations being studied.

The aim of the study was to analyze through high-throughput sequencing (HTS) of phylogenetic amplicons how three different fermentation methods (box, ground, and jute) affect the microbial populations in cocoa beans. Furthermore, the most suitable fermentation method was determined based on the observed changes in microbial communities. Focusing the attention on fungi, ground fermentation had a wider fungal community. *Pichia kudriavzevii* was observed in all three fermentation methods, while *Hanseniaspora opuntiae* was the most relevant yeast in jute and box and *Saccharomyces cerevisiae* dominated in the box and ground fermentation. Overall, the study revealed significant differences among the three fermentation methods, with the box method being the most favorable due to its limited fungal diversity and the presence of microorganisms that ensure a good fermentation.

Furthermore, this study enabled a comprehensive exploration of the microbiota in differently processed cocoa beans and a better understanding of the technological processes necessary to achieve a standardized final product.

***Starmerella bacillaris* - *Saccharomyces cerevisiae* interaction during sequential fermentations influences the release of yeast mannoproteins and impact the protein stability of an unstable white wine**

Luiza de Paula Dias Moreira^{1,2}, Davide Porcellato², Matteo Marangon^{3,1}, Chiara Nadai^{3,4}, Vinícius da Silva Duarte², Tove Gulbrandsen Devold², Alessio Giacomini^{1,3}, Viviana Corich^{1,3}

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Presenter: Chiara Nadai

Protein haze formation is the most common non-microbiological defect in white wines due to the aggregation of grape proteins during wine storage. Yeast cell wall components, particularly glycosylated proteins as mannoproteins, have a protective effect against haze formation, although their involvement remains poorly understood.

This study aimed at characterizing glycosylated proteins, and their polysaccharide fractions, released by *Starmerella bacillaris* and *Saccharomyces cerevisiae* during single and sequential fermentations, and to test their impact on the protein stability of a white unstable wine. Mannoproteins-rich extracts obtained from the supernatant of sequential fermentations showed an increase in the low MW polysaccharide fraction and improved the protein stability of a heat unstable white wine compared to the *S. cerevisiae* extracts.

Shotgun proteomic analysis on purified extracts revealed that the identified cell wall proteins exclusively found in the sequential fermentations were produced by both *S. bacillaris* (MKC7 and glucan endo-1,3-beta-D-glucosidase 1) and *S. cerevisiae* (Bgl2p). Moreover, the sequential fermentations significantly increased the expression of Scw4p and 1,3 beta-glucanosyltransferase similar to GAS5, produced by *S. cerevisiae*. They were found to be expressed during *S. cerevisiae* exponential growth phase, suggesting that the presence of *S. bacillaris* affected the correct cell wall assembly of *S. cerevisiae*, slowing down fermentation activity. All these findings demonstrated a strong interaction between *S. bacillaris* and *S. cerevisiae* that deeply influenced the mannoproteins-rich extracts composition. Finally, results suggested that some of the key proteins identified might play a key role in the observed increase in wine protein stability.

Deciphering Wine Yeast Community Dynamics: Insights from Machine Learning-Driven Approaches

Cleo Conacher^{1,2}, Bruce Watson^{2,3}, Florian Bauer¹

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Presenter: Cleo Conacher

The ecology of the wine yeast community (WYC) plays a crucial role in wine fermentation, yet fully understanding the dynamics within these communities remains a challenge. Current research aims to systematically harness natural WYCs to optimize fermentation performance and outcomes. However, implementing such strategies is challenging since there is a significant gap in predictive understanding of the mechanistic foundations of WYCs. Consequently, we have accumulated descriptive datasets that only superficially evaluate WYC, with minimal mathematical description or computation. In this context, machine learning (ML) emerges as a robust tool, capable of navigating this complexity, and offering the potential to transform these descriptive datasets into predictive models, thereby facilitating a deeper understanding of WYCs. This study presents a comparison of gradient boosted regression (GBR) and deep learning ML methods to evaluate the temporal population dynamics within a WYC. The models were trained on time-series data from experimental WYCs with step-wise increases in complexity, aiming to predict and analyse the dynamics of co-existing yeasts during growth in synthetic grape must. Model performance metrics were compared to assess each algorithm's ability to predict population changes throughout the fermentation process. Furthermore, comparisons were made of the insights gained from each model type. The results demonstrate the potential of ML to reveal novel insights into the temporal dynamics within complex WYCs. By leveraging the strengths of both GBR and deep learning approaches, researchers can develop a deeper understanding of WYC dynamics that can be applied in improving design and control of synthetic WYCs.

ID: 222; Stand: E06

Kazachstania yeasts may lower bread fructan content in extended dough fermentations.

Anna Wittwer, Rudi Appels, Kate Howell

The University of Melbourne, Australia

Presenter: Anna Wittwer

Kazachstania is a common yeast genus found in sourdough starters, and since sourdough bread tends to have a lower fructan content than non-sourdough bread, we are interested in the potential ability of Kazachstania yeasts to directly metabolise bread fructans. We used a Maximum Likelihood analysis to investigate the genetic similarities between Kazachstania yeasts derived from sourdoughs and those associated with other environments. Most sourdough-associated yeasts are found in one sub-clade of the genus. We performed several tests on a collection of *K. humilis* and *K. bulderi* yeasts, using inulin and fructo-oligosaccharides (FOS) as test carbohydrates. When grown on inulin- and FOS-supplemented media, we found that the *K. humilis* yeasts grew better than the *K. bulderi* yeasts on inulin, suggesting that the invertases of *K. humilis* may perform better on longer substrates. We then compared the ability of extracellular and cellular fractions of yeast cultures to metabolise FOS and inulin and found that FOS degradation was greater for the cellular fractions, suggesting that most of the enzymes responsible may be cell wall bound. Fermentation time in breadmaking can be a significant variable and our initial studies indicate that Kazachstania yeasts require longer than 12 hours to metabolise FOS, which indicates that longer fermentations may be necessary to make low-fructan bread. These data demonstrate that ability to break down fructans varies between Kazachstania strains and species, and that manipulation of fermentation parameters can contribute to the effectiveness of yeast metabolism in bread.

