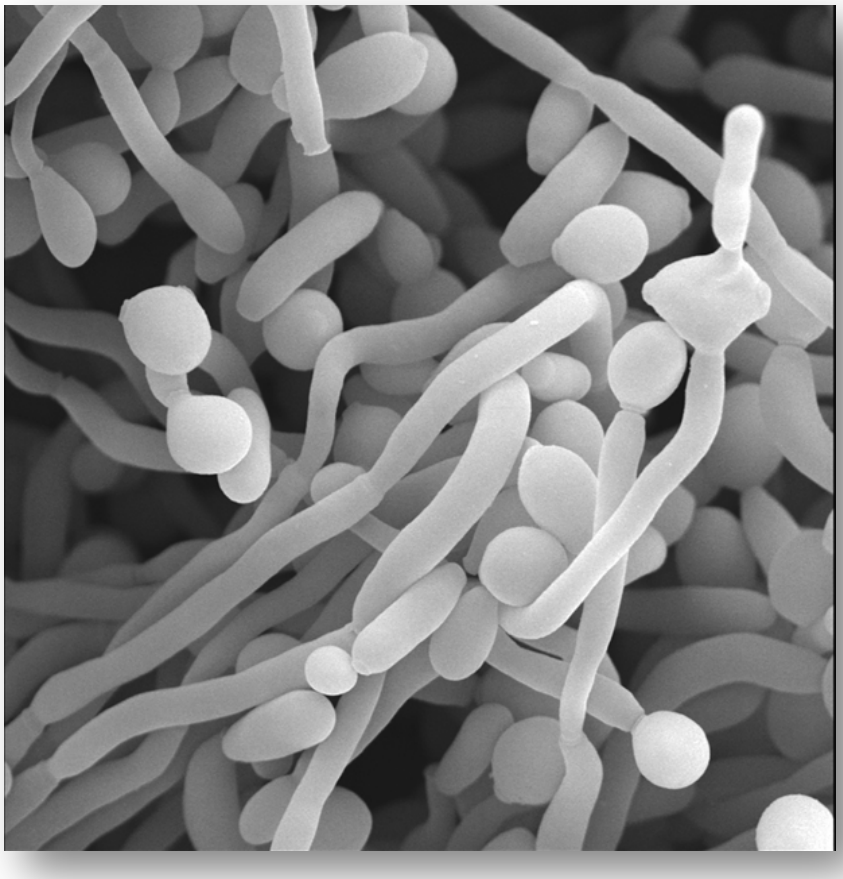
YEAST: PRODUCTS AND DISCOVERY 2017

A conference of the Australasian Yeast Group



Proceedings of the 7th Australasian Conference on Yeast: Products and Discovery

Mount Helen Campus, Federation University, Ballarat, Victoria, 29th November– 1st December 2017

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Cover art: Scanning electron micrograph (SEM) of *Candida albicans* biofilm, Courtesy Ana Traven

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YPD 2017: SCIENTIFIC PROGRAM

Wednesday 29th November

8.30 am - 9.30 am **Registration**

9.30 am - 10.30 am **Plenary lecture**

Chair: Ana Traven, Monash University

Rod Devenish, Monash University
Illuminating yeast mitophagy using Rosella – a pH biosensor

10.30 am – 12:00 pm **Session 1: Molecular Cell Biology**

Chair: Jiyoti Verma, Monash University

Harshini Weerasinghe, The University of Melbourne
Identifying the transcriptional response of the opportunistic, dimorphic human pathogen *Talaromyces marneffei* to growth within macrophages

Barbara Koch, Monash University
Discovering a new link between mitochondria and hyphal development in *Candida albicans*

Julianne Djordjevic, The Westmead Millenium Institute, Sydney
Why fungal pathogens up-regulate phosphate acquisition machinery in a phosphate-rich host: a paradox explained by pH and evolutionary rewiring of the phosphate signalling pathway

Carl Mousley, Curtin University
Reducing The Sweetness Of Protein Folding In The Endoplasmic Reticulum; A Sil1p Story

12:00 pm – 1:00 pm **Lunch**

1:00 pm - 2.30 pm **Session 2: Yeast in disease**

Chair: Dee Carter, The University of Sydney

Sophie Lev, The Westmead Institute Sydney
IP_{3-4} kinase arg1 regulates cell wall homeostasis and surface architecture to promote clearance of *Cryptococcus neoformans* infection in a mouse model

Megan Lenardon, The University of New South Wales
Candida albicans colonisation of the colon?

Timothy Tucey, Monash University
Glucose deprivation: how *Candida* exploits the host to evade innate immunity

Stefan Oehlers, The University of Sydney
Live imaging of host-cryptococcal interactions in the zebrafish infection model

2.30 pm -2.50 pm **Break**

2.50 pm - 4.20 pm **Session 3: Evolution**

Chair: Wieland Meyer, Westmead Institute Sydney

Mike McDonald, Monash University
Adaptation and recombination in experimental populations of *S. cerevisiae*

Wieland Meyer, Westmead Institute Sydney
DNA barcoding of human pathogenic yeasts

James Fraser, University of Queensland
Convergent microevolution of *Cryptococcus neoformans* hypervirulence in the clinic and laboratory

Richard Edwards, The University of New South Wales
Genomic and transcriptomic characterisation of evolved xylose metabolism in *Saccharomyces cerevisiae*

4.30 pm - 6.30 pm **Welcome drinks**

Thursday 30th November

9:00 am - 10.30 am **Session 4: Yeast Biotechnology**

Chair: Mark Bleackley, La Trobe University

Claudia Vickers, The University of Queensland

Engineering metabolic carbon flux regulation at the C10/C15 prenyl phosphate nodes for heterologous isoprenoid production in yeast

Sylvie Hermann-Le Denmat, Institute for Integrative Biology of the Cell, Université Paris-Saclay

Developing the new generation of beer yeasts

Simon Schmidt, The Australian Wine Research Institute

Survival of the fittest: The fight for dominance in grape juice and the genetics underpinning yeast strain performance

Kate Howell, The University of Melbourne

Yeasts from Australian sourdough starters: taxonomy, activity and interactions with bacteria to make bread of enhanced quality

10.30 am - 10.50 am **Break**

10.50 am - 12.40 pm **Session 5: Environmental Interactions and Ecology**

Chair: Lucia Zacchi, The University of Queensland

Anthony Borneman, The Australian Wine Research Institute

Wild wine: metagenomic analysis of microbial communities during wine fermentation

Sarah Knight, The University of Auckland

Regional microbial signatures positively correlate with differential wine phenotypes

Ben Schulz, The University of Queensland

Fungal Funk: exploring environmental yeast diversity to understand brewing biochemistry and to make better beer

