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POSTER ABSTRACT BOOK

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4B

Combination of microfluidics and fluorescence in situ hybridization to detect *Candida tropicalis* in urine samples

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Abstract

Candida tropicalis is the second most prevalent *Candida* species in urinary tract infections (UTIs). However, urine cultures take from 18 to 48 h to completely identify the microorganism, which may be sufficient for the progression of the UTI.

New developments in molecular biology, automation, and micro-electro-mechanical systems, could be transferred into *point-of-care* devices, providing a quick microorganism identification (24 h) on species level. In this work, we designed a portable microfluidic platform (chip), based on hydrodynamic forces (i.e., passive separation method that sorts cells by size, density, shape and deformability), combined with a molecular technique – fluorescence *in situ* hybridization (FISH), for the quick and specific detection of *C. tropicalis*.

The method was successfully tested in both *C. tropicalis* cell suspensions and artificial urine samples contaminated with *C. tropicalis.* For both cases, the initial concentration was 10E5 - 10E6 CFU/mL, described as the clinical *Candida* spp. concentrations in UTIs. Although some features still require optimization (e.g., time for probe hybridization/washing steps), preliminary assays showed cells with a strong fluorescence signal, which permitted a quick, sensible and specific *C. tropicalis* detection, within only 2-3 hours.

In the future it is expected that the chip might be applied for the quick detection of *C. tropicalis* in urine samples, which can be useful in healthcare centres.

7A

Beyond the Numbers – the Phenotypic Commonality in Aneuploidy and Antifungal Resistance

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Abstract

The rapid emergence of multi-drug resistant fungal pathogens has posed a considerable threat to our medical system. While fungal infections cause >1.5 million deaths each year, the pace of developing therapies has not increased in proportion since only three classes of antifungals are clinically available. One common challenge targeting drug resistance across fungal pathogens is the frequent occurrence of aneuploidy, a genome with random chromosome stoichiometries. This unbalanced genome state often occurs in clinical fungal isolates either transiently or persistently to increase the likelihood of appearance of adaptive mutations. Consequently, the resulting phenotypic variation potentiates the development of antifungal resistance. Despite the clinical importance, our understanding of how aneuploidy drives adaptive evolution is minimal.

Recently, we discovered that aneuploid cells are commonly under a hypo-osmotic stress state. This distinctive biophysical signature renders an altered cytoplasmic environment and cell surface dynamics in response to stress. As the underlying mechanisms driving the high adaptability of aneuploidy remain elusive, we investigate how the biophysical properties of aneuploidy contribute to the adaptive phenotypes. Furthermore, we explore the interactions between single-gene mutations and aneuploidy in a high-throughput genetic screen to elucidate the evolutionary trajectories of antifungal resistance.

11A

PKA isoforms are differentially required to respond to the toxic effects of diverse protein aggregation stresses

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Abstract

The cAMP/protein kinase A (PKA) pathway is a highly conserved signalling pathway responsible for sensitising cells to the presence of extracellular ligands such as nutrients. Yeast cells encode three isoforms of the PKA catalytic subunit (Tpk1-3) which phosphorylate common substrates to support cell growth and metabolism, but also exhibit isoform-specific roles in diverse processes. Recent studies have identified links between PKA signalling and the cellular response to stress conditions known to induce protein aggregation. However, the contributions of individual Tpk isoforms to the cellular defence pathways which mitigate the toxicity caused by protein aggregation are poorly understood. Here, we present findings which indicate that PKA isoforms are functionally non-redundant in the response to nascent protein misfolding stress (AZC) and heat stress. Using strains deleted for two Tpk isoforms, we show a requirement for Tpk1 or Tpk2 to tolerate both conditions and the chronic nuclear accumulation of the environmental stress response activator Msn2 in a strain expressing Tpk3 as the sole PKA isoform. In addition, we identify that Tpk3 specifically re-localises to morphologically different cytoplasmic granules upon AZC and mild heat stress exposure, while Tpk1 and Tpk2 do not. These foci do not co-localise with canonical stress granule or P-body markers, but show distinct co-localisation with the chaperone Hsp104, which is a disaggregase often used as an *in situ* marker to visualize protein aggregation. Together, these results provide evidence that PKA isoforms are differentially required to protect cells from the toxic effects of diverse protein aggregation stresses.

12B

A Fungal Foray in an NHP Gut Microbiome

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Abstract

The cynomolgus macaque, *Macaca fascicularis*, is a non-human primate (NHP) widely used in biomedical research as it shares behavioural, genetic, immunological and physiological similarities with humans. These similarities may extend to the enteric microbiome, with some microbial taxa common to both humans and NHPs. However, to date, the majority of these microbial surveys have focused on the prokaryome, and have largely ignored or overlooked the NHP gut mycobiome.

To address this shortfall, we have undertaken a region-by-region taxonomic survey of the cynomolgus intestinal mycobiota, from duodenum to distal colon, of ten captive animals of differing age. Using a high-throughput ITS1 amplicon sequencing-based approach, we found that fungi from the Ascomycota phylum dominate the cynomolgus enteric mycobiota. The budding yeast genus *Kazachstania* was most abundant, with *K. pintolopesii* and *K. telluris* highly prevalent, and the predominant species in many of the intestinal samples. However, while *K. pintolopesii* was present throughout the primate GI tract, *K. telluris* was found mainly in the small intestine.

In this study, *K. pintolopesii* was identified as the dominant enteric fungus in captive cynomolgus macaques. This contrasts with humans, where *Candida albicans* is a common member of the intestinal microbiota. To our knowledge, this is the first time *K. pintolopesii* has been identified as a primate gut commensal.

13A

Sc3.0 : An efficient tool to minimize the synthetic yeast genome

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Abstract
Recent advances in synthetic genomics now allow the synthesis of organisms' entire genomes. The most extensively altered genome built to date is the ongoing Sc2.0 project, that aims to create a fully synthetic *S.cerevisiae* yeast strain. Synthetic chromosome rearrangement and modification by Loxp-mediated evolution (SCRaMbLE) is a key addition within the Sc2.0 project. SCRaMbLE can efficiently shuffle the synthetic genome, evolving the synthetic yeast strains toward a desired property by diversifying strain genotype and phenotype.

We have shown that SCRaMbLE is a powerful mechanism for minimising the synthetic yeast genome. However, SCRaMbLE cannot delete sequences located within essential rafts, loxPSym flanked units harbouring an essential gene. Essential rafts can move within the genome but cannot be lost due to essentiality restrictions. Supplementing Sc2.0 strain with a Neochromosome housing essential genes would significantly increase the deletion power of SCRaMbLE, facilitating further compaction of the genome. Here, we describe the construction and characterisation of three versions of the Neo Essential chromosome 3 (NEC3), built using regulatory elements derived from various yeast species including *S. cerevisiae* , *S.paradoxus* and *S.eubayanus.*

We aim to generate a novel synthetic yeast with a minimal genome and hence with a multitude of potential applications in industry. Additionally, the Sc3.0 project will offer an opportunity to address various questions, previously intractable when using conventional methods. These include further quantification of how much of yeast genome is redundant, how much can be compacted and whether the current gene organisation is coincidence or inevitable?

14B

Regulation of actin nucleation by competitive binding of SH3 domains and actin at proline-rich regions

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Abstract

The spatiotemporal control of actin polymerisation is a critical mechanism underpinning organisation of many cellular processes. While SH3 domain-containing proteins have been reported to bring about changes in the actin cytoskeleton, the SH3 domains themselves have not been considered to be direct players in regulating actin dynamics.

