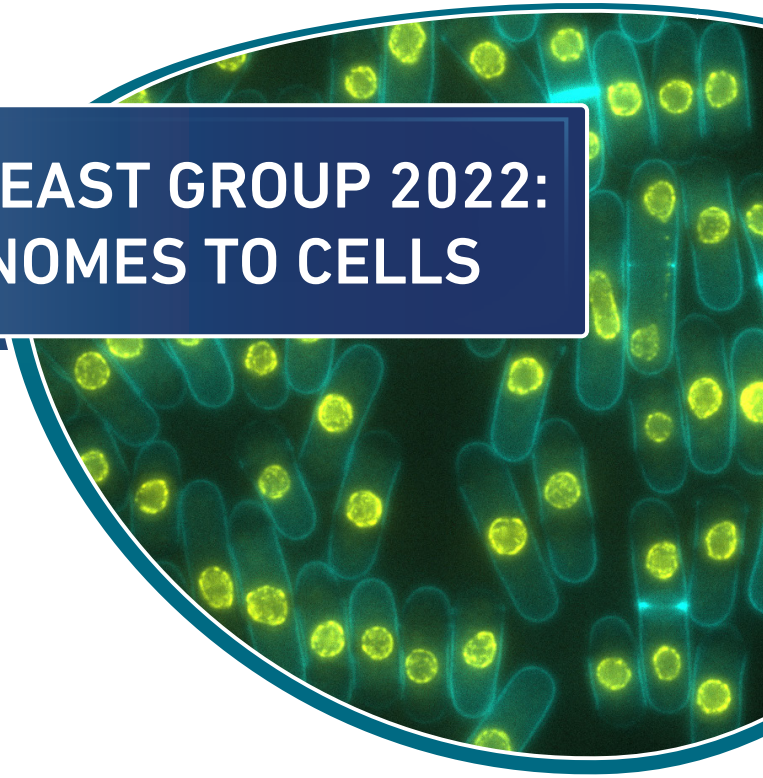
MICROBIOLOGY SOCIETY

BRITISH YEAST GROUP 2022: FROM GENOMES TO CELLS



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Poster book

P01

Mechanical stress generates unique cellular and transcriptomic responses in fission yeast

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Abstract

Living tissues are able to sense and respond to mechanical stress (MS) such as tension, compression, and shear pressure. A defective MS response leads to developmental abnormalities and is associated with tumor growth and metastasis. Unicellular organisms like yeasts are also exposed to MS due mainly to changes in turgor pressure induced by ion unbalance, which cause massive cell swelling or contraction, or to compressive forces, in conditions in which cell growth is physically constrained. However, how cells adjust homeostasis under MS is still poorly understood. Here, we apply increasing doses of MS by high-speed centrifugation to fission yeast cells upon surfaces of different stiffnesses to study genome-wide changes in gene expression. MS resulted in the rapid activation of p38 MAPK Sty1 and stress-induced transcription factor Hsf1 in a dose- and surface stiffness-dependent manner. Moreover, cells temporally downregulate growth rates and mitotic entry while maintaining their integrity intact. Consistently, we find changes in gene expression which are also dose- and surface stiffness dependent, and that include both genes involved in the core environmental stress response (CESR) and a unique transcriptional signature to MS. Our data suggest that, in addition to adjusting growth and the timing of mitotic entry, cells might rewire their metabolism during MS to regulate their viscosity and endomembrane system endurance as physical resilience mechanisms to compressive forces caused by MS.

P02

Advancing Pir-based yeast surface display by reshuffling and truncating Pir2-β-lactamase constructs

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Abstract

After synthesizing proteins, yeast *Saccharomyces cerevisiae* can retain them within the cell, secrete them into the medium, or bind them to the cell surface. In the third case, yeast anchors proteins by covalently binding them to β-glucan residues, thus allowing for surface display, a cell-engineering technique that can remake the entire cell surface into catalytically active living material. To immobilize proteins of interest closer to their N-terminus, the proteins are regularly fused with one of five Pir proteins (proteins with internal repeats), which covalently bind to β-1,3-glucan. However, beyond the requirement for glutamine in their characteristic internal repeats, the mechanism of attaching these proteins to the yeast cell wall remains unclear. Thus, the Pir-based surface display continues to rely on guesswork and intuition, which is less than efficient. To address this issue, we inserted β-lactamase at five positions in Pir2 (Hsp150) protein and followed its activity and Pir2-binding efficiency through enzymatic and immunochemical methods. Moreover, we constructed and tested additional six truncated Pir2-β-lactamase variants, thus determining the minimal portion of Pir2 required for efficient binding to the yeast cell wall. Finally, to enrich our experimental results with structural insights, we used a deep-learning Alphafold2-based algorithm to *in silico* predict the structure of Pir2-β-lactamase fusions. Therefore, we present and rationalize a novel set of practical guidelines for a reproducible, straightforward, and efficient Pir-based yeast surface display.