Alex Idnurm, The University of Melbourne

Isolation of shooting mutants in the basidiomycete yeast *Sporobolomyces*

Vlad Jiranek, The University of Adelaide

The indigenous microflora of *Eucalyptus gunnii*, a substrate for Australian Aboriginal fermentations

12.40 pm - 1.30 pm **Lunch**

1.30 pm - 3.00 pm **Session 6: Student presentations**

Chair: Alex Idnurm, The University of Melbourne

Hellem Cristina Silva Carneiro, Universidade Federal de Minas Gerais, Brazil
The agrochemical benomyl influences the morphophysiology and the capsular polysaccharides of *Cryptococcus gattii*

Danila Elango, The University of Queensland
Physiological regulation of the oligosaccharyltransferase

Aakash Gupta, The University of Melbourne
Role of cell wall components in the interaction with the host cell macrophages and pathogenicity of the dimorphic fungus *Talaromyces marneffei*

Anttoni Hakola-Parry, University of Sydney
Characterizing the antifungal properties of Australian honey

Chien-Wei (Max) Huang, University of Adelaide
Identification of Yeast Genes Affecting Production of Hydrogen Sulfide and Volatile Thiols from Cysteine Treatment during Fermentation

Aidan Kane, University of Sydney
Squalene synthesis inhibitors – a novel treatment strategy for yeast pathogens

Edward Kerr, The University of Queensland
Wild yeasts for tasty beer

Tom Lang, The University of Adelaide
Altering arginine catabolism highlights the importance of proline biosynthesis in wine-like fermentations

Tamayanthi Rajakumar, Victoria University of Wellington
Deubiquitination as a modifier of Niemann-Pick type C disease

Joanna Rothwell, University of Sydney
Using Synergistic Drug Combinations to Inhibit Pathogenic *Aspergillus* Species

Jeffrey Sheridan, Victoria University of Wellington
A novel high-throughput confocal microscopy assay for drug discovery

Brianna Steed, The University of Melbourne
SWItching it up: Purification of fungal SWI/SNF complexes reveals compositional differences from their yeast counterparts

Irma Tedja, The University of Melbourne
The effect of hybrid histidine kinase response regulator SRRA on growth of the dimorphic pathogen fungus *Talaromyces marneffei*

3.15 pm – 4.00 pm **Session 7: ECR presentations**

Chair: Rod Devenish, Monash University

Lucía F. Zacchi, The University of Queensland
Expanding the yeast toolbox to study the secretory pathway

Åsa Pérez-Bercoff, University of New South Wales
Investigating the evolution of complex novel traits using whole genome sequencing and molecular palaeontology

James A McKenna, La Trobe Institute for Molecular Science
Plant Defensins Rapidly Kill Candida Based Biofilms

4.00 pm – 7.00 pm **Free Time**

7.00 pm **Conference dinner**
Mitchell Harris Wines
38 Doveton St Nth
Ballarat Victoria 3350

Friday 1st December

9:00 am - 10.30 am **Session 8: Gene Expression and Signalling**

Chair: James Fraser, The University of Queensland

Janni Petersen, Flinders University

AMPK and Target of Rapamycin (TOR) integrate environmental signals to control cell growth and division

Bernhard Dichtl, Deakin University

Molecular genetic analyses of the yeast Set1C histone methyltransferase

Tamás Fischer, The Australian National University

RNA-DNA hybrids and RNase H activity are required for efficient DSB repair

Evelyn Sattleger, Massey University, Auckland

Deciphering the interaction between Gcn1 and Gcn2, proteins involved in many biological functions

10.30 am - 10.50 am **Break**

10.50am - 12.20 pm **Session 9: Systems and Synthetic Biology**

Chair: Gabriel Perrone, Western Sydney University

Traude Beilharz, Monash University

Building transcriptional landscapes using t-SNE based dimensionality reduction of public data

Oliver Rackham, Harry Perkins Institute of Medical Research, Perth

A synthetic biology pipeline for understanding antibiotic resistance and producing new antibiotics

Andrew Munkacsi, Victoria University of Wellington

Chemical genetic analyses of antifungal compounds in feijoa fruit

Heinrich Kroukamp, Macquarie University

Yeast 2.0: Construction Update and Progress Towards Industrial Application

12.20 pm - 12.30 pm **Concluding Remarks**
Rod Devenish

12.30 pm - 1.30 pm **Lunch and Departure**

Plenary Lecture

Chaired by **Ana Traven** (Monash University)

0-1

Illuminating yeast mitophagy using Rosella – a pH biosensor

Devenish RJ, Lucarelli GA, Dawson K and Prescott M

Department of Biochemistry & Molecular Biology, Monash University, Clayton campus, Melbourne, Victoria 3800, Australia

Autophagy is a fundamental pathway, conserved amongst eukaryotes, by which intracellular components and organelles are delivered to the lumen of the vacuole for degradation and recycling by the cell. Mitochondria that are damaged or no longer required are subject to mitophagy, a selective form of autophagy.
Our knowledge of how mitophagy is regulated and integrated into cell metabolism is incomplete and requires further investigation. To this end we developed Rosella, a dual-wavelength fluorescent protein biosensor. Rosella can be targeted specifically to mitochondria (m-Rosella) allowing mitophagy to be assessed by fluorescence imaging of cells, or by immunoblotting of the GFP component which can reveal its degradation within the vacuole.
Two facets of the use of m-Rosella will be reported:
1. Its use in a semi-automated, multi-well format imaging assay for library screena leading to the identification of genes whose over-expression leads to perturbation of mitophagy.
2. To follow the localisation of mitochondria in relation to the cell wall and thereby assess its importance for mitophagy.

ORAL PRESENTATIONS

Session 1. Molecular Cell Biology

Chaired by **Jiyoti Verma** (Monash University)

1-1

Identifying the transcriptional response of the opportunistic, dimorphic human pathogen Talaromyces Marneffei to growth within macrophages

Harshini Weerasinghe, Hayley E. Bujega and Alex Andrianopoulos

Genetics, Genomics and Systems Biology, the University of Melbourne, Royal Parade, Parkville VIC 3010, Australia

Fungal pathogens of animals and plants are a major concern with huge economic and public health consequences. The emergence of opportunistic fungal pathogens that cause human disease exacerbates the current problem. It is therefore essential to understand the mechanisms that fungi employ to survive within a host. *Talaromyces marneffei* is a dimorphic, opportunistic pathogenic fungus that infects immunocompromised individuals. *T. marneffei* grows as a multicellular hyphal form at 25˚C, capable of producing infectious conidia, which upon inhalation reach the alveoli of the lungs where they are phagocytosed by resident phagocytes such as alveolar macrophages. The transition to 37℃, which is the human host body temperature, induces the dimorphic switch to a pathogenic, uninucleate, fission yeast form. The yeast form is able to utilize macrophages as a niche from within which to avoid immune detection, by acquiring nutrients and withstanding macrophage-killing responses. To determine the transcriptional response of *T. marneffei* to the host environment, including those induced by growth at body temperature as well as to host-derived cellular signals, RNAseq analysis was used to create a transcriptomic profile of *T. marneffei* during in vitro growth at 25˚C (hyphal) and 37˚C (yeast) and during murine and human macrophage infection. Key nutritional and cell protective pathways that show common upregulation during the yeast growth phase were identified and these included carbon and nitrogen utilization, micronutrient uptake, melanin generation and oxidative stress protection. Additionally, several host responsive genes important for different aspects of establishment, viability and morphological maintenance of *T. marneffei* yeast growth in macrophages were identified and functionally validated. These findings revealed that *T. marneffei* yeast and hyphal forms have adapted specific metabolic programs tailored to the diverse environmental conditions encountered by each cell type. It also uncovered components of pathways that respond to host–specific rather than temperature specific signals.