Using budding yeast for in vivo cell assays, coupled with biochemical approaches, we have been able to reveal new insights into the interplay of SH3 domains, actin and a binding partner WASp/Las17. We have shown that SH3 domains and monomeric actin can bind to overlapping binding sites in the central poly proline region of Las17. Actin binding, and nucleation mediated by Las17, can be directly inhibited by the SH3 domains of an endocytic adaptor protein Sla1. Most effective inhibition requires all three Sla1 SH3 domains on a single contiguous peptide. Release of this inhibition is critical to allow the actin filament nucleation required to drive endocytic invagination. In vivo studies show that a single point mutation that compromises binding of one of the Sla1 SH3 domains, delays recruitment of the Arp2/3 complex to endocytic sites thereby leading to a reduced rate of endocytosis.

Overall our data allow us to propose a mechanism whereby a balance between SH3 binding and actin binding on Las17 can regulate de novo actin nucleation at the plasma membrane and ensure that it only occurs at appropriate sites.

15A

Intragenic complementation in the protein kinase domain in Ire1

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Abstract

In eukaryotic cells, secretory and transmembrane proteins fold in the endoplasmic reticulum (ER) before they exit the ER. Accumulation of unfolded proteins activates Ire1 to promote the unfolded protein response (UPR).In the yeast *Saccharomyces cerevisiae*, activation of Ire1 leads to splicing of the mRNA for the transcription factor Hac1. We have used b-galactosidase reporter assays to demonstrate expression of a UPRE-*lacZ* reporter gene that is positively regulated by the Ire1–Hac1 signalling pathway. In this study, the protein kinase domain was subjected to mutations to alter the catalytic aspartate D797 and lysine K799, which interacts with the terminal phosphate group of ATP, to alanine. Also, point mutations in the Mg^{+2} coordinating loop converted asparagine N802 and aspartic acid D828 to alanine. The expression of the UPRE-*lacZ* reporter gene in all single mutants K799, N802 and D797 was decreased compared to WT-Ire1. Under conditions of various ER stress inducing drugs the ability of the D797A mutant strain to survive was reduced in growth assays compared to other mutants in the Mg^{+2} coordinating loop and catalytic domain. D797-Ire1 mutant has less in b-galactosidase activity, survival of ER stress and *HAC1* splicing. Expression of Ire1 carrying double mutations D797A-N802A increased b-galactosidase activity significantly and restored growth compared to the single mutant D797A-Ire1. WT and protein kinase mutants express to the same level. This study suggests that introducing a second mutation such as K799A-Ire1 or N802A-Ire1 to the single mutant D797A-Ire1, restores signaling activity of Ire1.

21A

Quantitative genetic analysis of attractiveness of yeast products to Drosophila

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Abstract

The interaction between two model organisms, the fruit fly Drosophila melanogaster, and the budding yeast Saccharomyces cerevisiae, is widely perceived as mutually beneficial. The bridge of this biological connection is the volatile compounds from yeast fermentation, which can be attractive to the flies. The generation of volatile molecules derives from complex metabolism and different yeast will generate different combinations of volatiles. The attractiveness to fruit flies can therefore be considered a complex trait. In this study, six strains from SGRP were selected for fermenting commercial grape juice. The attractiveness of these ferments to a wild fruit fly line was then tested. Flies prefer the ferments of the Wine European (WE) strain over the North America (NA) strain. The preference assay was then applied to ferments of genotyped F1 progeny of WE crossed to NA for QTL mapping of genetic variation in attractiveness. GC-MS profiling of all ferments demonstrated that NA could be easily distinguished from other strains. 15 compounds were further quantified and the concentrations in F1 progeny ferments were correlated with genotypes using our r/QTL pipeline. Candidate genes from QTL mapping were tested by reciprocal hemizygosity analysis, validating the impact of two genes, PTC6 and YFL040W. The alleles of these validated genes from NA has better effect on the preference assay than the allele from WE, which is can be considered as antagonistic QTLs. The identification variation in these two genes underpinning metabolic differences, demonstrates how WE and NA have different attraction to one wild fruit fly line.

22B

YML003w, a gene of unknown function, is required for spontaneous and UV-induced mutations

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Abstract

YML003w. a gene of unknown function is defined on a truncated gene in S288C. The next annotated ORF, YML002w, is therefore not expressed in S288C. Previous studies suggest that these two genes is a large ORF in wild type strains named VRL1. Based on our previous study, we speculate YML003w might be involved in a DNA repair process and its null mutant is sensitive to DNA damage agents. No spontaneous or UV induced mutations are seen in the deletion of YML003w, while overexpression of YML003w results in an increase in spontaneous and UV-induced mutations compared to the wild type frequencies. We then created a series of double deletion strains with yml003w- and other DNA repair related genes. The deletion of YML003w results in loss of spontaneous mutations in most deletions, with the exception of rad5- and mms2-. UV sensitivity of many of these mutants is reversed in the absence of YML003w. Our findings suggest that YML003w plays an important role in mutagenesis and UV sensitivity and potentially interacts with Rad5 mediated pathways.

23A

Transcriptional Heterogeneity in Metabolically Cooperating Communities

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Abstract

Cell to cell variability within microbial communities can lead to resistance to environmental stresses, including treatment with antimicrobial drugs. One important source of variability in microbial communities is a cell's metabolic state. Complimentary metabolic states can underpin cooperation in interspecies and synthetic communities of microbes, however the extent of metabolic variation between individual cells in isogenic communities is not well understood. We gathered single cell gene expression data in yeast for isogenic cells, as well as in synthetic Self-Establishing Metabolically Cooperating Communities (SeMeCos) composed of cells with different metabolic capabilities (metabotypes). In the SeMeCos, where metabolic heterogeneity is known to occur, we see metabotypes cluster together based on their transcriptional signature. We have begun to characterize the transcriptional signatures of metabotypes in these clusters and compare them to previously collected data for metabotypes growing in isolation. We see some interaction between metabotypes and cell cycle markers. In order to characterize the extent and nature of metabolic heterogeneity in isogenic cultures, we search for these and similar signatures in cultures without metabotypes defined by plasmids.

24B

Investigation vacuole dysfunction in a yeast model of *SOD1* associated amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a motor neuron disease causing degeneration of motor neurons in the brain and spinal cord. Mutations in the gene *SOD1* that encodes the Cu/Zn binding enzyme superoxide dismutase are associated with 20% of familial ALS cases (fALS). Aggregation of mutated, unstable Sod1 causes fALS via toxic gain-of function. Research suggests a role for soluble mutated or misfolded Sod1 proteins in addition to larger insoluble aggregates that are often reported. Previous work from a yeast model of ALS established in our lab showed toxic effects of ALS-linked Sod1 mutations in yeast that were linked with metabolic dysfunction. This correlated with an inability to regulate amino acid levels coupled with a vacuole acidification defect. Vacuole acidification is maintained by the highly conserved V-ATPase pump. We show that Sod1 interacts with multiple subunits of the V-ATPase via a protein complementation assay. This work suggests a mechanism by which unstable forms of Sod1 may lead to a failure to regulate vacuole function generating metabolic dysfunction that may underpin the recognised toxic gain of function. The yeast vacuole is functionally akin to the mammalian lysosome. V-ATPase function and lysosomal dysfunction have recently been associated with ALS suggesting that the interactions we have determined may have relevance to the disease.