ID: 255; Stand: E07

Probiotic potential of some yeast sps. isolated from an ethnic beverage

BABITA RANA, GOPAL KRISHNA JOSHI

HEMVATI NANDAN BAHUGUNA GARHWAL UNIVERSITY, SRINAGAR GARHWAL, India

Presenter: Babita Rana

In the present era of IPR, this is of utmost importance to scientifically document and explore the commercial applications of ethnic food and beverage making practices prevalent in various part of the country. We have investigated probiotic potential of yeast sps. isolated from the fermentation broth of Gaingti which is a traditional alcoholic beverage commonly prepared in Jaunsar- Bawar tribal region of Uttarakhand, India. The investigation resulted in the identification of ten potent yeast strains with probiotic properties such as tolerance to bile salt and low pH, lysozyme resistance, antibiotic resistance, antimicrobial action, exopolysaccharide production, β -galactosidase, haemolytic and gelatinase activity. ITS (internal transcribed spacer) rDNA sequencing led to the identification of yeast strains as *Saccharomyces cerevisiae* BRGJ1, *Saccharomyces cerevisiae* BRGJ2, *Saccharomyces cerevisiae* BRGJ3, *Saccharomyces cerevisiae* BRGJ6, *Saccharomyces cerevisiae* BRGJ7, *Saccharomyces cerevisiae* BRGJ8, *Saccharomyces cerevisiae* BRGJ9, *Saccharomyces cerevisiae* BRGJ10, *Saccharomyces cerevisiae* BRGJ11, *Saccharomyces cerevisiae* BRGJ12, *Pichia kudriavzeii* BRGJ4 and *Suhomyces xylopsoci* BRGJ5.

Enhancing Fermentation Efficiency: Harnessing Ultrasound Irradiation for Rapid Process Optimization

Giulia Chicarella¹, Federico Ortenzi^{2,3}, Luca Buccarello², Alessandro Contaldo², Federico Montereali², Tommaso Mancini², Roberta Congestri³, Andrea Maselli⁴, Blasco Morozzo della Rocca⁵

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Presenter: Giulia Chicarella

The project aims to improve fermentation processes by utilizing ultrasound irradiation. Previous studies demonstrate a correlation between ultrasound and an enzymatic activity. Also frequency, power and the microorganism used can produce different responses. In this project we used a fermenter equipped with a transducer that emits ultrasound waves into the wort, and two boards, one internal to the lid and one external. Preliminary experiments comparing irradiated and non-irradiated samples revealed up to a 25% reduction in fermentation time without harming nor damaging the yeast cells. We performed different analyses including the cell count, for which we noticed a greater number of cells in the irradiated sample compared to the control, also confirmed by the optical density at 600nm that showed a greater absorbance in the treated one. To value the hypothesis of the increased transport of molecules, intracellular calcium was evaluated with a fluorescent indicator, also in this case an increase in calcium was noted after the treatment with ultrasounds. Temperature and pH were monitored in the process and no significant differences were reported between the two samples. Finally, the total content of phenols was also evaluated, which shows an increase with the treated, also advancing the hypothesis of a possible influence of ultrasounds on the organoleptic characteristics of the product. Further analysis and experimentation are required to confirm this hypothesis and gain deeper insights into the underlying mechanisms. Successful implementation of this technology can lead to more efficient and sustainable practices in the food and industrial sectors.

Improvement of a Method for Evaluating the Activation of the TORC1 Signaling Pathway in *Saccharomyces cerevisiae*

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Presenter: Eduardo I. Kessi-Pérez

Saccharomyces cerevisiae is the main species responsible for wine fermentation, one of the main problems being the deficiency of nitrogen sources in the grape must, which can cause stuck or sluggish fermentations. A great challenge is to identify the genetic basis underlying the phenotypic variability in nitrogen consumption and metabolism, with emphasis on the study of the TORC1 signaling pathway, given its central role in responding to nitrogen availability and influencing growth and cell metabolism. In addition, another interesting aspect to study is the effect that the domestication process could have had on TORC1 activation in relation to wine fermentation. In a previous work, we developed a method to assess the activation of the TORC1 pathway of a large number of strains, using the luciferase gene as a reporter, which allowed us to identify genes linked to this phenotype through QTL mapping using a recombinant biparental population. In the present work, we have improved this method to allow the phenotyping of strains belonging to the “1002 Yeast Genomes Project” population, which represents the most complete catalogue of genetic variation in *S. cerevisiae*. The improved method was tested on representative strains of two of the clean lineages described in the species (Sake 'SA' and Wine/European 'WE'), obtaining concordant results between both methodologies. This opens the possibility of using this new method to phenotype this entire population, which will allow us to assess the effect of domestication on TORC1 activation and identify new genetic variants linked to this phenotype.

Comparative transcriptional analysis of oenological yeast strains and their SUL mutants under varying sulfate concentrations during wine fermentation

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Presenter: Edoardo Bizzotto

In this study, we conducted an RNA-Seq analysis to investigate the transcriptional response of two oenological *Saccharomyces cerevisiae* strains under different sulfate concentrations. The strains, D20 and CE2P, were selected from a private collection according to their phenotypic characters, which include fermentation performance and their distinct properties regarding sulfate metabolism. While in controlled laboratory conditions D20 can reach the fermentation peak in 48 hours, CE2P peaks at 72 hours. Additionally, CE2P produces two times the SO₂ amount of D20. The two strains then had their sulfur assimilation genes SUL1 and SUL2 inactivated to investigate the specific metabolic alterations in the absence of the two main sulfate transporters. Starting from wild-type and mutated strains, two different concentrations of sulfates, 500 mg/L, and 1500 mg/L, were added to the must. By using grape must previously characterized, RNA was extracted at the fermentation peak. Considering the wild-type and mutant strains and the different concentrations of sulfates, we analyzed a total of eight different conditions. Our findings highlighted strain-specific differences in how genes are involved in sulfate assimilation. From the comparison with mutated strains at different sulfate concentrations, it was possible to highlight the use of alternative ways for the accumulation and disposal of sulfur metabolites in the cell. Overall, this study highlights the potential of RNA-Seq analysis in the study of gene expression and transcriptional mechanisms for investigating oenological yeast strains with the objective of improving the wine quality while reducing the concentration of undesired compounds in the final product.