1-2

Discovering a new link between mitochondria and hyphal development in *Candida albicans*

Barbara Koch, Adele Barugahare, Traude Beilharz and Ana Traven

Monash Biomedicine Discovery Institute, 23 Innovation Walk, Monash University, Melbourne, Australia

Mitochondria function as a master regulator of cell metabolism. They are not only the key energy provider, but also play fundamental roles in diverse metabolic pathways such as lipid homeostasis, carbon source adaption, cell wall integrity and drug resistance. For the opportunistic pathogen *Candida albicans*, the switch between yeast and hyphal growth is a key virulence factor. In this study, we investigated the roles of mitochondrial dynamics in hyphal growth using a small molecule inhibitor. Strikingly, the small molecule inhibitor repressed the yeast-to-hyphae transition and stopped ongoing filamentation. These effects on morphogenesis can be uncoupled from a general growth inhibition. RNAseq experiments of inhibitor-treated cells coupled with *Candida* mutant analyses, suggest the existence of a novel mechanism of action to repress hyphal growth. The inhibitor was protective to host cells, increasing the survival of bone-marrow derived macrophages in *ex vivo* macrophage-*Candida* infection assays, suggesting it has potential as a therapeutic. Our work has implications for uncovering novel biology that governs *C. albicans* filamentation. Furthermore, compared to the antifungal drugs used in the clinic at present, our study has relevance for the identification of new therapeutic avenues with a distinct mechanism of action. Since *Candida* species cause an estimated 400,000 life-threatening infections per year worldwide, and the mortality remains in the range of 40%, identifying new avenues for treatment is an urgent need.

1-3

Why fungal pathogens up-regulate phosphate acquisition machinery in a phosphate-rich host: a paradox explained by pH and evolutionary rewiring of the phosphate signalling pathway.

Julianne T Djordjevic[1,2,3] and Sophie Lev[1,2,3]

[1]Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, 176 Hawkesbury road, Westmead NSW 2145; [2]Sydney Medical School, Westmead NSW 2145; [3]Marie Bashir Institute for Emerging Infectious Diseases, University of Sydney, NSW.

The AIDS-related fungus, *Cryptococcus neoformans (Cn),* establishes a lung infection, but patients often present with life-threatening meningitis. Understanding how *Cn* disseminates to the brain is critical to inform new therapies, and blocking nutrient supply is one approach. *Cn* tolerates different host microenvironments (lung, blood and brain), which impact its ability to obtain nutrients. Host pH is mildly alkaline, particularly in blood where *Cn* exists predominantly as free cells. However, in lung and brain, acidic clusters (cryptococcomas) are more common. The macronutrient phosphate is essential for numerous cellular processes. During phosphate deprivation, the phosphate signalling (PHO) pathway is activated in fungi to up-regulate phosphate acquisition machinery. In *S. cerevisiae,* the Pho4 transcription factor regulates ~20 phosphate-responsive genes in consort with Pho2, but acts solo in *Cn* to regulate more genes (~130). We observed activation of the PHO pathway in *Cn* (upregulation of Pho4-dependent phosphate transporters) at or above physiological pH, even when phosphate was present. Furthermore, *Cnpho4*Δ exhibited a growth defect, reduced phosphate uptake and reduced dissemination to the brain in a murine model. Alkaline pH therefore mimics phosphate starvation in *Cn*, triggering upregulation of phosphate transporters to replenish phosphate stores and promote *Cn* growth, particularly in blood. Functional annotation of Pho4-dependent genes in *Cn* suggests that loss of Pho2 co-regulation, and a highly divergent Pho4 sequence, broadens target regulation in *Cn* as compared to *S. cerevisiae*, to include non-phosphate transporters. Evolutionary rewiring of Pho4 in *Cn* may therefore extend PHO pathway function to a role in acquisition of nutrients other than phosphate, thereby promoting virulence and disseminated infection.

1-4

Reducing The Sweetness Of Protein Folding In The Endoplasmic Reticulum; A Sil1p Story

Carl Mousley

Curtin University, Perth, Australia

BiP (Kar2 in yeast) is an essential Hsp70 chaperone and master regulator of endoplasmic reticulum function. BiP's activity is regulated by its intrinsic ATPase activity that can be stimulated by two different nucleotide exchange factors, Sil1 and Lhs1. Both Sil1 and Lhs1 are glycoproteins, but how N-glycosylation regulates their function is not known. Here we show that N-glycosylation of Sil1, but not of Lhs1, is diminished upon reductive stress. N-glycosylation of Sil1 is predominantly Ost3-dependent and requires a functional Ost3 CxxC thioredoxin motif. N-glycosylation of Lhs1 is largely Ost3-independent and independent of the CxxC motif. Unglycosylated Sil1 is not only functional but is more effective at rescuing loss of Lhs1 activity than N-glycosylated Sil1. Furthermore, substitution of the redox active cysteine pair C52 and C57 in the N-terminus of Sil1 results in the Doa10 dependent ERAD of this mutant protein. We propose that reductive stress in the ER inhibits the Ost3-dependent N-glycosylation of Sil1, which regulates specific BiP functions appropriate to the needs of the ER under reductive stress.

Session 2. Yeast in disease

Chaired by **Dee Carter** (University of Sydney)

2-1

IP_{3-4} kinase arg1 regulates cell wall homeostasis and surface architecture to promote clearance of *Cryptococcus neoformans* infection in a mouse model

Cecilia Li[1,2,3], Sophie Lev[1,2,3], Desmarini Desmarini[1], Keren Kaufman-Francis[1,3], Adolfo Saiardi[4], Ana P.G. Silva[5], Joel P. Mackay[5], Philip E. Thompson[6], Tania C. Sorrell[1,2,3,7] and Julianne T. Djordjevic[1,2,3,7]

[1]Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, 176 Hawkesbury road, Westmead NSW 2145, Australia; [2]Sydney Medical School-Westmead, The University of Sydney, Westmead NSW 2145, Australia; [3]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, NSW Australia; [4]Medical Research Council Laboratory for Molecular Cell Biology, University College London, Gower street, London WC1E 6BT, UK; [5]School of Life and Environmental Sciences, The University of Sydney, Camperdown, NSW 2006, Australia; [6]Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville , VIC 3052, Australia; [7]Westmead Hospital, Westmead, NSW 2145, Australia.