26B

Azoles influence Candida glabrata host-pathogen interactions

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Abstract

Invasive candidiasis is the most frequent health care associated invasive fungal infection and is commonly caused by *Candida albicans*. However, in recent years the incidence of infection caused by other antifungal-resistant *Candida* species – especially *C. glabrata* – has emerged as a serious concern. Although the targets of each antifungal drug class are known, there is still a big gap regarding how these drugs generally affect fungal cells in terms of variations in their metabolism, stress resistance, cell structure, and immune interactions. Our aim is to investigate how antifungal treatment alters fungal adaptive responses and immune interactions. Therefore, we used microscopy and flow cytometry to assess adaptive cell wall and morphological changes upon azole treatment of *C. glabrata* clinical strains isolated from human vaginal, faecal and blood samples. Additionally, we determined the effects of antifungal pre-treatment on fungal survival during macrophage challenge and virulence via a *Galleria mellonella* infection model. Flow cytometry analysis indicated that cell wall carbohydrate exposure was altered for several isolates during azole treatment. Interestingly, we observed that more *C. glabrata* CBS138 cells pre-treated with voriconazole were phagocytosed by macrophages at 2 hours post-challenge compared to the vehicle-treated control but maintained high intracellular numbers after 24 hours of infection. This trend in yeast intracellular survival was also observed for blood and vaginal isolates pre-treated with fluconazole and/or voriconazole. Altogether our results suggest that antifungal treatment alters *C. glabrata* morphological features and host-pathogen interactions without compromising survival of the pathogen, which may contribute to its ability to develop antifungal resistance.

27A

Mining and modeling the genome of yeast industrial hybrids

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Abstract

Saccharomyces pastorianus is an industrial natural yeast evolved from different hybridisation events between the mesophilic *S. cerevisiae* and the cold-tolerant *S. eubayanus*. This complex aneuploid hybrid carries multiple copies of the parental alleles alongside specific hybrid genes that encodes for multiple protein isoforms which impart novel phenotypes, such as the ability to ferment at low temperature. First, to study the genome plasticity of this hybrid, we developed HybridMine, an open-source tool for functional annotation of hybrid aneuploid genomes of any species by predicting parental alleles including paralogs. Next, we investigated the transcriptional signature of the different orthologous alleles in *S. pastorianus* CBS 1513 during temperature shifts in different culture media. We identified temperature-dependent media-independent genes and showed that 35% have their regulation dependent on extracellular leucine uptake, suggesting an interplay between leucine metabolism and temperature response. Moreover, the analysis of the expression of ortholog parental alleles unveiled that the majority of the genes express preferentially one allele over the other, and that *S. eubayanus*-like alleles are significantly over-represented among the genes involved in cold acclimatisation. Additionally, our expression data indicate that the majority of the protein complexes established in the hybrid are likely to be either exclusively chimeric or uni-specific, and that the redundancy is discouraged, a scenario which fits well with the stoichiometric balance-hypothesis. This study offers a first overview of the transcriptional pattern of *S. pastorianus* and provide a rationalisation for its unique industrial traits at expression level.

29A

The Role of Lipid Metabolism in Development of *Candida albicans* biofilms on Medical Devices

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Abstract

Tracheostomy tubes are medical devices utilized to provide short and long term mechanical ventilation and respiratory aid for patients in intensive care, but are often a site of mixed species biofilm growth within a patient. Biofilms may lead to device failure, and in more severe cases, dissemination and subsequent systemic infection that may prove fatal to a patient.

Previous works within the Gourlay lab have established *Candida albicans* as a primary coloniser of medical devices located in the respiratory tract, effectively scaffolding biofilm formation of further bacterial species to thrive where they otherwise may not. As such, the hindrance of *C. albicans* biofilm development may alleviate proliferation of bacteria, such as *Staphylococcus aureus*, upon the device.

One aspect of this work seeks to investigate previously uncharacterised lipid metabolic pathways within *Candida albicans* and their role in biofilm formation on medical devices. Microscopy and staining of neutral lipid have been used to establish the necessity for lipid mobilization in hyphal growth and, as such, biofilm development. CRISPR Cas9 has been used to generate *Candida albicans* mutants defective in lipid storage and mobilization, to determine potential therapeutic strategies for biofilm formation. Furthermore, using biofilm forming assays, the monounsaturated fatty acid palmitoleic acid has been identified as an inhibitor of biofilm development on tracheostomy tubing at the attachment phase. This may prove to be a promising prophylactic therapeutic for biofilm development upon medical devices.

30B

Investigating Gene Regulation by the Tup1-Cyc8 (Ssn6) Complex in the yeast *Saccharomyces cerevisiae*

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Abstract

The Tup1-Cyc8 (Ssn6) co-repressor complex is a powerful epigenetic regulator of genes in the yeast *Saccharomyces cerevisiae*. The highly conserved complex brings about a repressive chromatin structure at regulatory regions of its target genes or prevents the recruitment of the factors needed for activation of transcription. The *FLO* family of genes are repressed by the Tup1-Cyc8 complex, these genes encode the proteins required for flocculation, a stress response in yeast where the cells aggregate, or form flocs, to protect cells within the floc. Interestingly each mutant strain (*tup1*, *cyc8* and *tup1cyc8*) has a distinct flocculant phenotype. The *tup1* strain displays large, dense flocs compared to smaller, more dispersed flocs associated with the *cyc8* strain, whereas the *tup1cyc8* strain displays an intermediate flocculant phenotype. RT-qPCR showed that *FLO1,* considered to be the dominant member of this family of genes, is highly de-repressed in the *tup1* and *tup1cyc8* deletion strains. This suggests that Tup1 makes the dominant contribution to repression of this gene. However, this pattern is not seen at all target genes. The results of RNA-Sequencing show a core set of 429 genes significantly upregulated in all three mutant strains. These genes, on average, show the highest de-repression in the *cyc8* and *tup1cyc8* mutant strains. Indicating that Cyc8 makes the dominant contribution to repression at the majority of target genes. Together these results indicate that each of the subunits of the Tup1-Cyc8 complex may be dominant in bringing about repression at different sets of genes.

31A

Synthetic Biology In Pursuit Of The Minimal Cell Cycle

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Abstract

Background: Synthetic biology offers an alternative approach to answering fundamental research questions about biology by ‘learning from building’. In this work, we use the methodology from Synthetic Yeast Genome (Sc2.0) project to build and understand the minimal set of genes needed to achieve autonomous cell cycle oscillations in *Saccharomyces cerevisiae*.

Methods: For a proof-of-principle, we focused first on genes involved in the dynamics of cyclin waves. 4 pairs of B-type cyclin genes, and their regulators *FKH1*, *FKH2*, and *SIC1* were deleted by CRISPR from their native loci in the yeast genome and simultaneously re-assembled into a synthetic gene cluster in the same cell. To then explore the effects of removing combinations of genes from this cluster we used the Sc2.0 SCRaMbLE method, which uses Cre recombinase to delete and rearranges genes in the synthetic cluster.

Results: Growth tests of the 3, 5, 6, and 9 gene synthetic cluster strains showed no major growth defects following gene relocation. Our system allowed us to rapidly generate combinatorially diverse strains with different deletion profiles, and the frequency of gene loss and the growth rates of these strains were analysed and compared to computational models. Our results showed that the core cyclins (*CLN2, CLB5, CLB3, CLB2*) are able to reproduce the same oscillatory features without their counterparts.

Conclusions: We showed that the genetic complexity of the yeast cell cycle can indeed be reduced. Our ultimate aim is to use this synthetic approach to converge *in vivo* on the minimal eukaryotic cell cycle gene set.