P03

Genetic Determinants of Biofilm Formation in Fission Yeast

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Abstract

Although traditionally thought of as single-celled organisms, many yeast species can take on quasi-multicellular states by clumping together and adhering to surfaces in biofilms. Biofilms can represent a challenge in research and industrial yeast strains, where planktonic growth is typically favoured, and in combating pathogenic yeast where biofilms can enhance virulence and drug resistance. We observed that the parental strains from a previously described and fully sequenced Schizosaccharomyces pombe recombinant library showed strong differences in biofilm formation, especially when grown in minimal media. We used qualitative biofilm formation assays on the recombinant library to assess cell-to-cell adhesion as well as adhesion to other surfaces such as plastic or agar. We observed a range of phenotypes, some of which were more severe than the biofilm-forming parental strain. Based on these qualitative biofilm formation assays, we are establishing quantitative assays in order to perform Quantitative Trait Locus (QTL) analysis and identify some of the genes responsible for different aspects of biofilm formation in S. pombe. We will then compare any genes identified with genes associated with biofilm-related phenotypes in S. pombe and other yeast species. We also hope that the assays we develop are of use in future studies of biofilm formation in S. pombe and other strains of yeast. This research is an undergraduate summer project supported by a Genetics Society studentship.

P04

Improving the production of second generation biofuel through exploiting the natural diversity of the yeast *Scheffersomyces stipitis*

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Abstract

Bioethanol, produced by the fermentation of biomass by microorganisms such as yeast, can act as a renewable substitute for fossil based fuels such as petroleum, and help reduce greenhouse gas emissions. Bioethanol can be broadly classified into first and second generation, depending on the type of biomass material used, and microorganism used to ferment it. To overcome the issues of using feedstock for bioethanol production (first generation), lignocellulosic biomass can be used (second generation). *Saccharomyces cerevisiae*, a well-established fermenting organism, is not able to ferment pentose sugars present in lignocellulosic biomass and is therefore not the most suitable organism to produce second generation bioethanol due to incomplete fermentation of the biomass. The ascomycetous yeast *Scheffersomyces stipitis* (*S. stipitis)* has the highest native capacity for xylose fermentation of any known microorganism, and therefore has the potential to ferment the pentose sugars (e.g., xylose) present in lignocellulosic biomass. Different *S. stipitis* natural isolates vary in their ability to produce bioethanol, but the genetic basis underlying this remains largely unknown. To this end, we are analysing the genomic structure of a collection of *S. stipitis* natural isolates using a combination of sequencing techniques in order to link genotypic and phenotypic differences that may improve the production of second-generation bioethanol using this yeast. It is through this work, that a novel phenotypic switching system in *S.stipitis* has been discovered, which provides higher stress resistance to a range of inhibitors.

P05

Modulation of gene expression in *Candida albicans*

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Abstract

Candida albicans is a diploid yeast part of the CTG-clade, a group of yeast translating the CTG codon as serine instead of leucine. Many human pathogens can be found in this clade, however, *C. albicans* is the most successful opportunistic pathogen responsible for yeast infection ranging from recurring vaginal infection to severe systemic infection in immunocompromised patients. Additionally, *C. albicans* is a human commensal, so it is important for the fungi to adapt to its environment quickly to be able to live as a commensal or a pathogen. Such adaptation can be done by modulation of gene expression through epigenetic mechanism, including chromatin changes and gene silencing. We aim to investigate these mechanisms using a CRISPR-Cas9 system to delete several genes suspected to be involved in these pathways. Phenotyping analysis including yeast-to-hyphae transition and growth on different carbon sources don't show any differences between the wild type and the mutant strains. However, using quantitative PCR on genes found in telomeric region known to be assembled in heterochromatin, we were able to see an increase in gene expression in a series of knock-out mutants. To confirm the implication of these genes in the modulation of gene expression in different chromosomal region, our next step is to perform total RNA sequencing in wild type and mutants. Overall, these results will help us understand the adaptability of *C. albicans* in its environment.

P06

Studying the proteasome under proteotoxic stress

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Abstract

Cells must carefully control their protein levels so that the correct amounts are present at the right time and place to perform their functions. This control, called proteostasis, is achieved by balancing protein synthesis, folding, and degradation. Proteostasis is critical upon exposition to high temperature, or in oxidative stress conditions, and becomes less efficient during aging, causing chronic proteotoxicity and cell death. The cellular stress response helps overcome proteotoxicity by expressing protective factors such as chaperones, and proteasome subunits, showing that in addition to promoting protein refolding, cells might also trigger the assembly of new proteasome units to promote protein degradation.

Here we study proteasome activity and composition under proteotoxic stress induced either by heat shock treatment or by addition of the amino acid analog canavanine, which is incorporated into newly synthesized peptides, interfering with protein synthesis.

We find that protein misfolding induced in all these conditions results in the upregulation of proteasome activity and a boost of new proteasome units production. Deletion of chaperone ump1, involved in the regulation of 20S proteasome assembly, results in cellular deprivation of 20S proteasome pool while maintaining 26S and 30S pools. In addition, ump1D cells are unable to survive under proteotoxic stress and show premature aging relative to wildtype cells under quiescence. Thus our data show that proper levels of 20S proteasome are critical to maintain cell homeostasis under proteotoxic stress caused by transient protein unfolding and during aging.