We previously identified a series of inositol polyphosphate kinases (IPKs), Arg1, Ipk1, Kcs1 and Asp1, in the opportunistic fungal pathogen *Cryptococcus neoformans*. Using gene deletion analysis, we characterized Arg1, Ipk1 and Kcs1 and showed that they act sequentially to convert IP_3 to PP-IP_5 (IP_7), a key metabolite promoting stress tolerance, metabolic adaptation and fungal dissemination to the brain. We have now directly characterized the enzymatic activity of Arg1, demonstrating that it is a dual specificity (IP_3/IP_4) kinase producing IP_5. We showed previously that IP_5 is further phosphorylated by Ipk1 to produce IP_6, which is a substrate for the synthesis of PP-IP_5 by Kcs1. Phenotypic comparison of the *arg1*Δ and *kcs1*Δ deletion mutants (both PP-IP_5-deficient) reveals that *arg1*Δ has the most deleterious phenotype: while PP-IP_5 is essential for metabolic and stress adaptation in both mutant strains, PP-IP_5 is dispensable for virulence-associated functions such as capsule production and cell wall organization, as these phenotypes were defective only in *arg1*Δ. The more deleterious *arg1*Δ phenotype correlated with a higher rate of *arg1*Δ phagocytosis by human peripheral blood monocytes and rapid *arg1*Δ clearance from lung in a mouse model. This observation is in contrast to *kcs1*Δ, which we previously reported establishes a chronic, confined lung infection. In summary, we show that Arg1 is the most crucial IPK for cryptococcal virulence, conveying PP-IP_5–dependent and novel PP-IP_5–independent functions.

2-2

Candida albicans colonisation of the colon?

Megan Lenardon[1,2], Alan Walker[3] and Al Brown[2]

[1]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, 2052, Australia.
[2]Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.
[3]The Rowett Institute, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.

The major fungal pathogen of humans, *Candida albicans*, is a common cause of life-threatening hospital-acquired infections. About 40% of these infections are fatal even using current antifungal therapies. The source of these systemic infections is usually from the patient's own gastro-intestinal tract, which is generally colonised by *C. albicans*. In these cases, the fungal cells escape from the gut into the bloodstream when physical barriers or immunological defences become compromised. Surprisingly little is known about how *C. albicans* colonises the gastro-intestinal tract, and how it interacts with the gut microbiota. We have therefore developed a novel two-phase anaerobic fermentation system that simulates colon microenvironments which will be exploited to determine the adaptive mechanisms that enable *C. albicans* to persist in the colon in the presence of the gut microbiota.

2-3

Glucose deprivation: how *Candida* exploits the host to evade innate immunity

Timothy Tucey[1], Jiyoti Verma[1], Sarah Snelgrove[2], Tricia Lo[1], Michael Hickey[2], Traude Beilharz[1], Thomas Naderer[1] and Ana Traven[1]

[1] Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton VIC 3800

[2] Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Clayton 3168, Victoria, Australia

Candida albicans is the most prevalent fungal species causing human infections. To fight infections, macrophages undergo a metabolic shift whereby increased glycolysis fuels antimicrobial inflammation and killing of pathogens. We have devised live-cell imaging assays to monitor the *Candida*-macrophage interaction at unprecedented resolution. With this, we have shown that *Candida* co-culture with macrophages causes host cell death in two distinct phases termed Phase I and II. Phase I death lasts for the first 6-8 hours and results from NLRP3/caspase 1 inflammasome-dependent pyroptosis. Phase II death starts later, it is caspase 1-independent, and it kills the majority (60-70%) of the macrophage population under our experimental conditions, showing it is dominant over pyroptosis. In order to understand host and fungal factors that coordinate these distinct macrophage death pathways, we have performed dual RNAseq of Candida infecting murine bone-marrow-derived macrophages at time points corresponding to early and mid Phase I death, and early Phase II death. Our results suggest that *Candida* turns metabolic reprogramming into an Achilles' heel for macrophages. During *Candida*-macrophage interactions intertwined metabolic shifts occur, with concomitant up-regulation of glycolysis in both host and pathogen setting up glucose competition. *Candida* thrives on multiple carbon sources, but infected macrophages are metabolically trapped in glycolysis and strictly depend on glucose for viability: *Candida* exploits this limitation by depleting glucose, triggering mitochondrial catastrophe and rapid macrophage death. Using animal models, we show that macrophages die while responding to infection, and that glucose metabolism of the pathogen contributes to the impairment of host glucose homeostasis during infection, which promotes disease. Finally, glucose supplementation improves host outcomes in fatal candidaemia. Our results define a major microbial pathogenesis strategy that exploits nutritional vulnerabilities resulting from immunometabolic shifts.

2-4

Live imaging of host-cryptococcal interactions in the zebrafish infection model

Stefan Oehlers[1,2]

[1]Centenary Institute, Camperdown, NSW
[2]Sydney Medical School, Newtown, NSW

The human fungal pathogen *Cryptococcus neoformans* is capable of infecting a broad range of hosts, from invertebrates like amoebas and nematodes to standard vertebrate models such as mice and rabbits. Here we have taken advantage of the zebrafish platform to investigate host-pathogen interactions of cryptococcus with the zebrafish innate immune system, which shares a highly conserved framework with that of mammals. Through live-imaging observations and genetic knockdown, we establish that macrophages are the primary immune cells responsible for responding to and containing acute cryptococcal infections. By interrogating survival and cryptococcal burden following infection with a panel of fungal mutants, we find that virulence factors initially identified as important in causing disease in mice are also necessary for pathogenesis in zebrafish larvae. Live imaging of the cranial blood vessels of infected larvae reveals that *C. neoformans* is able to penetrate the zebrafish brain following intravenous infection. By studying a *C. neoformans FNX1* gene mutant, we find that blood-brain barrier invasion is dependent on a known cryptococcal invasion-promoting pathway previously identified in a murine model of central nervous system invasion. The zebrafish-*C. neoformans* platform provides a visually and genetically accessible vertebrate model system for cryptococcal pathogenesis with many of the advantages of small invertebrates. This model is well suited for higher-throughput screening of mutants, mechanistic dissection of cryptococcal pathogenesis in live animals, and use in the evaluation of therapeutic agents. I will discuss a new application of the platform in studying the vasculature as a druggable driver of cryptococcal pathogenesis.