32B

A high-throughput screening method for the discovery of *Saccharomyces* and non-*Saccharomyces* yeasts with potential in the brewing industry

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Abstract

Both *Saccharomyces* and non-*Saccharomyces* yeast strains are of great importance for the fermentation industry, especially with the flourishing of craft breweries, which are driving current innovations. Non-conventional yeasts can produce novel beverages with attractive characteristics such as flavour, texture, and reduced alcohol content; however, they have been poorly explored. A new method for screening the fitness of conventional and non-conventional yeast libraries utilising robotic platforms and solidified media representing industrial conditions is proposed. As proof of concept, a library formed of 6 conventional and 17 non-conventional yeast strains was distributed in 96, 384 and 1536 arrays onto a YPD agar medium. Following this, the library was replicated in different conditions mimicking beer and cider fermentation conditions. The colony size was monitored over time, and fitness values measured in maximum pixels/h and maximum biomass were calculated. Significant differences in growth were observed in between the different strains and conditions. As examples, *Candida milleri* Y-7245 displayed good performance in wort conditions, and *Kazachstania yakushimaensis* Y-48837 stood out for its performance in apple juice. The method is proposed to be used as a pre-screening step when studying vast yeast libraries. This would enable interested parties to discover potential hits for further study at a low initial cost. Furthermore, this method can be used in other applications where the desired screening media can be solidified.

33A

Predicting prions in pathogenic fungi

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Abstract

Prions drive significant phenotypic changes in yeast. These mechanisms generate reversible phenotypic heterogeneity that can provide evolutionary advantages in unpredictable environments. Thus far, yeast prions have primarily been studied in the model organism, *Saccharomyces cerevisiae*. Yet, pathogenic fungi contend with dynamic host environments that impose nutritional limitation and other stressors. To better understand prions in pathogenic fungi, we performed an *in silico* analysis to identify homologs of a subset of *S. cerevisiae* prions in *Candida glabrata*, *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. As expected, we found the closest homologs for *S. cerevisiae* prions *SUP35*/[*PSI*$^{+}$], *SWI1*/[*SWI*$^{+}$], and *SFP1*/[*ISP*$^{+}$] in the most closely related pathogens, *C. glabrata* and *C. albicans*. However, *C. albicans SWI1* and *SFP1* appear to have lost their prion-forming domains. PAPA, a prion prediction algorithm, returned significant scores for each *C. glabrata* prion homolog and for *C. albicans SUP35* suggesting these proteins may be capable of misfolding into prion-like conformations. *A. fumigatus* and *C. neoformans* also had at least one homolog of Sc*SUP35*/[*PSI*$^{+}$], though *C. neoformans SUP35* did not have a significant PAPA score. Intriguingly, only *C. glabrata* had a potential homolog of Sc*RNQ1*/[*PIN*$^{+}$], which promotes [*PSI*$^{+}$] propagation in *S. cerevisiae*. Upon further investigation, we found that *RNQ1* is largely absent from *Candida* species, but present in many post-Whole Genome Duplication species. Altogether our data suggest that a limited subset of *S. cerevisiae* prions appear to be conserved in fungal pathogens, especially *C. glabrata*, though their role in virulence is unknown.

35A

Implementing post-transcriptional gene expression circuits in yeast using RNA-binding-protein-degron fusions.

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Abstract

Synthetic circuitry in yeast is largely engineered using transcriptional components (promoters, transcription factors and terminators). However, there are potential advantages (e.g. speed of switching response, lower noise and linear response dynamics) to employing posttranscriptional components such as RNA-binding proteins, degrons, aptamers and aptazymes. This project explores the construction of posttranscriptional regulatory devices, focusing initially on translational switching based on fusions between RNA-binding proteins and switchable degrons.

Yeast cells were engineered using both chemical- and light- inducible protein degron fusions with the bacteriophage MS2 coat RNA-binding protein (MS2CP). These fusions were targeted to MS2 stem-loop (MS2SL) structures within the 5’ untranslated region of reporter gene mRNAs. Using the ymNeonGreen reporter gene meant that regulation of the target mRNA could be monitored down to the single cell level by means of flow-cytometry.

Our work has shown that the successful regulation of translation via a degron-repressor fusion is dependent on careful tuning of the structure and abundance of the protein fusion and on the abundance of the mRNA target, where achieving a suitable dynamic range is key. We have explored different ratios of repressor to target mRNA and find that the ratio needs to be finely calibrated in order to create an effective regulatory system. Ongoing work will complete the characterisation of this system and in parallel explore aptamer/aptazyme regulatory systems.

36B

Virulence traits and differential translocation of gut-derived *Candida albicans*

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Abstract

Candida albicans is an opportunistic pathogenic yeast commonly found as part of the gut microbiome, which is thought to be the major reservoir of *C. albicans* in humans. The ability of *C. albicans* to adhere to, invade and damage epithelium, along with subsequent escape from the gut lumen into the bloodstream, can lead to life-threatening disseminated fungal infections. Examining the interactions of *C. albicans* with the intestinal epithelial barrier is vital to understanding its ability to disseminate and cause systemic infections.

A set of seven *C. albicans* strains (six of gut origin: one healthy donor, one cancer patient, one Crohn's disease patient and three unknown health status; alongside 'non-gut' reference strain SC5314) was profiled for virulence traits. *C. albicans* strains were profiled for biofilm formation (crystal violet staining), hyphal formation (microscopy) and cytotoxicity (lactate dehydrogenase release; during coculture with confluent Caco-2 gut epithelial cells). Additionally, the ability of *C. albicans* strains to translocate across epithelial cell layers was assessed during coculture with differentiated Caco-2 monolayers on Transwell™ permeable supports.

Common virulence traits of hyphal formation and cytotoxicity were reduced in gut *C. albicans* compared to non-gut reference strain SC5314, while biofilm formation compared to SC5314 was variable. Translocation assays revealed that gut strains of *C. albicans* are capable of translocating across Caco-2 epithelial cell layers in greater numbers than non-gut reference strain SC5314, with the highest levels of translocation observed from a Crohn's disease isolate. These insights suggest that gut-specific adaptations may influence luminal escape and pathogenicity of *C. albicans*.

37A

Regulation and consequences of SNF1/AMPK complex control across different stress conditions

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Abstract

The capacity to adapt accordingly to the new cell requirements in a changing environment is crucial for organismal survival. The SNF1/AMPK protein kinase family is widely conserved in mammals, fungi, and plants, and in yeast, it represents a master regulator of cell adaptation. SNF1/AMPK plays roles in cell response to environmental stress, including carbon and oxidative stress; as well as in other important cellular processes and signalling pathways. The SNF1 complex is formed by three subunits, the Snf1 catalytic subunit, Snf4 gamma subunit and one of the three beta subunits, Gal83, Sip1 and Sip2. The mechanism of SNF1 complex activation following carbon stress has been widely studied where it functions as a master regulator of transcription enabling glucose-dependent repression/ derepression of gene expression that is central to the yeast Crabtree effect. However, relatively little is known in terms of the role of SNF1/AMPK in other stress responses.

In this study we have addressed the SNF1/AMPK complex integrity, protein-protein interactions and the impact of three upstream regulators Sak1, Tos3 and Elm1 in response to both glucose depletion and oxidative stress. These studies reveal a complex network of signalling interactions and highlight stress specific co-ordination of SNF1/AMPK function.