A predictive bioinformatics tool for biopeptide analysis and functional classification in yeast and bacterial fermented foods

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Presenter: Edoardo Bizzotto

Yeast is one of the most important and used microorganisms in fermentation processes. *Debaryomyces*, *Saccharomyces*, and *Zygosaccharomyces* species can be used in the production of biopeptides from food waste through microbial fermentation. Biopeptides are produced during fermentation through proteolysis and the diverse proteolytic abilities, together with the variability in substrates can significantly impact the quality and yield of the generated biopeptides. To address these challenges, in-silico bioinformatics analyses can be employed to evaluate suitable substrates and protease enzymes for the fermentation process. However, existing bioinformatics tools lack the capability to accurately predict yeast proteases, assess their performance on protein sequences within the substrate, and characterize biopeptide families. To overcome these limitations, we have developed FEEDS, an innovative biopeptide prediction and classification tool. FEEDS can predict the biopeptide families that can be produced based on the identified protease profiles in yeast genomes and the composition of the substrate undergoing proteolysis. Furthermore, the tool utilizes a machine-learning approach to assist in the functional classification of the biopeptides. Through in-silico testing conducted on over 1000 microbial genomes, we have gained insights into the performance of the tool and the machine learning models. The results obtained provide evidence for the effectiveness of biopeptide prediction, as FEEDS categorizes the majority of peptides originating from proteins of *Hordeum vulgare* and *Vitis vinifera*. These findings support conclusions drawn from biochemical tests documented in literature, further validating the importance of our biopeptide prediction tool for the in-silico preliminary assessment of microbial species and substrates selection.

Diversity of wine yeasts communities associated with social wasps occurring in the vineyard, at harvest time, in three DOCG areas in Tuscany.

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Presenter: Damiano Barbato

Recent studies on the yeast-insect associations demonstrated that populations of social wasps of the genera *Polistes*, *Vespa*, and *Vespula* act as a reservoir for the natural conservation of yeasts and as vectors capable of transferring such yeasts on the grapes. Therefore, this work was aimed to quantify the wine yeast communities occurring, at harvest time, on the exoskeleton, in the intestine of social wasps and on the surface of the grapes sampled in the vineyards of three companies of different DOCG areas in Tuscany: Chianti Classico, Brunello di Montalcino and Nobile di Montepulciano. In total, 256 yeast isolates belonging to 20 species frequently associated with the winemaking process and divided into 74 different strains were obtained. Independently of the oenological area, the yeast communities associated with the wasps showed similar levels of biodiversity and *Metschnikowia pulcherrima* was always found within the wasp gut as the prevalent yeast species. Several strains of this species showed β -glucosidase activity that can increase the aromatic complexity of the wine, and one strain produced the pulcherrimin pigment, which inhibited the development of the spoilage yeast *Brettanomyces bruxellensis*, proving to be a suitable biocontrol agent. A few *Kloeckera apiculata* and *M. pulcherrima* strains occurred also on the grapes. These findings confirmed the role of social wasps as yeast vectors in the vineyard and demonstrated that these insects are a natural reservoir of yeast strains to be exploited for biotechnological applications in oenology, such as for aroma improvement or the reduction of using chemical compounds.

Exploring wine yeast natural biodiversity to select strains with enological traits adapted to climate change

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Presenter: Manuela Côrte-Real

Wine is widely consumed throughout the world and represents a significant financial market. Unfortunately, climate change threatens many of the wine-growing regions. Winemakers aim to produce a consistent product every year; however, increases in environmental temperatures often lead to imbalanced grape physiology, altering the finished wine. As a consequence of climate changes, grapes at phenolic maturity will contain higher levels of sugar and spoilage microorganisms, resulting in wines with higher ethanol levels and volatile acidity, and decreased fixed acidity among other undesirable qualities. Grape must fermentation often depends on inoculated yeasts commonly hand-selected for specific properties; however, most yeasts often struggle to perform healthy fermentations or to complete fermentation under the aforementioned conditions. Thus, there is a need to identify yeasts that can yield wines with moderate ethanol and residual volatile acidity, from these challenging grape musts.

Herein, we characterized underexplored repositories of naturally occurring *Saccharomyces cerevisiae* strains isolated from wine environments, regarding adequate enological properties, as well as other less frequently investigated properties (e.g. osmotic stress, radiation-mitigating agents and fungicides resistance). Isolate's phenotypic data were organized in a biobank, and bioinformatics analysis grouped them according to their characteristics. Furthermore, we analyzed their potential to ferment musts with high sugar levels and uncovered several promising candidates. We expect that generating comprehensive robust information of the natural biodiversity will represent a step forward for knowledge-based decision in winemaking.

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ID: 70; Stand: E14

Enhancing Higher Education Learning of Yeast Biology: Valuable Resources in Educational Journals

Massimiliano Marvasi

Università degli Studi di Firenze, Italy

Presenter: Massimiliano Marvasi

In the last 30 years, there have been numerous proposals for curricula aimed at advancing students' knowledge of yeast biology in higher education. To enhance the learning of yeast biology, a diverse range of teaching strategies should be employed, including laboratory activities, workshops, flipped classrooms, and long-semester Course-Based Undergraduate Research Experience (CURE). CUREs, for example, involve whole classes of students in addressing a research question or problem that is of interest to stakeholders outside the classroom, providing an excellent opportunity for students to apply their knowledge and skills to real-world scenarios.

To show an example, the Journal of Microbiology & Biology Education, published by the American Society for Microbiology (ASM), reports several activities that can be used to teach yeast biology, covering both basic and applied science. Examples include: environmental yeast isolation to brewing, selection of auxotrophic isolates, generation simple gene knockout activities, CRISPR-Cas9 gene editing in yeast, microarrays, bio-art, modeling of 3D printed cells, fungal identification via DNA and MALDI-TOF, to cite some.

These resources are not only intended for biology students, but also for other actors such as nursing practitioners, medical and biotechnology students.

The educational journals are a valuable resource for undergraduate and graduate-level yeast education and are readily available to readers, as they are typically free of charge. By consulting these journals when developing a microbiology course that includes yeast and fungal biology, educators can enhance the learning experience and provide their students with valuable knowledge and skills.