Session 3. Evolution

Chaired by **Wieland Meyer** (Westmead Institute Sydney)

3-1

Adaptation and recombination in experimental populations of *S. cerevisiae*

Michael J. McDonald

Monash University, School of Biological Sciences, Clayton, VIC 3150

Evolution happens in any population of microbes, even when we don't want it too. Understanding adaptation is essential for the anticipation and mitigation of the possible deleterious effects of evolution. I will present recent work showing how high-throughput sequencing of experimental populations of the yeast Saccharomyces cerevisiae can provide insights into the mechanisms of adaptation. We varied the mode of reproduction (sex or asex) for replicate populations, and found that recombination sped up adaptation by fixing more beneficial mutations, and purging deleterious mutations from the population. We also sequenced metagenomes and multiple time points so that we could track the mutations as they arose and then segregated in the population. Our understanding of the evolution and ecology of natural communities comes from the "top down" approaches of community ecology and metagenome sequencing. Although experimental evolution can provide the genetic and phenotypic details of evolving populations, most microbial evolution experiments are in laboratory settings far removed from the actual conditions that microbes in the wild experience. This work is a small step towards connecting these two fields, so that the mechanistic insights possible in the lab are applied in experimental settings that better approximate natural and clinical environments.

3-2

DNA barcoding of human pathogenic yeasts

Meyer W[1], Irinyi L[1], Hoang M[1], Maszewska K[1], and Robert V[2]

[1]Molecular Mycology Research Laboratory, CIDM, Sydney Medical School-Westmead Hospital, MBI, The University of Sydney, Westmead Institute for Medical Research, Sydney, NSW, Australia, [2]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, e-mail: wieland.meyer@sydney.edu.au

Correct and fast identification of the causative agents of mycoses, especially yeast infections in the ICU, is of great importance to enable early diagnosis and targeted antifungal therapy. Increased phylogenetic knowledge of pathogenic yeasts is increasing our ability to delimitate species borders, enabling the development of accurate identification methods. DNA barcoding, based on the amplification of short DNA species specific signatures offers an accurate, fast, cost-effective, culture independent approach for species identification. The current primary fungal DNA barcode is the internal transcribed spacer (ITS) region. In 2015, an international consortium of medical mycology laboratories established the ISHAM-ITS database, the first quality controlled fungal barcode database for human and animal pathogenic fungi. Clinically important species show a low intra-species variability and a clear barcoding gap at inter-species level; consequently ITS sequencing can be reliably used for the identification of 75% of all species. However, for the remaining species an alternative barcode locus needs to be introduced to ensure reliable identification. A number of possible new loci were identified using whole genome sequencing data and possible primers were tested to be able to amplify the loci from a broad taxonomic range of fungi to ensure an accurate and reliable species identification in a clinical setting. Based on the general requirements of a barcode, such as amplification efficiency under standardized laboratory conditions, and the universality of the primers across different taxa, the translational elongation factor 1α (*TEF1α*) was proposed as official secondary barcode. The aim of current research is to generate *TEF1α* sequences for medically relevant species to complement the ISHAM-ITS database and to establish a new reference database for *TEF1α*. The intra- and inter-species variations of *TEF1α* locus compared to that of ITS region were evaluated. The *TEF1α* shows less intra-species and higher discriminatory power at inter-species level than the ITS, and improved the barcoding gap in some taxa.

3-3

Convergent microevolution of *Cryptococcus neoformans* hypervirulence in the clinic and laboratory

James A. Fraser

Australian Infectious Diseases Research Centre, School of Chemistry & Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

To gain insight into the evolution of virulence in the important fungal pathogen *Cryptococcus neoformans*, we investigated structural changes in genomic architecture such as inversions and translocations that distinguish the most pathogenic species of the *Cryptococcus* species complex, *Cryptococcus neoformans*, from the less clinically prevalent *Cryptococcus deneoformans* and *Cryptococcus gattii*. Synteny analysis between the genomes of the three *Cryptococcus* species (strains H99, JEC21, and R265) revealed that *C. neoformans* possesses surprisingly few unique genomic rearrangements. All but one are relatively small and are shared by all molecular subtypes of the species. In contrast, a large translocation peculiar to the C. neoformans type strain H99 was found in all tested subcultures from multiple laboratories, suggesting that it has possessed this rearrangement since its isolation from a human clinical sample. Building on this observation, we investigated these laboratory subcultures further, making two important discoveries. First, we identified a mutation in the gene *Low Mating Performance 1* (*LMP1*) that compromises mating and virulence. One of the H99 subcultures bearing this mutation was used for the first C. neoformans gene deletion set that has been widely used by the community. Second, we revealed that the most commonly used H99 laboratory subcultures belong to a mutant lineage of the type strain that is hypervirulent. The pleiotropic mutant phenotypes in this H99L (for “Laboratory”) lineage are the result of a deletion in the gene encoding the SAGA Associated Factor Sgf29, a mutation that is also present in the widely-used H99L-derived KN99a/α congenic pair. At a molecular level, loss of this gene results in a reduction in histone H3K9 acetylation. Remarkably, analysis of clinical isolates identified loss of function *SGF29* mutations in *C. neoformans* strains infecting two of fourteen patients, demonstrating not only the first example of hypervirulence in clinical *C. neoformans* samples, but also parallels between in vitro and in vivo microevolution for hypervirulence in this important pathogen.

3-4

Genomic and transcriptomic characterisation of evolved xylose metabolism in *Saccharomyces cerevisiae*

Richard J. Edwards[1], Åsa Pérez-Bercoff[1], Gustave Severin[1], Tonia L. Russell[2], Psyche Arcenal[3], Anna Sophia Grobler[3], Paul V. Attfield[3] and Philip J. L. Bell[3]

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The conversion of xylose to ethanol is vital for second generation biofuel production. Microbiogen Pty Ltd have have successfully evolved a Saccharomyces cerevisiae (Baker's yeast) strain ("MBG11a") that grows efficiently on xylose as a sole carbon source, unlike wild strains. We are using PacBio whole genome sequencing, deep Illumina population resequencing, and RNA-Seq to identify genes involved in the evolution of this new metabolic activity. Comparing our draft MBG11a assembly to the S288C yeast reference genome reveals over 115,000 variants, including over 14,000 coding changes across 3360 genes. In addition, MBG11a has several large insertions and deletions (approx. 63 kb missing and 164 kb extra, excluding Ty element changes) compared to S288C. To identify loci with important consequences for xylose utilisation, MBG11a was mated with a MBG11a/S288C hybrid strain that could not grow on xylose or galactose minimal media. Haploid offspring were competed on glucose, galactose and xylose minimal media and populations sequenced to identify MBG11a alleles changing in frequency in response to selection. Two candidate loci with unique protein-coding variants were identified in regions under significant positive selection on xylose minimal media. One showed classic signs of gene duplication followed by neofunctionalisation, while the other was identified as a master regulator of transcription. RNA-Seq analysis of MBG11a was used to identify genes with significantly increased expression on xylose versus glucose minimal media. This highlighted two further candidate genes, previously shown to substitute for key xylose metabolic proteins. Future work will focus on confirming whether the identified proteins are necessary and/or sufficient for S. cerevisiae to grow efficiently on xylose. Understanding how new biochemical pathways evolve in a sexually reproducing population is a complex and largely unanswered question. We have also sequenced populations at seven key time points during the evolution of xylose metabolism. By sequencing and assembling reliable full genomes of the founding ancestors, we will trace how mutations have interacted with existing genetic variation during evolution of this novel phenotype.