P07

Anillin/Mid1p Interacts with the ESCRT-associated Protein Vps4p and Mitotic Kinases to Regulate Cytokinesis in Fission Yeast

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Abstract

Cytokinesis is the final stage of the cell cycle which separates cellular constituents to produce two daughter cells. Using the fission yeast *Schizosaccharomyces pombe* we have investigated the role of various classes of proteins involved in this process. Central to these is anillin/Mid1p which forms a ring-like structure at the cell equator that predicts the site of cell separation through cytokinesis/septation in fission yeast. We demonstrate a direct physical interaction between Mid1p, and the endosomal sorting complex required for transport (ESCRT)-associated protein Vps4p, a genetic interaction of the *mid1* and *vps4* genes essential for cell viability, and a requirement of Vps4p for the correct cellular localization of Mid1p. Furthermore, we demonstrate *in vitro* phosphorylation of Mid1p by human aurora A and the polo-like Plk1 kinases, a genetic interaction of the *mid1* and the aurora kinase *ark1* genes is essential for cell viability, and that Ark1p is also required for the correct cellular localization of Mid1p. We mapped the phospho-sites of Mid1p by human aurora A and Plk1 kinases and assessed their importance in fission yeast using *in vivo* mutational analysis. Combined these data suggest a physical interaction between Mid1p and Vps4p important for cytokinesis and identify phosphorylation of Mid1p by aurora and polo-like kinases as being significant for this process.

P08

Resolving a metabolic paradox - Inorganic sulfur fixation via a new homocysteine synthase allows yeast to cooperatively compensate for methionine auxotrophy

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Abstract

The assimilation, incorporation, and metabolism of sulfur is a fundamental process across all domains of life, yet how cells deal with varying sulfur availability is not well understood. We studied an unresolved conundrum of sulfur fixation in yeast, in which an organosulfur-auxotrophy caused by deletion of homocysteine synthase Met17p is overcome when cells are inoculated at high cell density. We discovered that an uncharacterized gene YLL058Wp, herein named Hydrogen sulfide utilizing-1 (HSU1), acts as a homocysteine synthase and allows the cells to substitute for Met17p by re-assimilating hydrosulfide ions leaked from met17Δ cells into O-acetyl-homoserine and forming homocysteine. Our results show that cells can cooperate to achieve sulfur fixation, indicating that the collective properties of microbial communities facilitate their basic metabolic capacity.

P09

Chromatin phosphoproteome through the cell cycle

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Abstract

With every cell cycle the cell must orchestrate essential chromatin processes such as gene expression, DNA replication, chromosome protection, condensation and segregation. How the cell coordinates these processes globally with respect to cell growth and environmental changes remains a mystery. The master regulator of the cell cycle, cyclin-dependent kinase (CDK, Cdc2 in fission yeast), directs essential chromatin processes in part through direct phosphorylation of chromatin-associated substrates. We are using cell cycle synchronization and ChEP (Chromatin Enrichment for Proteomics), SILAC and TMT mass spectrometry to investigate the chromatin phosphoproteome through the cell cycle.

P10

The regulation of CDK activity by the Cks protein Suc1

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Abstract

The eukaryotic cell cycle is ordered by the activity of cyclin dependent kinases (CDKs). CDKs are active when bound to a cyclin, and can also bind to a Cks protein, forming a tripartite complex. Cks proteins are evolutionarily conserved across eukaryotes, and at least one paralog is essential for viability. Cks proteins have been implicated in diverse functions across different experimental systems, including in cell cycle regulation. However, a systematic characterisation of their role in regulating CDK activity *in vivo* is lacking.

The fission yeast *Schizosaccharomyces pombe* has one Cks protein, Suc1. We show that deletion of *suc1* can be rescued by human and *Saccharomyces cerevisiae* Cks proteins, demonstrating that the essential function(s) of Cks proteins are conserved from yeast to humans. By manipulating Suc1 expression levels we demonstrate that Suc1 is a dose-dependent inhibitor of cell cycle progression. However, a critical minimal concentration of Suc1 is required for viable cell division. To investigate the essential function(s) of Suc1, we use a novel temperature-sensitive allele to ablate Suc1 function in asynchronous cultures and reveal a complex mixture of terminal phenotypes. Several of these are indicative of defects in the transition into, and progression through, mitosis. Using a fluorescent reporter for CDK activity *in vivo,* we find that CDK activation at the G2/M transition is impaired at the restrictive temperature. This suggests that a major essential function of Cks proteins is in the activation of CDK at the onset to mitosis, which we are now characterising further.

P11

Regulation of germination initiation by nutrient sensing pathway in fission yeast spores

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Abstract

Yeast cells live in unpredictable natural environments and thus often face with unfavorable conditions such as nutrient starvation. Under such conditions, yeast cells protect themselves by entering into a non-dividing and stress resistant state, called spores. Fission yeast *Schizosaccharomyces pombe* undergoes meiosis and sporulation in the absence of nitrogen source. The fission yeast spores are highly tolerant to nutrient starvation, heat shock, and even desiccation. By nutrition refeeding, the spore breaks dormancy and returns to the actively dividing vegetative state. This process is called germination. Germination must be tightly initiated because spores could be endangered if germination takes place under the unsuitable conditions. However, the molecular mechanism underlying the spore germination remains still unclear. In this study, we focused on the cAMP/PKA pathway, a nutrient sensing pathway, because germination is triggered by the addition of the nutrient, glucose. Previous genetic analysis had shown that this pathway is essential for germination initiation in fission yeast. First, we established the deletion strains lacking *pka1*, cAMP-dependent protein kinase catalytic subunit, and *cyr1*, an adenylate cyclase. More than 90% of these mutants failed to germinate after 1 day, while the wild-type strain completed germination in approximately 12 hours. We are currently attempting to obtain suppressor mutants that can normally germinate even without cAMP/PKA pathway.