38B

CO_2 enhances the ability of *Candida albicans* biofilms to form, overcome nutritional immunity and resist antifungal treatment

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Abstract

C. albicans is the predominant human fungal pathogen and frequently colonises medical devices, such as voice prosthesis, as a biofilm. It is a dimorphic yeast that can switch between yeast and hyphal forms in response to environmental cues, a property that is essential during biofilm establishment and maturation. One such cue is the elevation of CO_2 levels, as observed in exhaled breath for example. However, despite the clear medical relevance, the effect of CO_2 on *C. albicans* biofilm growth has not been investigated to date. Here, we show that physiologically relevant CO_2 elevation enhances each stage of the *C. albicans* biofilm forming process; from attachment through maturation to dispersion. The effects of CO_2 are mediated via the Ras/cAMP/PKA signalling pathway and the central biofilm regulators Efg1, Brg1, Bcr1 and Ndt80. Biofilms grown under elevated CO_2 conditions also exhibit increased azole resistance, tolerance to nutritional immunity and enhanced glucose uptake to support their rapid growth. These findings suggest that *C. albicans* has evolved to utilise the CO_2 signal to promote biofilm formation within the host. We investigate the possibility of targeting CO_2-activated processes and propose 2-deoxyglucose as a drug that may be repurposed to prevent *C. albicans* biofilm formation on medical airway management implants. We thus characterise the mechanisms by which CO_2 promotes *C. albicans* biofilm formation and suggest new approaches for future preventative strategies.

39A

The role of mitochondria and the electron transport system (ETS) in *Cryptococcus neoformans* pathogenesis

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Abstract

Cryptococcus neoformans is an opportunistic pathogenic fungal strain which causes cryptococcosis and cryptococcal meningitis in humans. It is estimated that there are up to 600,000 AIDS related cryptococcal meningitis cases per year, with most cases surfacing in sub-Saharan Africa and countries such as Brazil and Thailand. For infected HIV patients, the prognosis is often poor; patients have a 10-week mortality, although combination treatment with antifungal drugs such as Fluconazole, Amphotericin and Flucytosine is possible if detected in the early stages of infection. However, recent studies have shown that this infection is heteroresistant to Fluconazole, which poses a problem. Here we investigate the role of mitochondria and the electron transport system (ETS) in *Cryptococcus neoformans* with the aim of developing this as a potential drug target. Our results indicate that ETS inhibition is effective at preventing *C. neoformans* proliferation and rapid loss of viability. Unlike other yeasts, ETS inhibition does not trigger resistance by the upregulation of an alternative oxidase enzyme (AOX) suggesting a vulnerability to respiration inhibitors. Our data suggests that AOX may perform a function outside the ETS in *C. neoformans* and that the development of new fungal specific inhibitors against the ETS of this fungal pathogens holds promise for the development of future theraputics.

40B

Quantitative genetics of yeast hybrids: harnessing the power of biodiversity for industrial applications

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Abstract

Hybrids between different *Saccharomyces* species are commonplace in nature and in industrial settings, where they underwent centuries of selection in harsh fermentative conditions. Through hybridization we are able to combine advantageous traits and increase genetic diversity without resorting to GMO products. However, the study and the development of inter-species hybrids has been hindered by both their sterility and their genetic makeup.

Thus, to accelerate evolution, it is of the foremost importance to untangle the genomic complexity of the industrial hybrids.

In this study we generated a novel platform to study inter-species hybrids by crossing geographically distant strains from different species. The allotetraploids generated exhibited high spore viability, allowing continuous multigenerational breeding and generated a phenotypically diverse progeny.

Pooled F12 segregants exhibiting extreme traits were then tested as a platform to perform large scale mapping of QTL, thanks to their varied genomic heritage. Through state-of-the-art genetic techniques we identified species-specific and hybrid-specific features responsible for traits of biotechnological interest such as maltose, low temperature, and acetic acid. Furthermore, we investigated the effect of the mitochondria on the QTL landscape, by comparing QTL regions mapped in hybrid progeny derived from parental lines with different mitochondria.

Our platform has proven successful in both harnessing the power of biodiversity for industrial application and in bringing yeast hybrids in the realm of quantitative genetics.

45A

Functional and transcriptional profiling of non-coding RNAs in yeast reveal context-dependent phenotypes and in trans effects on the protein regulatory network

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Abstract

Non-coding RNAs (ncRNAs) are increasingly being shown to play pivotal roles in the transcriptional and post-transcriptional regulation of genes in eukaryotes. Stable Unannotated Transcripts (SUTs) and Cryptic Unstable Transcripts (CUTs) have been shown to affect nearby genes by physically interfering with their transcription (*cis*), or interact with DNA, proteins or other RNAs to regulate the expression of distant genes (*trans*). Here, we carried out a large-scale screening on the ncRNA *Saccharomyces cerevisiae* deletion collection and provide evidence for SUT and CUT function. Phenotypic data on 372 ncRNA deletion strains in 23 different growth conditions were collected, identifying ncRNAs responsible for significant fitness variations. Transcriptome profiles were collected for 18 haploid ncRNA deletion mutants and 2 essential heterozygote ncRNAs showing a high correlation between altered phenotypes and global transcriptional changes, in an environmental dependent manner. By analysing the expression network new functional ncRNAs acting *in trans* by modulating transcription factors were identified. Furthermore, we described the impact of SUTs and CUTs in modulating coding genes in response to different environmental conditions, acting on important biological processes such as ethanol tolerance, mitochondrial function, and respiration (SUT125, SUT126, SUT035, SUT432), plasma-membrane fluidity and sterol biosynthesis. (CUT494, SUT530, SUT468) or rRNA processing (SUT075 and snR30). Overall, this data captures and integrates the regulatory and phenotypic network of ncRNAs and protein-coding genes, support the notion of the involvement of ncRNAs in fine-tuning cellular expression via regulation of transcription factors, as an advantageous RNA-mediated mechanism that can be fast and cost-effective for the cells.

46B

The role of chromosomal duplication in the acquisition of fluconazole resistance in *Candida glabrata*

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Abstract

Candida glabrata is the second most prevalent fungal human pathogen, carrying a high mortality risk in immuno-compromised patients. As only a few anti-fungal drug classes are available, acquisition of resistance during treatment is a major concern. Chromosomal duplications are a known mechanism of resistance to fluconazole (a first-line fungistatic drug) in several pathogenic fungi, such as *Candida albicans* and *Cryptococcus neoformans*, but are rarely reported in *C. glabrata*. Instead, gain-of-function mutations in the transcription factor *PDR1* are considered the predominant mechanism of resistance in this yeast. We sequenced survivors on supra-inhibitory concentrations of fluconazole, using lab strain BG2 and YJBT-194 (a related clinical isolate), to study "breakout" mechanisms of resistance. 28% (6/21) of BG2 and 78% (11/14) of YJBT-194 non-petite survivors had chromosomal duplications, but only 14% (3/21) and 0% (0/14) (respectively) had mutations in *PDR1*. BG2 survivors had disomies of ChrE (where *ERG11*, the target of fluconazole, is located) as well as disomies of ChrIL (where *SNQ2*, an azole efflux pump, is located), while YJBT-194 survivors had disomies of ChrIL only. ChrIL disomy was unstable, reverting to the euploid state at a rate of ~1%, while ChrE disomy was considerably more stable, with a reversion rate of less than 0.1%. These results highlight the prevalence of chromosome duplication as a mechanism of drug resistance in *C. glabrata*. Accordingly, aneuploidy may be an evolutionary stepping-stone towards the irreversible resistance of *PDR1* gain of function mutations, and may be a "missing link" in the process of resistance acquisition during treatment in the clinic.