Session 4: Yeast Biotechnology

Chaired by **Mark Bleackley** (La Trobe University)

4-1

Engineering metabolic carbon flux regulation at the C10/C15 prenyl phosphate nodes for heterologous isoprenoid production in yeast

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Monoterpenes and sesquiterpenes have numerous industrial applications; however, production and harvesting from natural sources is commonly unfeasible and/or uneconomical at the scales required. Where chemical synthesis is possible using petrochemical precursors, again it is commonly uneconomical/unfeasible; moreover, there is a shift towards more environmentally friendly sustainable production routes. Metabolic engineering in microbes can provide such routes where the technoeconomic and lifecycle analysis is favourable. However, significant engineering is required to achieve the yields/rates/titres required for industrial application. We have developed a number of novel systems and synthetic biology tools to assess and control carbon flux around the C10 and C15 prenyl phosphate precursor nodes in the yeast terpenoid production pathway. In particular, this talk will focus on novel genetic circuit regulatory mechanisms and protein-mediated degradation approaches to control carbon flux through metabolic pathways that compete with the desired production pathways, thereby improving monoterpene/sesquiterpene product yields/rates/titres.

4-2

Developing the new generation of beer yeasts

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The worldwide booming craft beer industry is continuously looking for innovation and uniqueness in its beverage production. As a major contributor to flavour and character profile, yeasts affect the style of beer being brewed and are a source of craft beer innovation. The majority of yeasts used in brewing process within New Zealand are however imported commercial strains, resulting in an absence of *terroir* or place identity. In an effort to develop new strains with novel and local characters, we have isolated wild NZ yeasts from natural habitats around the Auckland area. From this, we have identified a large number of different isolates of ascomycetous yeasts, including *Saccharomyces paradoxus* and *Wickerhamomyces anomalus.* Preliminary trials in collaboration with a craft brewery suggest these strains are ill-suited to commercial beer production. Therefore, we are putting isolates through a program to adapt them to the brewing environment, mimicking the beer yeast domestication that has occurred for commercial strains. Working closely with craft breweries, our approach will develop local yeast strains that are suitable for commercial brewing operations.

4-3

Survival of the fittest: The fight for dominance in grape juice and the genetics underpinning yeast strain performance

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The complex interaction between yeasts and their environment is brought sharply into focus when wine fermentations fail to complete. Retrospective analyses of such failures are difficult or impossible because of the many combinations of factors that may lead to this undesirable outcome. These factors include choice of yeast strain, of which there are many, grape juice composition and winemaker intervention. The relationship between yeast strain genetics and its interaction with the grape juice environment is the subject of this work. 200 commercial wine yeasts, or yeasts isolated from wine, were sequenced to evaluate wine yeast genomic diversity. A representative subset of 94 strains was created, each with a unique DNA bar code introduced into the HO locus by homologous recombination. Competition experiments were used to evaluate differential fitness in response to environmental challenges enabling the parallel determination of fitness profiles in a range of industrially relevant media formulations. Environmental variables commonly associated with poor fermentation performance, such as sugar concentration and temperature, were not strong discriminating factors of yeast strain fitness. Copper and sulphite concentration, and nitrogen availability were, however, powerful contributors to fitness variations between wine yeast strains. Fitness based predictions of performance were evaluated using single inoculum fermentations. These experiments showed a high concordance between pooled culture fitness and individual strain performance profiles. The genetic determinants of copper tolerance in wine yeasts have been identified through breeding of high-fitness and low-fitness individuals and bulk segregant analysis to identify genetic determinants of fitness.

4-4

Yeasts from Australian sourdough starters: taxonomy, activity and interactions with bacteria to make bread of enhanced quality

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Yeast and bacterial communities inhabit a sourdough starter to make artisanal bread. This bread is increasingly sought after by consumers because of its distinctive flavour, attractive aroma and health properties of reduced gluten sensitivity. It is likely that microbial interactions provide some of these positive outcomes, and if characterised could be used to produce bread of superior health qualities. We surveyed sourdough starters from Victoria and identified *Saccharomyces cerevisiae* and *Kazachstania exigua* yeasts. When these yeasts were used to ferment wheat flour in an extended time fermentation, the bread had a heterogeneous crumb structure, a deeper colour and a distinctive chemical aroma profile than those made with commercial baker's yeast. When bread was made combining these yeasts individually and in combinations with lactic acid bacteria also isolated from these sourdough starters, including *Lactobacillus plantarum*, *Lb. brevis*, *Lb. rossiae*, *Lb. casei*, the bread aroma profiles and crumb structure were more distinctive, with compounds associated with *sour* aromas produced, and preferred by sensory panels. We assayed the protein structure of these breads by LC-MS and found that the low-molecular weight range of gliadin and glutenin were more diverse in mixed culture fermentations. The interactions of the yeasts and bacteria are likely to influence the production of enzymes which lead to the modification of gliadin and lead to an altered gluten content. This may mean that the bread is suitable for consumption for people on a low-gluten diet. The use of defined mixed cultures in commercial breadmaking, by exploiting the microbial diversity of artisanal starters can produce bread with distinctive and attractive aromas, crumb structure which is appreciated by sensory panels and has altered and potentially enhanced health properties.

Session 5: Environmental Interactions and Ecology

Chaired by **Lucia Zacchi** (The University of Queensland)

5-1

Wild wine: metagenomic analysis of microbial communities during wine fermentation

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Wine is a complex beverage, comprising thousands of metabolites that are produced by yeasts and bacteria acting on grape must. To ensure a robust and reliable fermentation, most wines are produced by inoculating grapes with specific commercial strains of the wine yeast Saccharomyces cerevisiae. However, there is a growing trend back to the historical practice of performing uninoculated or 'wild' fermentations, in which only those yeasts and bacteria that are naturally associated with the grapes or winery are used. Wild ferments show a far more complex progression of microbial species than inoculated wines and, accordingly, a more complex taste and aroma profile. As such, differences in these resident microflora between vineyards and wineries are therefore thought to have a key role in defining regional expression of wine characteristics. In order to map the microflora of spontaneous fermentation, metagenomic techniques are being used to monitor the progression of microbial species in large numbers of wild fermentations from across the major winemaking areas of Australia. Notable differences between regions, vineyards and wineries were apparent and these can be broadly defined by the resulting microbial composition of the wild ferments.