Parrotfish microbiome and its role in the coral reef ecosystem

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Presenter: Andrea Quagliariello

Coral reef islands are a unique landforms composed by sediments derived from the surrounding coral reefs, which are particularly sensitive to climate change. Recently, parrotfishes have been identified as the main actor in the formation of such islands. Particularly, excavator parrotfish species contribute for >80% of the new coral sediment production, which constitutes the substrate for reef islands formation. To date, how parrotfish microbial symbionts reacts to coral ingestions and their role in sediment formation remains elusive. To this end, we collected both oral and rectal microbiome samples from 8 different species of parrotfish, as well as corals and seawater samples at the Maldives islands (Magoodhoo reef). Preliminary analyses, based on 16S rRNA amplicon sequencing, show that the gut ecosystem is richer than the oral one in term of bacterial diversity and that both are poorly influenced by coral microbiome. Moreover, the gut ecosystem is characterized by a strong phylogenetic diversity between the excavator and scraper species, suggesting that the different feeding behaviour, as well as the different intake of coral in their diet shape the composition of the host microbiome.

Study of Caspofungin sensitivity of strains of *Candida spp.* in the intensive care unit of the Fattouma Bourguiba University Hospital in Monastir, Tunisia

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Presenter: Sameh Belgacem

Introduction: *Candida* fungal infections are increasingly common in intensive care units. Caspofungin is the first-line treatment for the management of these infections. Our objective is to study the sensitivity of *Candida spp.* to caspofungin and to evaluate the effect of overconsumption of this antifungal on the evolution of minimum inhibitory concentrations (MIC).

Materials and methods The study involved 223 strains of *Candida spp.* isolated from the CHU Monastir intensive care unit during the period from January 2018 to May 2021. The MICs were determined according to the Clinical & Laboratory Standards Institute (CLSI) reference method. The strains categorized as resistant were sequenced at the level of the FKS genes and the relationship between the MICs and the consumption of caspofungin was attributed using the SPSS software.

Results: The prevalence of resistance in the intensive care unit was 5.8%. One-third of *Candida glabrata* isolates tested were categorized as resistant, of which only one strain had cross-resistance to anidulafungin. This strain carried an F625del deletion in the HS1 region of the FKS1 genes. The evolution of MIC values was significantly correlated with the consumption of Caspofungin for *Candida albicans* and *Candida tropicalis*.

Conclusion: there was probably an overestimation of resistance to caspofungin by referring to the threshold values established by CLSI taking into account the absence of mutation in the FKS regions and the absence of cross-resistance to Anidulafungin.

SACCHAROMYCES CEREVISIAE AS A SURROGATE HOST TO STUDY (+)RNA VIRUS REPLICATION

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Presenter: Sergio Giannattasio

Positive-strand RNA [(+)RNA] viruses are agents of important diseases in humans, animals and plants, including COVID-19. Regardless of the host, the replication of all (+) RNA viruses occurs in association with the host endomembrane system. Based on this common replication mechanism, we have used the yeast model to express the replication-associated proteins of one plant (carnation Italian ringspot virus, CIRV) and one human (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) (+)RNA virus to decipher virus-membrane interactions, with the final aim of identifying host factors co-opted for viral replication, to develop new antiviral strategies.

We showed that CIRV p36 expression in *S. cerevisiae* strain YPH499 (i) increased necrotic cell death and concomitantly decreased regulated cell death in response to acetic acid; (ii) decreased respiratory yeast cell growth; (iii) altered the mitochondrial network; (iv) decreased oxygen consumption due to respiratory chain complex impairment. SARS-CoV-2 non-structural proteins nsp3 and nsp4, but not nsp6 expression, significantly reduced cell growth. Viability was significantly reduced in nsp4-expressing cells. nsp3, nsp4 and nsp6 sedimented in a membrane-enriched fraction, indicating the association of SARS-CoV-2 nonstructural proteins with cell membranes. Immunofluorescence analyses showed that nsp3, nsp4 and nsp6 localized to the endoplasmic reticulum in *S. cerevisiae* cells, and not to mitochondria or Golgi apparatus. Targeting properties of SARS-CoV-2 non-structural proteins expressed in yeast are maintained and mirror those in their natural hosts. *S. cerevisiae* is confirmed as amenable and safe surrogate model host to study (+)RNA virus replication and possibly identify novel druggable targets to develop antiviral strategies.

Functional analyses of missense *COQ7* pathogenic variants and evaluation of new options for the treatment of CoQ₁₀ deficiency using yeast *S. cerevisiae*

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Presenter: Martina Magistrati

Primary CoQ₁₀ deficiency is a group of inborn errors of metabolism determined by defects in CoQ₁₀ biosynthesis. Mutations in the *COQ7* gene, encoding the mitochondrial 5-demethoxyubiquinone hydroxylase, have been identified in patients presenting CoQ₁₀ deficiency. Yeast *Saccharomyces cerevisiae*, a widely used model organism to study mitochondrial diseases, has been exploited to validate and characterize the newly identified and previously reported mutations thanks to the presence of the ortholog gene *CAT5*. In particular, human variants (p.Arg54Gln, p.Ile66Asn, p.Tyr149Cys, p.Arg107Trp, p.Val141Glu, and the compound p.Leu111Pro+p.Thr103Met) have been studied by creating the equivalent yeast strains (*cat5*^{R57Q}, *cat5*^{I69N}, *cat5*^{Y154C}, *cat5*^{R112W}, *cat5*^{V146E}, and *cat5*^{K108M+L116P}); all variants have been validated and can be divided into severe mutations or leaky mutations based on the oxidative growth defect and the residual respiratory activity of the strains.

No effective treatment exists for CoQ₁₀ deficiency; supplementation of 2,4 dihydroxybenzoic acid (2,4-diHB) was previously shown to rescue the oxidative growth defect of *cat5Δ* yeast strain if the CoQ₁₀-biosynthesis complex was stabilized by *COQ8* overexpression. We observed that the supplementation with 2,4-diHB, with or without *COQ8* overexpression, rescues the oxidative defect of both the leaky and severe mutants. Furthermore, the defective CoQ production of *cat5*^{Y154C} and *cat5*^{V146E} strains leads to a reduction in the respiratory complex subunits (RC) levels, and this defect is completely rescued by the “combo” treatment (2,4-diHB + *COQ8*).