47A

TORC1 regulates actin dynamics to control proteasome homeostasis

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Abstract

The conserved protein kinase complex TORC1 is a central regulator of protein homeostasis. TORC1 is active under nutrient replete, unstressed conditions and promotes bulk protein synthesis, cell growth, and proliferation. Many stresses inactivate TORC1, including nutrient starvation. When TORC1 is inactivated, bulk protein synthesis is inhibited, and stress-responsive protein synthesis activated. Degradation of damaged or unwanted proteins is increased through autophagy activation and enhanced proteasome assembly.

The 26S proteasome is composed of the 20S core particle (CP), capped with one or two 19S regulatory particles (RP). Following TORC1 inhibition, the Mpk1 kinase is activated. Mpk1 facilitates increased translation of proteasome regulatory particle assembly chaperones (RPACs), leading to increased proteasome assembly. The mechanisms underlying the RPAC translation increase remain unclear, however.

Here, we show that actin depolarisation upon TORC1 inhibition causes RPAC mRNAs to associate more with actin patch structures. Increasing this interaction enhances translation further. We identified Ede1 as a protein which binds translating RPAC mRNA and show that Ede1 is required to increase RPAC mRNA association with actin patches, RPAC translation and proteasome assembly upon stress.

48B

Quantitative Trait Loci for protein secretion in *Komagataella phaffii*

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Abstract

Background

Komagataella phaffii (old name: *Pichia pastoris*) is a yeast species widely used for protein production in industry. Almost all previous research on it has been conducted using derivatives of a single isolate, CBS7435. We aimed to use QTL analysis to identify genomic variants in other natural isolates that could improve its industrial performance.

Methods

We used a fungal beta-glucosidase (BGL) as a test secreted protein. We integrated a *BGL* gene, expressed from a constitutive *pGAP* promoter, into the same genomic locus in the lab strain CBS7435 and two unrelated natural isolates of *K. phaffii*. Strains were crossed, sporulated and 1000 random segregants from each cross were phenotyped for BGL secretion. To map QTLs, 30 superior and 30 inferior segregants were individually sequenced and aligned to the CBS7435 reference genome. Candidate genes within QTLs were identified by Reciprocal Hemizygosity Analysis and causative SNPs were pinpointed by CRISPR/Cas9 editing.

Results

We identified a strong QTL prominent in both crosses with a twofold effect on the level of secretion of BGL, at which CBS7435 has the superior allele. We also identified weaker QTLs unique to each cross, at which the natural isolates harbour alleles that improve protein secretion. We dissected one of these QTLs to identify a SNP that significantly increases BGL secretion when introduced into CBS7435 by CRISPR/Cas9 editing.

Conclusion

This work reveals QTL analysis as a powerful method for dissecting the polygenic basis of enhanced protein secretion in *K. phaffii* and a potentially valuable platform for improving its industrial performance.

50B

GAL-to-*MAL* suppression: a new example of catabolite repression beyond glucose in *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae consumes multiple different carbon sources and prioritises glucose in a phenomenon called glucose repression. Although the mechanism behind this repression is clear, it remains unknown whether and how cells prioritise a non-glucose carbon source over another. Here we provide evidence that cells favour galactose consumption over palatinose, a maltose-like sugar. We observed diauxic growth in galactose-palatinose mixtures, which is robust to a wide range of concentrations. Consistently, we found that the expression level of the *MAL* genes induced by palatinose is significantly lower when galactose is present and that consumption of palatinose is prevented by constitutive expression of the *GAL* regulon, through deleting *GAL80*. To understand the mechanism behind such *GAL*-to-*MAL* suppression, we systematically deleted other *GAL* genes in the *gal80Δ* strain. We confirmed that the suppression is not caused by high expression of the enzymes encoded by *GAL1*, *GAL10* and *GAL7*, but, surprisingly, is relieved by deletion of *GAL2*, encoding for the galactose permease. Our results show that yeast prioritise carbon sources other than glucose and suggest that catabolite repression is more complex than thought.

52B

A novel role for the high-osmolarity glycerol (HOG) pathway in the regulation of tumour associated lipogenesis

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Abstract

Enhanced *de novo* lipogenesis is an important metabolic hallmark of cancer cells, in part attributed to dysregulation in the signalling pathways which control expression of lipogenic genes. Understanding the important pathways which contribute to aberrant lipogenic signalling in cancer will potentially reveal new targets for anti-cancer therapies. The p38 MAPK pathway is important for regulating various aspect of cellular biology, but a role in lipogenesis is yet to be characterised. Here, evidence is presented which reveals a potential new role for the *S. cerevisiae* MAPK Hog1p, the functionally and structurally conserved homolog of human p38 as a central regulator of lipogenesis. Genomic deletion of HOG components (*HOG1*, *MSN2* and *MSN4*) causes significant reduction in neutral lipid accumulation compared to wildtype cells. Double deletion of the *S. cerevisiae* diglyceride-acetyltransferase *DGA1* and *HOG1* results in a significant reduction in neutral lipid accumulation comparable to that of respective single delete cells, by Nile red assay. Further, Dga1p possesses four potential phosphorylation sites, recognisable by Hog1p, which appear to be required for neutral lipid accumulation, measured by Nile red assay. This suggests Hog1p is a potential protein kinase for Dga1p activation. Preliminary evidence suggests that Hog1p is required for normal expression of *DGA1* when cells are accumulating lipid, measured by western blotting. Future work will investigate the role of Hog1p in regulating the expression of *DGA1,* and consolidate a novel protein interaction between Hog1p and Dga1p during lipogenesis.

54B

NECROTIZING FASCITIS IN NEONATE BY LICHTHEIMIA RAMOSA : A CASE STUDY

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Abstract

Zygomycetes of the order Mucorales can cause life-threatening infections in humans. These mucormycoses are emerging and associated with a rapid tissue destruction and high mortality. We present a rare fatal case of cutneous mucormycosis in a premature neonate admitted with neonatal sepsis and necrotizing fasciitis. He was diagnosed with Lichtheimia ramosa infection. He was managed surgically along with Amphotericin B. Patient survived due to prompt diagnosis and appropriate management. The low birth weight, prematurity, respiratory distress, administration of corticosteroid and broad spectrum antibiotics were noticed as the risk factors for this case which had led to fungal infection. Early diagnosis is critical in prevention of morbidity and mortality associated with the disease.

55A

Cellular aggregation of *Candida auris* likely does not determine its virulence

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Abstract

Candida auris is a human fungal pathogen responsible for nosocomial infections of immune-compromised patients worldwide with high mortality rates (>60%). High levels of resistance to antifungal drugs and environmental persistence mean these infections are difficult to treat and eradicate from the healthcare setting. Epidemiological and genomic studies have identified five geographical clades (I-V), which display phenotypic and genomic differences. A common phenotype, primarily observed in clade III strains, is the capability to grow as aggregates. Aggregates are clusters of cells that cannot be dispersed by chemical or mechanical means. Previous studies of aggregative and non-aggregative isolates from different clades have shown that aggregation potentially affects virulence. However, it is difficult to deduce from such experimental setups whether the observed differences in virulence depend on the capability to aggregate or due to other strain/clade-specific traits. We show that aggregates comprise a heterogenous population of cells. Fluorescent staining of the cell wall shows that aggregates, unlike non-aggregating cells, contain cells of normal size and shape alongside large round cells with higher chitin levels. We also identified growth conditions where aggregative strains will exclusively grow as single yeast cells. This is allowing us to dissect whether aggregation is a virulence trait by comparing the same isolate in its aggregating and non-aggregating form. Using transcriptomics, we compared strains grown under aggregating and non-aggregating conditions, observing differential expression of genes involved in cell budding specific to the aggregating strain. Pre-growth conditions significantly change *in vivo* virulence independent of a strain’s ability to aggregate.