P12

Investigating the role of MCA1 and PIM1 proteases within the stress and PCD response of *Saccharomyces cerevisiae*

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Abstract

Stress is experienced by all organisms. Once a certain threshold has been reached in times of excessive stress, the programmed cell death (PCD) pathway is activated. PCD occurs in organisms during their developmental stages or when a particular type of stress, whether it be biotic or abiotic, affects cells. When the cells experience stress, this PCD pathway is triggered as a mechanism to minimise the damage to the cell and its neighbours. Yeast is extensively used as a model to understand the cellular processes within eukaryotes and conservation of these pathways is seen across eukaryotes. Proteolysis is a major pathway to resolve cell damage by removing or modifying proteins.

To better understand these mechanisms, proteases MCA1 and PIM1 were further investigated in response to stress Deletion strains of *Saccharomyces cerevisiae*; were grown in the presence of various stresses, at different concentrations and durations of stress exposure. Growth and viability were determined after treatments.

The results will show how the presence of H2O2, NaCl, and heat stress will impact the deletion strains', ΔMCA1, and ΔPIM1 growth in comparison to the wild type BY4741. This will provide insight into how these family proteins are involved in the stress and PCD response. More widely, the knowledge that is obtained can then be transferred to multicellular eukaryotes, such as plants that have homologs of these yeast genes. Further exploration of this, could show how plants deal with different sources of stress as sessile organisms.

P14

Genestorian: A web application for model organism strain collections

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Abstract

Genotypes of model organism strains are recorded by researchers as manually entered text, loosely following an allele nomenclature that becomes increasingly obsolete with the growing diversity of genetic modifications. When consulting a laboratory's strain database or publication, it can be hard or impossible to understand how a strain was generated. Documentation of plasmid history is better, as it is possible with proprietary software. However, proprietary tools do not allow exporting the history of plasmids in an Open Source machine-readable format, preventing the integration of collections with other informatic tools and limiting the usability of data produced by researchers.

Genestorian is a project that aims to produce:

- Open standards to document the generation of plasmids and strains in a machine-readable format, with an emphasis on interoperability, adhering to FAIR principles.

- Easy to use web tools for experimental researchers to document strain and plasmid generation in their collections by leveraging those open standards.

Genestorian is in active development. If you are interested, please contact us during the poster session or at manuel.lera-ramirez@ucl.ac.uk. We are very keen on feedback from potential users. Visit our website https://www.genestorian.org/ to see demos of the web applications or contribute to the project (there are ways to contribute if you don't know how to code).

P15

Using CRISPR-Cas9 to study the evolutionary role of genome instability in *Candida albicans*

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Abstract

Candida albicans is a typical member of the human microbiome which can also cause a range of infections from superficial to systemic. The ability of *C. albicans* to survive the broad range of conditions it encounters in the human host is partially attributed to its genomic instability. This instability generates diversity and allows selection of fitter genotypes which can thrive in the hostile host microenvironments. Consequently, *C. albicans* clinical isolates have diverse karyotypes. *C. albicans* chromosomal rearrangements often occur around repetitive elements including the Major Repeat Sequence (MRS), a conserved repeat array of unknown function occurring on seven of the eight *C. albicans* chromosomes.

This study aims to establish a cause-and-effect relationship between MRS-driven chromosome rearrangements and generation of novel, fitter genotypes. To this end, we have developed a CRISPR-Cas9 approach to generate double strand breaks within the MRS, inducing chromosome rearrangements in unstressed standard laboratory growth conditions. These unstable strains have then been evolved in clinically relevant stresses, including antifungal drugs. Long-read genomic sequencing will allow us to link improved fitness with specific novel genotypes.

This study, for the first time, will assess whether MRS-driven genome instability is advantageous for the adaptation of *C. albicans* to environmental stress.

P17

Pin4 coordinates transcriptional and post-transcriptional stress responses in yeast