Overall, the present study enables to expand the phenotypic spectrum of *COQ7*-associated primary CoQ₁₀ deficiency and leads to the identification of a novel potential treatment for CoQ₁₀ deficiency.

Gut mycobiota and risk stratification in pediatric Crohn's disease. Comparative immunophenotyping of *Saccharomyces cerevisiae* and *Candida spp.* strains

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Presenter: Monica Di Paola

Investigation of the gut fungal communities in animal models of Inflammatory Bowel Diseases (IBD) showed a controversial role of *Saccharomyces cerevisiae* and *Candida spp.* in health and disease. Current approaches on gut microbiota investigation involved in IBD pathogenesis failed to achieve the expected goal since the analyses were conducted to the species level, while recent evidence show that the determinants of the inflammatory or tolerogenic response, associated to the disease, are at the strain level. To understand how the gut microbial communities, especially fungi, influence or are influenced by gut inflammation, we performed a phenotypic characterization and studied the immunomodulatory potential of yeast strains isolated from fecal samples of Crohn's disease (CD) patients stratified for low or high risk for aggressive disease evolution. For this purpose, we screened *S. cerevisiae* and *Candida spp.* strains isolated from CD patients. We compared the cytokine profiles, obtained upon stimulation of Peripheral Blood Mononuclear Cells (PBMCs) and Dendritic Cells with different strains, and evaluated the relationship between strain's cell wall sugar amount and immune response. We observed a strain-dependent rather than species-dependent differences in immune responses. Differences in immunogenicity correlated with the cell wall composition of *S. cerevisiae* intestinal strains. Interestingly, CD patients responded differently to "self" and "non-self" strains, eliciting pure Th1 or Th17 cytokine patterns. Starting from identification of predictive microbial signatures driven by risk of aggressive disease evolution, our results will be preliminary to develop of targeted and personalized therapeutic approaches for precision medicine.

Transitional human mycobiome in Africa: the impact of rural-to-urban transition in the gut mycobiome of households from Burkina Faso

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Presenter: Sonia Renzi

Urbanization represents the global change with the highest impact on human lifestyle, socio-economic status and health. Rural-to-urban transition and Westernization produced dramatic changes in diet, affecting the intestinal microbial community composition and changing disease epidemiology, with an increase in the incidence of non-communicable diseases. In the past years, the bacterial fraction of the transitional gut microbiome was investigated in different populations worldwide, while the shaping of intestinal fungal communities following the human transition are almost unexplored. Here, we show the effect of human transition from rural to urban environments on the gut mycobiota composition of households from Burkina Faso, living in areas at different levels of urbanization (rural, semi-urbanized and urban areas). We observed that households living in rural villages showed a greater intestinal mycobiota diversity compared to the urbanized and wealthy urban families from the capital city Ouagadougou and from Italy. We observed a differential abundance gradient of the 33 detected fungal ASVs associated with rural-to-urban transition. We also identified 12 fungal species, known for the impact on human health, affected by urbanization gradient. Our results shed light on yet well-explored transitional mycobiota, suggesting a legitimate role of the fungal community, similarly to bacteria, in adaptation to rural-to-urban transition and overall in the hypothesis of hygiene.

ID: 63; Stand: E21

Role of ferric reductase-related proteins in fungal heme acquisition

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Israel Institute of Technology- Technion, Israel

Presenter: Sunanda Dey

Candida albicans is a commensal organism that can cause life threatening systemic candidiasis in immunocompromised patients. Like all systemic pathogens, *C. albicans* needs to scavenge iron in the iron-scarce host environment. Like many microbial pathogens, *C. albicans* has evolved mechanism to utilize the abundant hemoglobin heme as iron source. This mechanism involves extracellular soluble and cell surface-anchored CFEM- domain hemophores that can extract heme from hemoglobin or albumin, and transfer it to the cell surface, where two ferric reductase (FRE)-related membrane proteins, Frp1 and Frp2, participate in heme uptake. Based on this observation, we also screened predicted *S. cerevisiae* FREs and found that Fre5 is involved in heme acquisition as well, suggesting that the function of FRE-like proteins in heme transport might be widespread. Our results suggest that in *C. albicans*, the function of Frp1/2 may be to capture heme from the CFEM hemophores and internalize it. Here, we attempted to identify specific amino acids within the Frp1/2 protein structure that interact with heme. We took advantage of the AlphaFold 2 protein structure predictions and looked for potential heme-iron coordinating residues (His, Tyr, Cys, Met) properly positioned to function as part of a heme-binding site. We performed site-directed mutagenesis at these sites in Frp1 and analyzed mutant protein localization, ability to utilize hemoglobin-heme and sensitivity to toxic heme analogues. We found that Frp1 is localized in the membrane, ER and vacuole of *C. albicans* and we identified several residues that are important for Frp1's function in heme acquisition.

ID: 65; Stand: E22

***In vitro* evolution of a *Candida auris* clade II strain to caspofungin resistance selects for *FKS1* hotspot I mutations**

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Presenter: Alexander Lorenz

The human fungal pathogen *Candida auris* has rapidly emerged as a global healthcare threat, causing nosocomial outbreaks with high mortality rates. Resistance to all classes of antifungals has been reported in the species and some isolates are pan-resistant, making effective treatment increasingly difficult. Especially, resistance to echinocandins is a massive issue in this yeast, as caspofungin and related antifungals are the treatment of choice for systemic *C. auris* infections.

This study was undertaken to get a mechanistic understanding of caspofungin resistance in *C. auris*. We *in vitro* evolved a clinical caspofungin-sensitive *C. auris* clade II isolate to a high level of resistance. Resistant derivative strains were whole-genome sequenced. The resistant *C. auris* isolates harbour mutations in the *FKS1* gene altering a conserved residue (S639) also mutated in caspofungin-resistant clinical isolates; demonstrating that *FKS1-S639P* and *FKS1-S639Y* mutations are sufficient to confer resistance to caspofungin and other echinocandins. A comparative phenotypic analysis of these caspofungin-sensitive and -resistant strain pairs which are otherwise isogenic will be presented; a particular focus of this analysis was cell wall ultrastructure and organisation.

ID: 72; Stand: E23

Delving into the association of *Saccharomyces cerevisiae* with social wasps: commensal or passenger?