5-2

Regional microbial signatures positively correlate with differential wine phenotypes

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The history of mankind and yeasts, particularly Saccharomyces cerevisiae, has been intertwined for centuries in our pursuit to create fermented products such as wine. Today wine is a valuable economic commodity as well as a cornerstone of many social occasions. The diversity of flavours and aromas varies not only by grape variety but also by geographic origin, intriguing and engaging consumers and adding to the wines value. Often coined terroir, these differences are traditionally considered to arise from variations in local climates, soils and topography and the potential for microbes to contribute to these differences has largely been ignored until recently. To understand whether microbes may contribute to terroir, we must first understand their ecology and population biology. After all, if the communities and populations of yeasts do not differ between geographic regions, the opportunity for them to contribute regionally distinct flavours and aromas to wines does not exist. Focusing on the dominant fermenting yeast species S. cerevisiae, we genetically characterised the New Zealand population associated with vines and wines. Across different geographic regions we unveiled a complex pattern of differentiation mixed with varying levels of migration. Selecting isolates from these geographic regions, we fermented standardised Sauvignon Blanc juice and analysed the chemical composition of the resulting wines. Using statistical analyses, we were able to demonstrate that the chemical profiles of the wines produced varied significantly depending on where the yeast were originally isolated from, thus providing the first objective evidence of a microbial aspect to terroir. These results reinforce the need to better understand what forces shape microbial communities in our vineyards not just to ensure their sustainable management and vineyard longevity, but also to enhance wines local identity.

5-3

Fungal Funk: exploring environmental yeast diversity to understand brewing biochemistry and to make better beer

Ben Schulz

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Abstract not provided.

5-4

Isolation of shooting mutants in the basidiomycete yeast *Sporobolomyces*

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Fungal species that reproduce as yeasts face challenges for their successful dispersal in the environment. Amongst a subset of the basidiomycete yeasts, a unique mechanism of dispersal has evolved: this involves the formation of a specialised daughter cell, secretion of sugars from two parts of the cell, water condensation, and the fusion of the two drops of liquid to create the energy for propulsion into the air. The same mechanism of spore release is found in mushroom-forming species, yet the genes behind this process are largely unknown. These genes and their associated functions as required for ballistospore formation and release can be identified using the haploid yeast Sporobolomyces sp. IAM 13481. Approaches include forward genetic mutagenesis screens, a focused set of analyses on sugar biosynthesis and utilisation pathways, and using amoeba as a selective pressure. These methods have revealed that carbon availability regulates spore production and shooting, which requires the synthesis of sugars like fucose, which are not produced by ascomycete species. Impairing morphology of the cell, through loss of a number of highly conserved pathways, impacts on the ability to produce the specialised yeast for dispersal. Knowledge about these processes required for ballistospore formation may provide new directions to address the spread of problematic basidiomycete species.

5-5

The indigenous microflora of *Eucalyptus gunnii*, a substrate for Australian Aboriginal fermentations

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It is not widely recognised that Australia's Aboriginal people used plant materials in order to make flavoured 'cordials' or specifically as a base for fermentation. This study focusses on the now endangered cider gum, which is native to the remote Central Highlands of Tasmania. Itself of interest because of its unique ability to grow at the harsh edge of frost hollows, the cider gum also produces a sugar-rich exudate, which leaks from sites of frost, insect or animal damage. Australia's Aboriginals recognised the value of this sugar source, seeking out such trees, actively tapping them to allow its accumulation and spontaneous fermentation. With the support of Aboriginal authorities we have conducted the first study of this natural reservoir of yeasts. Unfermented samples were rich in maltose, glucose and fructose, whereas fermented sap contained high concentrations of gluconic and acetic acid as well as moderate concentrations of glycerol and ethanol. A myriad of yeast isolates and DNA extracts were also recovered. Amplicon-based ITS phylotyping has revealed a great diversity of fungal sequences between trees, sampling sites and dates. Notably, *Saccharomyces* is quite rare, while 25% or more of amplicons do not align with known fungal genomes, suggesting novel yeast species. Examples from the most recent and more detailed characterisation of selected isolates will be presented. It is hoped the work will help capture and preserve this unique and potentially endangered biological and cultural resource.

Session 6. Student Presentations

Chaired by **Alex Idnurm** (The University of Melbourne)

Short (5 minute) presentations by students who are not given talks in main sessions

6-1

The agrochemical benomyl influences the morphophysiology and the capsular polysaccharides of *Cryptococcus gattii*

Hellem Cristina Silva Carneiro[1,3], Rafael Wesley Bastos[1], Vanessa Silva de Carvalho[1], Glauber Ribeiro de Sousa Araujo[2], Susana Frases I[2], Dee Carter[3], Daniel Assis Santos[1]

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The increase in the global human population requires greater agricultural productivity, which is accompanied by an increased use of agrochemicals. The widespread use of various pesticides may cause environmental contamination and alter resident microbial communities. Benomyl is a broad-spectrum fungicide from the benzimidazole group and is used as a systemic protector of soil and foliage in crops of soy, cotton, corn, and rice, among others. This agrochemical is a mitotic inhibitor that binds to β-tubulin and depolarizes microtubules from the fungal cytoskeleton. *Cryptococcus gattii* is an environmental yeast and is one of the main etiological agents of cryptococosis, causing disease in both healthy and immunocompromised individuals. As *C. gattii* is found associated with plants it may be exposed to agrochemicals that could can change aspects of fungal virulence. The aim of this work was therefore to study the influence of benomyl on the cellular morphology and the capsular polysaccharide of *C. gattii*. Benomyl led to smaller cell and capsule size, decreased cellular electronegativity and increased surface/volume ratio. In addition, the distribution of the polysaccharide fibers increased from 0–2,500 nm in the wild type strain to 2,500–4,200 nm following benomyl exposure. Benomyl exposure also increased the effective diameter and the Zeta potential of secreted polysaccharides. In conclusion, exposure of *C. gattii* to benomyl modified the polysaccharide capsule, which is a major cryptococcal virulence factor. We now plan to study the molecular mechanisms underlying the morpho-physiological and virulence changes in *C. gattii*.

Keywords: cryptococosis; agrochemicals; virulence.

Financial support: CAPES, CNPq and FAPEMIG.