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Abstract

Organisms must constantly cope with variable and stressful environments. Cells adapt to stress by altering protein expression at both the transcriptional and translational levels, and RNA-binding proteins (RBPs) are at the heart of these responses.
We previously examined global dynamics in Saccharomyces cerevisiae in response to glucose withdrawal and heat shock. Over short time courses, each stress induced rapid remodelling of the RNA-protein interactome without corresponding changes in RBP abundance. In the case of glucose withdrawal, an almost uncharacterized RBP, Pin4, showed greater than 2-fold change in total RNA binding by 16 min.
To map Pin4 binding sites on RNAs, we developed and applied a new variant of UV-crosslinking approaches, reCRAC. We identified over 1,000 mRNA targets. Pin4 predominately binds the 3' UTR regions of mRNAs, which are important for mRNA stability and translation. Following glucose starvation, Pin4 binding to specific mRNAs was lost. Loss of Pin4 slowed growth, even on glucose medium, and this was exacerbated following transfer to non-fermentable glycerol/ethanol medium. Consistent with the observed preferential binding to mRNAs for mitochondrial proteins, pin4Δ strains were hypersensitive to oxidative stress. Replacement with phospho-mimics blocked Pin4 function, supporting the model that phosphorylation is inhibitory, possibly by interfering with RNA-binding.
Unexpectedly, RNA-sequencing data showed that yeast lacking Pin4 fail to mount a transcriptional response to glucose withdrawal, and are unable to upregulate stress-specific mRNAs. Overall, our analyses indicate the importance of the interplay between transcriptional and posttranscriptional stress responses, and indicate a key role for Pin4 in their coordination.

P18

EMC disruption affects mitochondrial function by altering sterol homeostasis

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Abstract

Mitochondrial function is highly conserved in eukaryotes as it is essential to maximize the biological fitness of any eukaryotic cell. In fact, the defective function of this organelle represents the cellular basis of some serious hereditary diseases in humans. Therefore, the characterization of the genes involved in the correct mitochondrial structure and function is essential to understand and treat these diseases. Using fission yeast as a biological model, we are characterizing the function of the *oca3* gene, the ortholog of EMC2 in humans. This gene is a component of the EMC complex of the Endoplasmic Reticulum (ER) membrane. It has been proposed that this complex participates in the membrane contacts of the ER and the mitochondria and is related to sterol homeostasis. However, it has only been related to the function of the reticulum in humans and the biological functionality of these contacts is not known in detail. Oca3 overexpression causes lethality. Its deletion, like that of the other components of the complex, is viable although conditional on temperature. The lack of function of Oca3 produces aberrant aggregations of mitochondria related to a significant decrease in oxygen consumption, loss of mitochondrial DNA and asymmetric and deleterious segregation in meiosis. These data suggest a role in mitochondrial stability, dynamics, and function not previously described.

P19

Assessment of natural yeast strains for increased tolerance to inhibitor compounds encountered during fermentation of lignocellulosic residues to produce bioethanol

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Abstract

Determining effects of biomass- derived inhibitors on yeast performance will enable development of strategies for improved fermentation performance. Yeast from tropical sub-Saharan regions have received minimal attention and may harbour natural strains that may display increased tolerance toward inhibitor compounds. In the present study, efforts were made to ascertain the effects of some biomass-derived inhibitors on growth rate and ethanol production potential of selected natural yeast strains. Fufural (10.4, 20.8, 31.2mM) acetic acid (16.7, 33.3, 50mM), formic acid (5.43, 10.9, 16.3mM) were studied. Of all the yeast strains studied, yeast strains designated Y5338, Y5250, Y5542 were selected for their ability to display multiple inhibitor tolerance. Results revealed growth rates of yeast strains did not decrease significantly ($p < 0.05$) for all inhibitor/concentrations when compared to control. An average of 2 h delay was observed in lag phase of yeast strains in the presence of acetic and formic acid when compared to the control. Whilst in the presence of 3g/L fufural lag phase of Y5338 had a 3 h delay, lag phase of Y5250 and Y5542 doubled when compared to controls. Higher ethanol yields were observed in the presence of formic acid across all strains. However maximum ethanol yield of (8.3 g/L) was observed for Y5542 in the presence of 5.43mM formic acid and a lower ethanol yield (4.1 g/L) was observed for Y5338 in the presence of 31.2mM of furfural. Natural yeast strains may have interesting gene targets for increased tolerance to inhibitor compounds relevant for lignocellulosic fermentation.

P20

Optimizing Extract Recovery from Sorghum using Combinations of Exogenous Crude Microbial Amylase, Pullulanase and Glucoamylase produced from Yeast Strains

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Abstract

Crude enzyme extracts obtained from yeast isolates from cassava mill area for optimizing extract recovery from sorghum or malted sorghum was studied. Enzymes from yeast are gaining attention due to the advantages derived from them. Aside from yeast having short generation time, diverse enzymes can also be obtained from them. Most important, yeasts from which crude enzymes could be extracted for use in industrial processes abound in many natural environments. The present study examined the performance of crude enzyme extracts (amylase, pollulanase and glucoamylase) isolated from yeasts strains (SP40 and SS12) obtained from a cassava mill area. The crude enzyme extracts were used in mashing grain sorghum or malted sorghum using various enzyme combinations to study their effectiveness on the quality of wort specific gravity (SG), reducing sugars (RS) and free amino nitrogen (FAN). The decantation mashing that protected the enzymes and gelatinized sorghum starch during the mashing process was more effective in producing wort of higher SG, RS and FAN products. As with the control commercial enzymes studied in comparison with the crude enzymes, lower doses of the crude enzyme extract also produced higher wort SG, RS and FAN products. The activities of the crude amylase, pullulanase and glucoamylase from SP40 and SS12 were similar as both yeast strains produced mainly starch hydrolysing enzymes. The crude enzymes compared well with commercial enzymes, especially using lower doses of the crude enzyme extract to produce wort SG, RS and FAN products and this will save costs for the industry.