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Presenter: Silvia Abbà

Yeasts rely on animal vectors to spread in the environment. Recent studies showed that social wasps (*Polistes*, *Vespa*, and *Vespula* spp.) play a relevant role in yeast ecology.

The social wasps' cycle begins in spring when overwintered and fertilised females establish new nests. It has been shown that foundresses can harbour yeasts from autumn to spring and transmit them to their offspring and nestmates thanks to trophallaxis, allowing vertical and horizontal transmission. During summer, wasps feed on ripe grapes, releasing the yeasts on the berries, representing a suitable environment for *S. cerevisiae* with high concentrations of fermentable sugars.

These observations confirmed the role of wasps as a natural reservoir of yeasts during winter and as vectors, contributing to their survival, diversity and dispersion in the environment. Nevertheless, despite the long-lasting colonization by yeasts of the gut of hibernated wasps being proved, we do not know if this association is equally stable with active wasps or if the yeast is maintained in the wasp nest through a handover mediated by trophallaxis. Are yeast cells commensals or passengers of active wasps?

To answer this question, we fed *Polistes* spp. wasps with recognisable *S. cerevisiae* strains and assessed the presence of yeast cells over time in both wasp faeces and guts. These data, indicating the maximum time spent by yeasts in the intestine of active insects, provide us further hints on the natural yeast-wasp association.

Iron overload impairs the antifungal activity of caspofungin against *Candida albicans*

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Presenter: Andreia Pedras

Invasive fungal infections (IFIs) caused by *Candida* spp. are the most common fungal diseases among hospitalized patients receiving immunosuppressive or intensive antibacterial therapies. Echinocandin drugs are widely used to treat IFIs, as they act on the fungal cell wall. Iron overload - often associated with diseases (hemochromatosis, cancer), viral infections or medical conditions requiring specific treatments (chemotherapy, repeated blood transfusion) - is considered a serious risk factor for IFIs. We found that iron overload also decreases the activity of the echinocandin drug caspofungin against the most prevalent fungal species that causes nosocomial IFIs - *Candida albicans*. Flow cytometry analyses showed that *C. albicans* recovers from the fungicidal effect of caspofungin by more than 50% when cultures are supplemented with iron. Scanning electron microscopy analyses revealed that the disruption of the cell wall integrity by caspofungin is prevented by iron. Using checkerboard assays, we confirmed the existence of a clear antagonism between iron and caspofungin in *C. albicans* reference and clinical strains. This effect was observed in other clinically relevant fungal species known to cause IFIs. Other echinocandins (micafungin and anidulafungin) also antagonize with iron, although to a lesser extent. In addition, we observed that *C. albicans* biofilm formation is no longer impaired by caspofungin when combined with iron. Iron overload also impaired caspofungin activity in vivo against a *C. albicans* clinical strain in the *Galleria mellonella* model, leading to a great decrease in larvae survival. Our findings may have important implications for the treatment of invasive candidiasis associated with medical settings of iron overload.

A novel pathosystem with the model plant *Arabidopsis thaliana* for exploring molecular basis of *Taphrina* infections

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Presenter: Agate Auzane

Pathogenic yeasts have mostly received attention in the context of human health, however, both true yeasts and dimorphic fungi can pose a significant threat to plants. Among phytopathogenic fungi, members of the genus *Taphrina* stand out with many agronomically important pathogens such as the causative agent of peach leaf curl disease *T. deformans* and *T. pruni* that causes seedless, deformed plums. As the infections are opportunistic and most hosts are woody, studying *Taphrina*-plant interactions has been challenging. Here we describe a novel *Taphrina* pathosystem with the genetic model plant *Arabidopsis thaliana*. *Taphrina* strain M11 was isolated from wild arabidopsis plants and has been reproducibly found on arabidopsis in other studies. Infection of arabidopsis with *Taphrina* M11 resulted in disease symptoms including subtle leaf curling, reminiscent of leaf deformation symptoms caused by other *Taphrina* species. Genomic analysis revealed features associated with host interactions, including putative effectors, plant hormone biosynthesis pathways, and potential MAMPs recognized by the plant innate immune system. Notably, yeast-like fungi in the genus *Taphrina* lack the best described fungal cell wall MAMP – chitin – however, their chitin-free cell walls elicit immune response in arabidopsis. To understand the recognition of these cell walls by plant immunity, we have performed reverse genetic screening of candidate receptors and analysed *Taphrina* cell wall composition. In conclusion, we have established a model system for studying interactions of *Taphrina* yeasts with plants. Use of this system will help to define genetics of *Taphrina*-plant interactions.

ID: 124; Stand: E26

Conserved nucleases repress pathogenic proliferation of the L-A RNA mycovirus

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Presenter: Jie Gao

L-A is a double-stranded RNA virus that chronically infects the budding yeast *S. cerevisiae*. While it has historically been considered a benign resident of the cytoplasm, we showed that L-A is pathogenic in cells lacking multiple antiviral systems. Disrupting parallel-acting antiviral genes results in rampant L-A proliferation, which causes lethal heat intolerance in the host cell. We leveraged this conditional pathogenic phenotype to screen for novel antiviral host factors in yeast and recovered distinct functional groups that collaborate to limit L-A proliferation. Two parallel-acting antiviral factors were investigated in greater depth: Nuc1 and Rex2, both highly conserved RNA exo/endonucleases with enigmatic functions. Using molecular and cell biological approaches, we find that the antiviral functions of Nuc1 and Rex2 require nucleolytic activity but are carried out in the mitochondria and nucleus respectively. Neither appear to contact the site of viral replication in the cytoplasm, and efforts are underway to identify the downstream targets of Nuc1 and Rex2 with RNA immunoprecipitation and sequencing approaches. Preliminary data suggest that these nucleases act through an indirect mechanism that limits the availability of pro-viral host RNA species, evocative of other RNA viruses that co-opt host RNA species for their replication. Combined, our findings serve to highlight the novel antiviral mechanisms of conserved host factors and advance budding yeast as a powerful model for exploring antiviral innate immunity.