56B

Pentose utilisation in Saccharomyces

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Abstract

The usage of lignocellulosic biomass for second-generation bioethanol needs the advancement of microorganisms that can produce ethanol by metabolising fermentable sugars obtained from the hydrolysis of the lignocellulose such as glucose, xylose and arabinose. This study focused on the ability of different wild *Saccharomyces* species to grow on pentoses by screening a large set of strains. Also, QTL analysis was applied using an F12 AIL population together with the 4 parental strains in order to identify possible alleles linked to pentose metabolism. Weak growth on arabinose resulted in it being excluded and so we focused only on xylose. Results indicate that the right arm of chromosome XV plays the main role in xylose utilisation in some *Saccharomyces cerevisiae strains*. Bioinformatics analysis of chromosome XV sequence revealed the existence of a 65 Kb subtelomeric introgression in the WE strain genome harbouring putative proteins related to sugar transport and sensing as well as stress-resistance genes. Results obtained in this study indicate that for the improvement of xylose fermentation further analysis should be applied towards the yeast sensing-signalling system to enhance the ability to sense xylose as a fermentable carbon source. The impact of carbohydrate metabolism gene families located in the subtelomeres should also be explored more. Future work will be focused on validating the introgressed ORFs to identify their direct impact on xylose utilisation.

57A

Non-*cerevisiae de novo Saccharomyces* yeast hybrids with a heterotic maltotriose growth

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Abstract

Lager beer is produced with the domesticated hybrid yeast *Saccharomyces pastorianus* created by the fusion of an ale *Saccharomyces cerevisiae* with the cryotolerant *Saccharomyces eubayanus*. The discovery of the latter species settled the debate on the origins of the hybrid and logically, generating *de novo S. pastorianus* hybrids is of interest with several examples reported. Furthermore, the increasing biodiversity of the *Saccharomyces* genus inspired researchers to exploit this diversity and use further unconventional species for the construction of hybrids with enhanced brewing characteristics. In the search for novel flavours and aromas, it has been demonstrated that *S. eubayanus* is dispensable in *Saccharomyces* hybrids by applying alternative cryotolerant species. On the other hand, maltotriose utilization, another important brewing trait thought to be inherited by *S. cerevisiae* in *S. pastorianus*, is yet poorly investigated for other members of the genus.

The aim of the current project is to identify non-*S. cerevisiae* maltotriose consumer candidates in order to construct *de novo* hybrids with a heterotic maltotriose utilization. To this end, nearly 200 strains from the 8 different species of the *Saccharomyces* genus collected from various environments were screened for growth on maltotriose. Candidate *Saccharomyces* strains showed slow growth on this sugar after a long lag phase. Remarkably, *de novo* hybrid combinations with *S. eubayanus* resulted in heterotic and comparable growth profile to the well-known *S. pastorianus* CBS1513 strain. These findings indicate that *S. cerevisiae* could be replaceable for the maltotriose utilization phenotype in *Saccharomyces* hybrids to produce modern lager brews with novel flavours.

59A

Characterising the Role of Mif2 Phosphoregulation by Cyclin-Dependent Kinase in Budding Yeast

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Abstract

Kinase-mediated protein phosphorylation plays an essential role in cell cycle progression. In *Saccharomyces cerevisiae*, a single cyclin-dependent kinase (CDK), Cdc28, is known to be the driving force of the cell cycle. Cdc28 activity is tightly regulated by binding to different cyclins at specific cell cycle stages, which recruit the kinase to its substrate proteins. An important target of CDK phosphorylation is the kinetochore, a large multi-protein complex that attaches chromosomes to the spindle microtubules during cell division. Kinetochore malfunction can lead to errors in chromosome segregation, a leading cause of birth defects and result in aneuploidy - an incorrect number of chromosomes - which is a hallmark of cancer cells. The yeast kinetochore consists of at least 60 different proteins which are organised into several subcomplexes, many of which contain specific sites for CDK phosphorylation. However, the impact of specific phosphorylation events on cell cycle progression is often unclear.

Using the Synthetic Physical Interactions (SPI) method, we have identified a number of potential CDK-phosphoregulated candidates at the budding yeast kinetochore, including the conserved centromeric protein Mif2. We further characterised the effect of Mif2 phosphorylation by CDK using phospho-mutants of potential CDK sites. Our data suggests that CDK phosphorylation stabilises Mif2 to ensure kinetochore integrity during mitosis and that dynamic phosphoregulation of Mif2 is important for normal progression through mitosis.

60B

Identification of novel factors that regulate the stability of the Myosin V, Myo2 receptors, during peroxisome and vacuole inheritance

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Abstract

Eukaryotic cells contain various membrane-enclosed compartments known as organelles. It is crucial for cells to inherit organelles during cell division. The transport of organelles to their allocated locations in dividing cells is critical for the maintenance of a full complement of organelles over many cell generations. Defective organelle inheritance and maintenance may result in metabolic defects or cell death. In *Saccharomyces cerevisiae,* organelle movement during asymmetric cell division is dependent by the Myosin V motor, Myo2. During this movement, Myo2 is recruited by cargo-specific receptors, Vac17 for the vacuole, Inp2 for the peroxisome. Upon delivery of organelles, in the newly forming bud, the receptors are broken down and the organelles are deposited. Understanding how these receptors bind to Myosin and accomplish the delivery of organelles to the bud is a major question to unravel. Using various genetic approaches, we have found that the kinase Kin4 and its paralog Frk1 regulate peroxisome and vacuole transport by preventing premature proteasome-mediated degradation of Myosin receptor proteins in the mother cell.

63A

Novel interactors of spindle checkpoint component Mad3 in meiosis

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Abstract

Meiosis is a specialised cell division process where one round of DNA replication is followed by two rounds of division, leading to formation of four gametes from one cell. In meiosis I, homologous chromosomes are paired together, while sister chromatids co-segregate to the same pole. The spindle assembly checkpoint (SAC) is a surveillance mechanism that monitors improper kinetochore-microtubule attachments and arrests the cells in metaphase until all homologs are bioriented, thus ensuring proper chromosome segregation during anaphase.

In my PhD, I have used *Saccharomyces cerevisiae* as a model to further investigate the necessity of individual SAC components Mad1, Mad2 and Mad3 in prolonging the duration of metaphase I when cells undergo meiosis in the absence of kinetochore-microtubule attachments or when there is lack of tension between homologs as opposed to duration of metaphase I in unperturbed meiosis.

I used mass spectrometry to identify interactors of Mad2 and Mad3, where I identified that Mad3, but not Mad2, strongly interacts with Stu1 and Slk19. Stu1 is essential for spindle stability and elongation in every cell cycle while Slk19 is necessary for FEAR activation. Stu1 and Slk19 also work together to promote kinetochore capture by microtubules. This had led me to consider that Mad3 works in conjunction with Stu1 and Slk19 to execute a SAC-independent role in meiosis. I have dissected the interaction domains of Mad3-Stu1-Slk19 and am now exploring the functions(s) of this complex in context of previous observations.

65A

FungiDB: An integrated functional genomics platform for fungi and oomycetes.