P21

Core Control Principles of the Eukaryotic Cell Cycle

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Abstract

The most widely accepted model for how CDKs control the cell cycle is that S-CDKs and M-CDKs are catalytically specialized with significant different substrate specificities to execute S-phase and mitosis. A second model is that S-CDKs and M-CDKs are redundant with each other, such that both act as sources of overall cellular activity which rises through the cell cycle phosphorylating S-phase substrates at a low CDK level and mitotic substrates at a high level. Using multiplexed phosphoproteomics assays of in vivo S-CDKs and M-CDKs we show that surprisingly their substrate specificities are remarkably similar. Normally S-CDK cannot undergo mitosis but can do so efficiently when PPI is removed from the SPB, where some mitotic substrates are localised which become better phosphorylated in the absence of PPI. We propose that the core cell cycle engine which temporally orders the cell cycle events of S-phase mitosis is largely based on a quantitative increase of CDK activity through the cell cycle combined with minor qualitative differences in substrate specificities of S-CDK and M-CDK.

P22

Yeast proteomic response to amino acid supplementation and media composition

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Abstract

Yeasts have evolved to optimize the use of nutrients available in their environment while retaining the capacity to face 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 better understand how *S. cerevisiae* shapes its metabolism in response to different media, we used high-throughput data-independent proteomics to measure *S. cerevisiae* proteomes upon supplementation with 18 individual amino acids (AA) or a mix of these AA.
In total, we quantified 3013 proteins across 20 different conditions in biological quadruplicates. To gain further insight into the diversity of proteome adaptation in complex media, we also measured the proteomes of the 1011 wild isolate collection in rich (SC) and minimal (SM) media, leading to a combined dataset of 623 proteins in 753 strains.
These datasets were used to identify differentially expressed proteins associated with the media-specific and core response to supplementation, and to study the reorganization of metabolic fluxes, notably in AA biosynthetic 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 pathway most often perturbed. Comparison between SC and SM media in the wild isolates collection showed that proteins associated with central carbon metabolism had the most diverse profiles, possibly reflecting metabolic adaptation to the strains' preferred environment.

P23

Questioning the Nonsense: What mechanism(s) connect pre-mRNA splicing with Nonsense Mediated mRNA Decay in fission yeast?

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Abstract

Nonsense-mediated mRNA decay (NMD) is a translation-linked mechanism believed to selectively destroy aberrant transcripts bearing premature translation termination codons (PTCs) in the cytoplasm. So far, the current and available models of NMD till date do not satisfactorily explain the molecular mechanisms of NMD in any organism. Pertinently, none of the proposed models can fully explain why and how the presence of an intron in a gene can enhance NMD. Unlike in mammalian cells, where it has been shown that the exon junction complex (EJC) of proteins serves as the nexus between pre-mRNA splicing and NMD, studies in other organisms have challenged this argument, and have revealed evidence that an EJC-like complex is not involved in splicing-dependent NMD in several eukaryotes. Building on our previous report, wherein we demonstrated that NMD is EJC-independent and could be enhanced by splicing in S. pombe, using GFP reporter constructs with and without introns and with PTCs in the coding sequence, we aim to provide answers to the fundamental question of what links pre-mRNA splicing in the nucleus to translation and consequently NMD in the cytoplasm. Our recent evidence suggests that the RNA helicase and EJC factor, fal1, previously thought to be essential is heat sensitive. Our current efforts are now channeled towards developing a genome-wide genetic screening system to screen for novel trans-acting factors that may be involved in splicing dependent NMD in S. pombe and determining the putative roles of the NMD factors in RNA processing.

P24

The role of DNA repair proteins in eccDNA formation in Saccharomyces Cerevisiae

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Abstract

It was once assumed that DNA deletion products were not maintained by the host cell, but it is now known that they may persist and have significant effect on cell phenotype in the form of extrachromosomal circular DNA (eccDNA), which can have a variety of physiological effects, including roles in cancer tumor evolution and as an immunostimulant. It is suggested that the level of eccDNA is determined by its formation rate, its ability to replicate and whether the eccDNA provides a selective advantage to its host cell. However, the mechanism of eccDNA production is not fully elucidated. It is believed that DNA damage followed by repair is a significant source of eccDNA generation. In this project, Saccharomyces cerevisiae yeast mutants deficient in DNA repair proteins were grown from single cells to populations. Their eccDNA content was sequenced and analysed to estimate eccDNA formation rate within each genotype, as well as their formation mechanism. The three main DNA repair mechanisms investigated are homologous recombination (HR), non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ). Determining the contribution of each pathway will increase the knowledge of how cells and cell populations may rapidly evolve and generate genetic diversity on which evolution can act, whether this is a yeast population adapting to limited nutrients or a cancer cell that becomes resistant to chemotherapy. Knowing which proteins and pathways are responsible for eccDNA may furthermore allow for targeted manipulation of eccDNA formation, which could possibly aid in the treatment of cancer or inflammatory conditions.

P26

Epigenetics of stress-induced genome instability in the human fungal pathogen *Candida albicans*

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Abstract

Candida albicans is a widely studied human fungal pathogen, responsible for millions of infections and nearly half a million deaths annually. These disastrous effects on human health have been linked to *C. albicans* ability to alter its genome, which may allow selection of fitter genotypes with advantageous phenotypic traits, such as increased virulence or antifungal resistance.