ID: 129; Stand: E27

Yeast complementation assays as pre-screening tool for membrane channel functionality - Characterization of urea and water permeability of *Helicobacter pylori* UreI

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Presenter: Anna Stoib

Yeast complementation assays test the functionality of exogenous proteins expressed in deletion strains by observing growth or survival. Only if the exogenous protein is substituting the function of the deleted endogenous protein, yeast cells can survive/grow under the specific assay conditions. We utilized yeast complementation assays as pre-screening tools to test functional differences, of various affinity tag positions, compared to the WT protein, for further *in-vitro* characterization. Moreover, the assays can be utilized for qualitative characterization of solute permeabilities and pH gating of urea and water for proteins like *Helicobacter pylori* UreI, an inner membrane pH gated urea channel. To test the urea permeability a urea uptake deficient *Saccharomyces cerevisiae* strain (YNVW1 ddur3) expresses our constructs and grows in liquid media at different pHs with urea as sole nitrogen source. For water as solute the samples are rapidly frozen and the survival is determined with FDA and PI, with water permeable channels increasing the survival rate. Our results emphasize the need of *in-vivo* characterization as not only the overall tag position, but also a minor amount of N-terminal amino acids, influence *HpUreI*s functionality. On the contrary, the pH dependent water permeability was similar for all tag constructs. In general, this type of cost-effective yeast complementation assays can be adapted for testing a broad range of solutes and used as a pre-screening tool for affinity tag positions or protein mutations before quantitative *in-vitro* protein characterization.

Multiomic analysis in colorectal-cancer models: the effect of red meat based versus pesco-vegetarian diets on the gut mycobiota and metabolic profile

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Presenter: Niccolò Meriggi

Colorectal cancer (CRC) is the third cause of cancer related death worldwide and its incidence is strongly related to dietary habits. In this study we analyzed the gut fungal communities and relative metabolites on CRC risk to determine whether reduced intake of red/processed meat can reduce the level of toxic metabolites. We assessed variations in the incidence of tumors and tumor-related markers, changes in intestinal fungal communities and metabolic profiles according to the different dietary treatment by using two animal models, i.e., the animal model of colon carcinogenesis (Pirc rats mutated in the Apc gene) and AOM -induced germ-free (GF) rats treated with faecal microbial transplantation (FMT) from Pirc rats. Pirc rats were fed with a control diet (CTR), a high-risk meat-based diet (MBD) and with a low-risk pesco-vegetarian diet (PVD). An additional arm was represented by a tocopherol-supplemented MBD (MBD-T) diet, which may mitigate CRC risk. Pirc rat feces were then used for FMT in AOM-induced GF rats, all fed with control diets (CTR) only. We demonstrated that the pesco-vegetarian diet produced a significant decrease in the number of tumors and tumor-related markers, furthermore, changes in mycobiota structure as well as metabolic profiles were highlighted. Our results thus demonstrate how the diet can modulate the gut mycobiota and influence the risk of colorectal cancer and highlights the importance of integrating multiomic approaches for understanding the effects of diet on the gut mycobiome.

Systems level dissection of transcriptional responses to Dendritic cells uptake of different *S.cerevisiae* strains reveals differences between strains of human and vineyard origin.

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Presenter: Duccio Cavalieri

The human microbiome is increasingly considered as a key player in health and disease. The fungal component of the microbiome is often underscored and less frequently considered in metagenomic studies. Yeasts of the ascomycetes genus are frequently associated to regulation of the immune balance and can serve as an indicator of correct microbial homeostasis in the human gut microbiome. Here we use RNA sequencing and immunophenotyping to describe how cells of different strains of *S.cerevisiae* from human and wine origin differently regulate gene expression following the engulfment into the dendritic cell. Symmetrically we investigated how the dendritic cells differently regulate gene expression and immune responses by comparing the cytokine and transcriptional profiles of human monocyte-derived dendritic cells (DCs) exposed to the different strains. The main result indicate that *S.cerevisiae* strains isolated from human gut differentially express pathways associated to Chitin and sterol metabolism and this differential gene expression is associated to production of IL6 and vacuole acidification pathways in the dendritic cell.

Characterization of the community of meristematic fungi and black yeasts associated to white marbles of the Florence Cathedral

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Presenter: Domenico Celi

Rock inhabiting fungi (RIF) represent one of the most damaging groups of microorganisms causing deterioration of outdoor exposed monuments and difficult to remove due to their ability to cope with several stresses. The most representative groups belonging to RIF are meristematic fungi and black yeasts. Meristematic fungi exhibit isodiametric cellular expansion, resulting in a minimal surface-to-volume ratio. Meanwhile, black yeasts reproduce by forming daughter cells through either yeast-like multilateral or polar budding. In this work, the community of meristematic fungi and black yeasts inhabiting the external white marble of the Cathedral of Santa Maria del Fiore and causing its darkening was studied. Twenty-six different strains were isolated from two differently exposed sites of the Cathedral and characterized. The phylogenetic analysis based on the sequences of ITS and LSU rDNA regions showed that isolates belong to six different genera: *Knufia* and *Lithohypha* (class Eurotiomycetes), *Coniosporium*, *Paradevriesia*, *Vermiconia* and *Aureobasidium* (class Dothyeomycetes), indicating a wide diversity in the limited marble sampling areas. Some strains belonging to different genera were also investigated for their tolerance to environmental stressors and their interaction with stone. Eight strains were tested for thermal preferences and showed to grow as mesophilic/cryophilic microorganisms. Nine out of ten strains resulted able to produce organic acids and dissolve carbonate substrates. Their sensitivity to essential oils of thyme and oregano and to a common commercial biocide was also tested. The efficacy of the essential oils against RIF growth suggests a promising direction for developing a treatment with minimal environmental impact.