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Abstract

FungiDB (https://fungidb.org) is a free online resource for data mining and functional genomics analysis for fungal and oomycete species. FungiDB is part of a family of knowledgebases called The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org) that integrates genomic, transcriptomic, proteomic, and phenotypic datasets, and other types of data for pathogenic and nonpathogenic, free-living and parasitic organisms, hosts, and vectors of human disease. FungiDB is one of the largest VEuPathDB databases containing hundreds of genomes including yeast, filamentous fungi and oomycetes, and it offers a user-friendly web interface with embedded bioinformatics tools that support custom in silico experiments leveraging the integrated public data, and also datasets in the private My Workspace and VEuPathDB Galaxy interfaces. With FungiDB, you can browse genomes & examine gene record pages, create search queries to mine omics scale datasets, annotation, and the results of in-house analyses (protein domains, orthology predictions via OrthoMCL.org, metabolic pathways, etc.). Expert knowledge about phenotypes, publications, and other gene-centric data can be captured by the user comments and bulk data submission systems. New datasets can be nominated for integration via emailing to FungiDB help desk or social media. FungiDB also offers a range of tutorials and webinars for novice and expert users.

FungiDB is a component of the NIAID Bioinformatics Resource Centers and is supported in part by NIH HHSN75N93019C00077 and the Wellcome Biomedical Resources #212929 & 218288 grants.

* *presenting on behalf of the entire VEuPathDB Bioinformatics Resource Center*

66B

Microfluidics for single-cell noise measurements in budding yeast

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Abstract

The combination of microfluidics and time-lapse microscopy is a powerful one, with the potential for the generation of time-series data for single-cell measurements across a whole population of cells. This project aims to employ these tools for the development of technologies and strategies that facilitate the study of stochastic events at the single-cell level. A key focus of the work is the development of microfluidic and image analysis techniques.

Such developments within this project are being applied to study the specific example of noise within the expression of the yeast GCN4 transcriptional regulator. This is activated during amino acid starvation, through a novel post-transcriptional regulatory system. The majority of research into gene expression noise thus far has focused on transcriptional noise, so the impact of post-transcriptional regulation on noise is relatively poorly understood.

Biological systems show many apparently deterministic behaviours at the macroscopic level, but their underlying mechanisms are often stochastic in nature. Noise in gene expression generates heterogeneity across clonal cell populations, and is an important factor in many cellular processes (e.g. circadian rhythms). Even within the well-known “repressilator” synthetic gene circuit, noise had a significant impact on the circuit output. An appreciation of stochasticity in gene expression is therefore crucial for a complete understanding of biology, and will also be essential for the successful design of future synthetic biological systems.

67A

Synthetic genetic interactions of non-coding RNA in Saccharomyces cerevisiae

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Abstract

Over the past years evidence has been emerging for non-coding RNAs (ncRNAs) to play essential roles in the cell. Recent studies have identified a diverse catalogue of ncRNAs in yeast with only a handful ascribed a function. Previously, we generated and performed a large scale environmental phenotypic screening of the single ncRNA deletion mutant collection in *Saccharomyces cerevisiae* and identified ncRNA responsible for significant fitness changes. Here, a double ncRNA deletion mutant library was generated to study ncRNA-ncRNA interactions. The synthetic genetic array (SGA) methodology was used to shed a light on their functions by detecting mutant colonies with phenotype different than expected. Data was analysed from 5 different experimental conditions to reveal environmentally dependent functions. Over 1000 significant ncRNA epistatic interactions were observed. Positive epistasis accounted for ~90.2% and only ~5.1% and ~4.7% were synthetic sick or lethal, respectively. An increase of negative interactions was recorded in different stresses, suggesting that these ncRNAs may have environmentally-dependent functions or may be differentially transcribed in different conditions. The vast majority of ncRNA-ncRNA interactions did not correlate with the epistasis of their neighbouring genes, suggesting that the ncRNA SGA network is independent from the protein one. We also focused on the two U3 paralogues SNR17A and SNR17B responsible for processing of 18S rRNA. As expected, they share the majority of genetic interactions in standard rich media, however a large number of unique epistatic interactions were found in other conditions, suggesting that SNR17A and SNR17B may have sub-functionalised under different environmental pressures.

68B

Single-cell analysis shows that flavin-based yeast metabolic cycles are robust and respond to nutrient changes

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Abstract

The yeast metabolic cycle (YMC) is a biological rhythm present in budding yeast. This biological rhythm is linked to the cell division cycle and entails oscillations in oxygen consumption, intracellular metabolite concentrations, and cellular events. Most studies on the YMC have been based on bulk-culture experiments, and the behaviour of the YMC in individual cells is unclear. In particular, it is unknown both how nutrient changes affect the frequency of the cycles and what is its underlying molecular mechanism. I aim to characterise the YMC in single cells and its response to perturbations. Specifically, I use microfluidics to trap individual yeast cells and record the intensity of flavin fluorescence, a component of the YMC.

To analyse the thousands of flavin time series produced, I have developed a computational pipeline. Feature extraction followed by graph-based geometric clustering shows promise in classifying time series into groups that are experimentally relevant. I plan to quantify their relationship with oscillations of the cell division cycle.

My results show that cycles of flavin intensity synchronise with budding in both prototrophic and auxotrophic strains. Furthermore, the YMC is prolonged in synthetic minimal media, with its fewer nitrogen sources. These observations suggest that the YMC is robust and responds to nutrient changes.

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PRS genes, a paradigm for gene duplication and its potential for yeast research?

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Abstract

The *S. cerevisiae* genome contains five highly homologous *PRS* genes, each capable of encoding PRPP (phosphoribosyl-pyrophosphate) synthetase, a key enzyme essential for the synthesis of purine and pyrimidine nucleotides. *PRS1* and *PRS5* may have arisen from the prototype Prs-encoding genes, *PRS2*, *PRS3* and *PRS4* by duplication followed by acquisition of non-homologous regions (NHRs), shown to link primary metabolism with intracellular signalling. The *PRS* genes are highly conserved from yeast to human. We have created genocopies in *PRS1* associated with the human neuropathies, Arts syndrome and CMTX5 which caused increased caffeine sensitivity and elevated Rlm1 expression in the mutated strains. The insertions in Prs1 and Prs5 serve as a physical link to the cell wall integrity (CWI) pathway and explain why cell viability requires specific heterodimers for the provision of PRPP and the maintenance of CWI integrity. The interaction between Prs1 and Mpk1/Slt2, a component of the CWI pathway was demonstrated by immunoprecipitation. Prs3 contains a NLS (nuclear localisation site), deletion of which causes caffeine sensitivity, reduced Rlm1 expression and loss of the Prs1/Prs3 heterodimer. The contribution of Prs5 to CWI is shown by increased phosphorylation of Mpk1/Slt2 following mutation of the three phosphosites located within NHR5-2. We now intend to investigate the signalling pathway connecting the conserved master regulator TOR (target of rapamycin) with PRPP synthetase, thus adding a new dimension to the application of yeast research in the discovery of novel therapeutic targets for the treatment of human neuropathies, CMTX5 and Arts syndrome.

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Isotope labelling and proteomics reveal novel types of metabolic heterogeneity in yeast colonies

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Abstract

Microbial communities often find themselves in complex and dynamic nutritional environments with many ways of obtaining a certain required metabolite, either via direct uptake or via synthesis from the ensemble of available nutrients. Which metabolic strategy cells adopt is not at all easily measured but has important consequences for growth and other phenotypes such as stress resistance. We present a novel, proteomics-based method that can determine the producer/consumer status for amino acids with sub-population-level resolution. It reveals that nutrient gradients in colonies result in a complex metabolic community where spatial arrangement, metabolic phenotype and gene expression are linked.

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