We have discovered the first genome-instability hotspot in the *C. albicans* genome: the TRE (TLO Recombination Element), a 300bp element located downstream the subtelomeric TLO genes. TRE stability is controlled by chromatin state. However, very little is known about mechanisms underlying subtelomeric-associated instability.

Our hypothesis is that host-driven environmental changes unlock genomic instability at TRE elements via alteration of chromatin states, leading to gene expression rewiring and DNA damage. Intrinsic DNA damage could be triggered, for example, by stalled replication forks during transcription-replication interferences, or by R-loop formation. In this project, we combine genetic and genomic approaches to determine why TRE are sites of genome instability, focusing on whether and how R-loops have a role in increased TRE-related instability.

P27

Genome regulation in S. pombe spores and their response to stress

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Abstract

Spores are a dormant stage of Schizosaccharomyces pombe that are highly resistant to many types of stress. Little is known about the genetic regulation that occurs within dormant spores, which appear to remain dormant with no morphological changes until they are fed. To determine how spores maintain dormancy, a transcriptome time course was done with spores of different age, compared to vegetative cells. The transcriptome was dramatically reprogrammed in spores compared to vegetative cells. Moreover, principal component analysis (PCA) on the transcriptome data readily separated these different time points, indicating a dynamic transcriptome during the spores' life. We also find that transcription in fully developed spores can still be modulated in response to stressful environmental changes. Surprisingly, these stress responses are 'remembered' in vegetative cells derived from the stressed spores, as reflected in altered gene expression and chronological lifespans compared to vegetative cells derived from unstressed spores.

We looked for similarities in transcriptome regulation between spores and other dormant stages: quiescent fission yeast cells, dauer stage in nematode worms, and embryonic diapause in turquoise killifish. In general, there was a weak positive correlation at the level of single genes that are differentially expressed, but a much stronger similarity of functional categories that are regulated. For example, genes involved in cell-cycle and proliferation pathways are down-regulated while genes functioning in autophagy, stress response and protein translation are universally up-regulated during dormancy. This analysis suggests that cellular processes are involved in dormancy that are conserved from microbes to vertebrate.

P28

Proteomic and metallomic responses of *Saccharomyces cerevisiae* to perturbations of environmental metal availability

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Abstract

A small subset of metal ions is essential for many biochemical processes inside all living cells. Although decades of careful experimentation in various model systems has led to the discovery of numerous functions of the essential metals, systematic studies that investigate the interactions of metal ions with various components of living cells remain scarce. Here, we describe a systems-scale study to determine the consequences of a perturbation in extracellular metal availability on the growth rate, intracellular metal concentration and protein abundance of a simple eukaryote - *Saccharomyces cerevisiae*. The results reveal novel inter-dependencies of the concentration of each essential metal on the availability of the others and system-wide changes in protein abundance. The protein abundance changes reveal widespread effects of perturbed metal availability on the *S.cerevisiae* metabolic network. We then generate novel hypotheses about protein function and protein-protein interactions using ensemble clustering and pairwise protein abundance correlations across the dataset. Our work is a novel resource for the yeast community that can be investigated further in future to explore various aspects of perturbed metal availability and to generate or test new hypotheses about the role of metal ions in relation to specific proteins or pathways.

P29

A Co-transcriptional Regulatory Mechanism Tightly Controls Gene Expression during Stress in Budding Yeast

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Abstract

The Nrd1-Nab3-Sen1 (NNS) complex terminates transcription and orchestrates gene reprogramming during as *Saccharomyces cerevisiae* adapts to low nutrients. Some of the mRNAs that are terminated by the NNS complex during glucose starvation are simultaneously targeted for transcription initiation, such as *PIC2*, a mitochondrial copper transporter. To understand the role of NNS-mediated suppression, we have compared the Pic2 levels and phenotype of strains coding for wild-type and *PIC2* without its RNA-binding sites for NNS, both in standard and low concentrations of raffinose, a non-fermentable carbon source. Using quantitative PCR and fluorescence assays, we confirmed that impairing NNS regulation of *PIC2* increases its expression. With single-cell continuous-flow microfluidics, we revealed that *PIC2* transcriptional noise was higher in mutants and showed that Pic2 levels correlated with larger cell size and defective cell division. Further, we demonstrated that Pic2 overexpression leads to mitochondrial hyperpolarization and increases cells' resistance to oxidative stress. Our results are consistent with the NNS complex suppressing stochasticity and so preventing abnormally high expression of stress-specific target genes during adaptation. Through this regulatory mode, the NNS complex may either modulate the delay between exposure to environmental stress and the beginning of adaptive gene expression or allow a rapid increase in transcription levels when the stress signal reaches a particular threshold.

P30

Distinct roles of Scy1p and Cex1p in traffic and adhesion

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Abstract

Background

In mammals the SCY1-like family of protein pseudokinases (SCYL) have been implicated in intracellular trafficking and RNA interactions. In yeast Cex1p, the ortholog of SCYL1, has been linked to COPI coated vesicles, little is known about Scy1p, ortholog of SCYL2 which interacts with the Ent3p ortholog epsinR.