ID: 267; Stand: E31

artiMATE - artificial neural networks of satellite imagery, fungal metagenomes and high-throughput interaction studies

Giovanni Schiesaro, Michael K. Jensen

DTU Biosustain, Denmark

Presenter: Giovanni Schiesaro

Soil microbiomes are responsible for fundamental processes of our global ecosystems and are indispensable for the cycling of carbon, nitrogen, and phosphorus critical for the survival of animals and plants. Major open science efforts have been initiated to map spatiotemporal OTU abundances showing that soil microbiomes share mathematically describable relationships with their environment and that the ecosystems maintain a persistent microbial community. Despite this, two main challenges prevail. First, there is a scarcity of high-quality well-annotated satellite images that have been cross-referenced to soil microbiome sampling sites, and second, complex ML algorithms often value higher accuracy and performance at the sacrifice of meaningful biological information. The project artiMATE (artificial neural networks of satellite imagery, fungal metagenomes, and high-throughput interaction studies) has the fundamental goal to identify new pest management drug targets and plant growth-promoting natural chemistries. To do so, artiMATE will mine and categorize publicly available imagery and DNA data sets to develop artificial neural networks (ANNs) that can: i) predict abundances of operational taxonomic units (OTUs) as a function of image data, ii) predict the environment as a function of OTUs at sampling sites, iii) use causal inference and active learning to predict the environment in those locations where metagenomic data are not available, iv) guide interpretation of ANNs and validate co-existence of OTUs by experimentally screening in yeast large libraries of their chemotactic components.

ID: 239; Stand: E32

Yeast metagenomics: analytical challenges in the analysis of the eukaryotic microbiome

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Presenter: Benedetta Cerasuolo

Even if their impact is often underestimated, yeasts and yeast-like fungi represent the most prevalent eukaryotic members of microbial communities on Earth. They play numerous roles in natural ecosystems as well as in association with their hosts; they are involved in the food industry and pharmaceutical production, but they are also a cause of diseases in other organisms, making an understanding of their biology mandatory. The ongoing loss of biodiversity due to overexploitation of environmental resources is a growing concern in many countries. It therefore becomes crucial to understand the ecology and evolutionary history of these organisms to systematically classify them. In order to do so, it is essential that our knowledge of the mycobiota reach a level similar to that of the bacterial communities. To overcome the existing challenges in the study of fungal communities, the first step to be taken should be the establishment of standardized techniques for the correct identification of species, even from complex matrices, both regarding wet lab practices and bioinformatic tools.

ID: 331; Stand: E33

Utilizing yeast complementation assays to elucidate the pH gating mechanism of *Helicobacter pylori* inner-membrane urea channel Urel

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Presenter: Sahar Shojaei, Anna Stoib, Andreas Horner

Over 50% of the world population suffers from chronic gastric infection with *Helicobacter pylori* which is linked to peptic ulcer disease and stomach cancer. Yet, the efficacy of the common therapy including antibiotics and proton pump inhibitors is decreasing. An alternative drug target could be *H.pylori* pH-gated inner-membrane urea channel Urel, which is pivotal for the survival of the pathogen in the acidic environment of the human stomach. However, despite *in vivo* studies and high-resolution structures in the open and closed state, HpUrels gating mechanism is still elusive. Using yeast complementation assays, we tested point mutations of charged residues and periplasmic variants carrying changes at the N- or C-terminus or within periplasmic loops 1 (PL1) and 2 (PL2). The functionality and pH gating behavior of these constructs are compared to the wildtype protein in the physiologically relevant pH range from 4.0-7.0. To do so, our constructs are expressed in an urea uptake-deficient strain of *Saccharomyces cerevisiae* (YNVW1 *ddur3*), and the resulting cells are grown in liquid media with urea as the sole nitrogen source at different pH levels. Under these conditions, the survival rate is increased if the channels are permeable for urea. Our results emphasize the importance of PL1 in the pH gating mechanism and illustrate the significance of the N- and C-terminus for the stability of the open state. Overall, yeast complementation assays can be a powerful tool for testing exogenous protein functionality and examining qualitative differences between protein variant activity for a broad range of solutes.

Restoring the immune response and inhibiting *Candida* virulence: The promising role of *Saccharomyces cerevisiae* in vulvovaginal candidiasis

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Presenter: Ilse Palmans

Vulvovaginal candidiasis (VVC) is the most prevalent form of candidiasis affecting 75% of females worldwide. VVC arises from an imbalanced vaginal microbiome, *Candida*'s virulence, or dysregulated immune responses. To address the limitations of current treatments and increasing resistance, we propose *Saccharomyces cerevisiae* as a probiotic intervention for VVC.

Screening 70 strains, we selected the top 10 *S. cerevisiae* strains with desirable characteristics against various *Candida* strains. These selected strains demonstrated variable inhibitory effects on *Candida* growth, hyphae formation, adhesion, and toxicity toward vaginal epithelial cells. Interestingly, certain strains exhibit the potential to impede *Candida* virulence across all investigated traits. To assess the impact of *S. cerevisiae* on immune dysregulation during VVC, we investigated its effects on cytokine release, macrophage inflammasome activation, and neutrophil activation. Several *S. cerevisiae* strains decreased the secretion of pro-inflammatory cytokines (IL-8 and IL-1 α) while increasing the release of an anti-inflammatory cytokine (IL-1Ra). Furthermore, these strains reduced IL-1 β secretion by macrophages, decreasing neutrophil recruitment and activation.

Our findings highlight the probiotic potential of *S. cerevisiae* for VVC by impeding *Candida* virulence and reducing inflammation.

Exploring the Impact of Stressed Conditions on Skin Physiology: Skin Microbiome Analysis in Submariners of the Italian Navy

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Presenter: Marta Nerini

Stressed conditions, such as space missions and military operations, can significantly affect skin physiology and wound healing. This study, conducted in the frame of the EXPOSOME SIGNATURE-NEPTUNE Project selected by ESA and funded by ASI, aimed to investigate the skin fungal and yeast microbiome during missions under stressed conditions by sampling submariners of the Italian Navy using patches. DNA extraction from the patches, both before and after the missions, was compared, including the use of 3M 10cm x 20 cm patches. The DNeasy PowerSoil Pro Kit (QIAGEN) was adapted for DNA extraction, with modifications to develop an appropriate extraction buffer. Lysozyme and Proteinase K were used, posing a challenging task in buffer development. Through testing various reagent combinations and measuring DNA concentration via Qubit Fluorometer, the most efficient buffer composition was determined as SDS 10%, CD1 (starting reagent of the DNeasy PowerSoil Pro Kit), and Lysozyme (20mg/ml). Following extraction, ITS PCR was performed. This study provides technical insights to further study the effects of stressed conditions on skin physiology and the skin microbiome, contributing to our understanding of the challenges faced in demanding environments and potential strategies for maintaining skin health.

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