Methods

To investigate possible defects in the trafficking of proteins in strains lacking SCY1 and CEX1, we immunoprecipitated 35S-labelled CPY, assayed for invertase activity and analyzed the cycling of Sec22p between ER and Golgi by Western Blot and immunofluorescence. Possible interaction partners of Scy1p and Cex1p were identified by yeast-two-hybrid assays. Furthermore, we examined the influence of the SCY1/CEX1 gene deletion on cell/cell- and cell/surface-adhesion using plate wash assays and RT-qPCR.

Results

We generated deletion mutants scy1Δ, cex1Δ and cex1Δ scy1Δ to analyze their role in intracellular trafficking. Neither CPY nor invertase transport was impaired but cex1Δ and cex1Δ scy1Δ failed to cycle Sec22p. In a yeast-two-hybrid assay only Scy1p was able to interact with the clathrin coated vesicle adaptors Ent3p and Ent5p, hinting on a distinct cellular function of Scy1p and Cex1p. Furthermore, cells lacking Scy1p lost the ability to invade agar plates as well to flocculate and show reduced expression of the flocculin Flo11p.

Conclusion

The cellular and molecular function of SCYL is debated. Here we present evidence, that the yeast orthologs Scy1p and Cex1p play different roles in intracellular trafficking.

P31

The role of Mid1 and the ESCRT protein Vps4 in regulating cytokinesis in fission yeast

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Abstract

Abnormality in the regulation of cytokinesis has been recognized as a mechanism in the pathogenesis of cancers and neurogenerative disorders. Endosomal sorting complex required for transport (ESCRT), polo-like kinase, aurora kinase, and anillin-like proteins are established key regulators of cell cycle-related events in both fission yeast (Schizosaccharomyces pombe) and higher eukaryotes (humans).

In this study, viable double gene mutants of the S. pombe genes vps4, ark1, plo1, and mid1 were identified and characterized by tetrad analysis in yeast strains. Growth assays, and image analysis of synthetic growth phenotypes of the double mutant strains in comparison with wild-type (WT), and mid1Δ (mid1 deletion) yeast strains revealed changes in growth rate and colony formation of some double mutants. Mutation of the serine 332 and 531 amino acids in Mid1 to alanine as phosphoresistant mutants, revealed defective growth and changes in morphology suggesting a role of phosphorylation in this process. We also showed a direct physical interaction between Mid1p and Vps4 which is essential for the correct cellular localization of Mid1p.

This study provides insights into the synthetic phenotypes and colony morphology of vps4Δ-mid1 double, plo1-mid1 double and ark1-mid1 double gene mutants suggesting that each three proteins could be a regulator of Mid1 and interact with Mid1 to control aspects of growth and cytokinesis during cell cycle progression. Furthermore, these results suggest a physical interaction between Mid1p and Vps4p is important for cytokinesis. Combined these observations will be useful in further understanding the processes that regulate cytokinesis in fission yeast and humans.

P32

Integration of extrachromosomal circular DNAs is a frequent source of structural variation in the eukaryotic model Saccharomyces cerevisiae

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Abstract

When an eukaryotic cell divides, the chromosomes are segregated evenly to the daughter cells in a process orchestrated by their centromeres. Accordingly, genes and alleles are distributed equally, preventing sudden gene loss or copy number changes. DNA circularization, however, frees the DNA from the centromere control, which opens new possibilities for genes to spread, replicate or get transcribed. Circular DNAs are a common feature of eukaryotic genomes and have been shown to drive adaptive processes in yeast and in human tumors. However, what it means for a gene to be on a circle has largely been unexplored, mainly due to lack of model systems. Here, we generate endogenous circular DNAs that can be selected and be maintained in a population of cells. We study the effects of circularization on cell growth, segregation, gene expression and gene mobility. We show how circularization is able to change the segregation rate of genes and increase their copy number heterogeneity. We also find that circles can reintegrate back into the linear genome and generate structural mutations more frequently than translocations. Finally, we observe that circularization is able to free genes from transcriptionally inactive areas and allow their expression. Collectively, our results show a new way in which genes can bypass the restrictions imposed by chromosomes on segregation and copy number, resulting in substantial phenotypic changes after very few generations.

P33

Identification of a meiosis-specific chromosome movement pattern induced by persistent DNA damage

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Abstract

Chromosome movements aimed to promote pairing and recombination between homologues during meiotic prophase follow a fixed pattern which can be modeled through cytoskeleton force dynamics. However, these movements present frequent microvariations that remain unexplored. Their periodicity or stochastic nature is unknown yet, making it difficult to investigate their biological significance. Using Schizosaccharomyces pombe as a model organism exhibiting dramatic meiotic nuclear oscillations, we propose a computational analysis of 3D time-lapse live-fluorescence information which allowed to identify and characterize microvariations in chromosomal trajectory and morphology during meiotic prophase. Additionally, we show evidence suggesting that these microvariations and interruptions in oscillations are due to the presence of persistent DNA damage during chromosome movement, as an intrinsic nuclear mechanism to govern them. We predict that our approach will be useful for the discovery of new and still undisclosed patterns in chromosome movements during gametogenesis.

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