6-2

Physiological regulation of the oligosaccharyltransferase

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Asparagine (*N*)- linked glycosylation is a common and important post-translational modification (PTM) present in all three domains of life - archaea, eukaryota and bacteria. It has essential roles including facilitating protein folding, stability and function. It specifically refers to the attachment of carbohydrates onto asparagine (Asn) residues, typically within the consensus sequon N-X-S/T, where X cannot be proline. Biosynthesis of the donor glycan substrate for *g*lycosylation is a multi-enzymatic process catalysed by the Alg (asparagine-linked glycosyltransferase) enzymes (Alg1-14). The sequential activity of the Alg enzymes results in a 14-sugar oligosaccharide ($Glu_3GlcNAc_2Man_9$) that is transferred onto a protein acceptor substrate by the oligosaccharyltransferase (OTase). Defects in the *N*-glycan biosynthetic pathway cause changes in glycan occupancy, structure, and changes in protein abundance. While the oligosaccharyltransferase (OTase) is the central enzyme in *N*-glycoprotein biosynthesis, physiological regulation of the enzyme is poorly understood. To test for the presence of a regulatory mechanism controlling OTase activity in response to glycosylation stress, we used SWATH-MS to quantify site-specific changes in glycan occupancy in a yeast model system. We compared site-specific glycosylation in yeast with defects in LLO structure (*Δalg6*) and abundance (tunicamycin-treated cells). We identified a subset of sites that were inefficiently glycosylated in tunicamycin-treated cells but which remained efficiently glycosylated in Δ*alg6* cells. This suggested that LLO structure regulates site-specific OTase activity to ensure glycosylation is optimally targeted in conditions of glycosylation stress.

6-3

Role of cell wall components in the interaction with the host cell macrophages and pathogenicity of the dimorphic fungus *Talaromyces marneffei*

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Talaromyces marneffei is an opportunistic, human pathogenic fungus endemic to Southeast Asia. Being a member of the dimorphic group of fungi it exhibits two different cellular morphologies at different temperatures which has evolved to colonize their respective environment. At 25°C *T. marneffei* grows in a multicellular hyphal or mold form that can undergo asexual development to produce conidia (infectious agent). At 37°C *T. marneffei* grows in a unicellular yeast form that divides by fission and these yeasts are the pathogenic form found inside infected hosts. The hyphal-to-yeast transition of *T. marneffei* is considered to be crucial for its survival in the host and pathogenesis. To avoid recognition by the immune cells it resides within the host macrophages, where the yeast form can evade the host immune system and withstand macrophages acidic environment. Cell wall being the most extracellular component forms the first line of interaction between the fungus and the host cell. Cell wall comprises different Pathogen-Associated Molecular Patterns (PAMPs) on them which are recognized by specific proteins called Pattern Recognition Receptors (PRRs) present on the surface of the immune cells. Our study focuses on these different cell wall components of *T. marneffei*, which are differentially expressed in different morphological state and to observe their contribution in recognition and escape from murine macrophages.

6-4

Characterizing the antifungal properties of Australian honey

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Manuka honey produced from *Leptospermum scoparium* and *Leptospermum polygalifolium* plants possesses unique antibacterial activity, however only limited research on the antifungal potency these honeys have been undertaken. Fungal infections are a global health issue, affecting over 300 million people, and limited treatment options are available. *Cryptococcus* species have been found to have susceptibility to *L. polygalifolium* honeys that appears independent from its antibacterial activity, and while honey could not be used to treat cryptococcosis it may contain therapeutic lead compounds that could be further developed. Some honeys types also produce high levels of H_2O_2 via bee-derived glucose oxidase that is also antimicrobial. This study aimed to 1) determine the nature of the antifungal compound/s in *L. polygalifolium* honey, and 2) assess the spectrum of activity of *L. polygalifolium* honey and high-H_2O_2 honey towards different fungal strains and species, including yeasts and moulds. The antifungal compound/s in *L. polygalifolium* honey was found to be is small (<1000 Da), was inactivated by heating to 70^{O}C, and was stable over time when the honey was diluted. Species in the *Cryptococcus gattii* complex were all susceptible *L. polygalifolium* honey, and the dermatophytic mould pathogen, *T. rubrum* was particularly susceptible to high H_2O_2-honey. It appears likely that honey causes a general stress response in yeasts and moulds due to its attack from multiple angles, including osmotic and oxidative stress. Overall, the antifungal compound/s within *L. polygalifolium* honey, and the use of high H_2O_2-honey provide potenital avenues as unique antifungal treatments.

6-5

Identification of Yeast Genes Affecting Production of Hydrogen Sulfide and Volatile Thiols from Cysteine Treatment during Fermentation

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An early burst of hydrogen sulfide (H_2S) generated by yeast from cysteine could contribute to the formation of the desirable varietal thiols which contribute to the tropical aromas in varieties such as Sauvignon Blanc. *TUM1* was for the first time identified to play a critical role in the early production of H_2S from cysteine (Huang *et al.* 2016). Overexpressing *TUM1* elevated production of H_2S, while its deletion reduced the H_2S by half. Moreover, deletion of either *MET17* or *MET2* led to an additional delayed burst of H_2S, suggesting that a portion of the H_2S generated from cysteine is fed directly into the sulfate assimilation pathway. Triple deletants of *STR2*, *STR3* and individual *MET* genes, were shown to require both *MET17* and *TUM1* to bypass the transsulfuration pathway and grow on high concentrations of cysteine as the sole sulfur source. These results illustrate that cysteine is not converted to sulfate or sulfite, but rather to sulfide via a novel pathway requiring the action of Tum1p. Further investigations revealed that deleting genes involved in cysteine uptake such as *LST4, LST7, AGP1*, *GNP1*, *MUP1*, *STP1* and *DAL81* all resulted in reduced production of H_2S from cysteine (Huang *et al.* 2017). In conclusion, these findings not only have advanced our understanding of yeast cysteine catabolism, but also could be applied to develop better yeast strains to enhance tropical aromas of wines that appeal to many consumers.

References:

Huang CW, Walker ME, Fedrizzi B, Roncoroni M, Gardner RC and Jiranek V. The yeast *TUM1* affects production of hydrogen sulfide from cysteine treatment during fermentation. *FEMS Yeast Research* 2016;**16**:fow100. https://doi.org/10.1093/femsyr/fow100

Huang CW, Walker ME, Fedrizzi B, Gardner RC and Jiranek V. Yeast genes involved in regulating cysteine uptake affect production of hydrogen sulfide from cysteine during fermentation. *FEMS Yeast Research* 2017;**17**:fox046. https://doi.org/10.1093/femsyr/fox046

6-6

Squalene synthesis inhibitors – a novel treatment strategy for yeast pathogens

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Annually, up to 900,000 deaths are caused by infection with yeasts and yeast-like fungi. Fluconazole (FLC) is an antifungal triazole used to treat these dangerous infections. It is cheap, easy to administer in low-income regions, and has excellent bioavailability. FLC is losing efficacy, however, due to the emergence of resistant isolates. Squalene, a precursor for ergosterol, is produced by the mevalonate pathway, upstream from the action of FLC. Squalene synthesis inhibitors may therefore synergize with FLC and the combination might retard the acquisition of resistance. Isolates of yeast pathogens *Cryptococcus neoformans, Cryptococcus gattii, Candida albicans* and *Candida glabrata* were tested for susceptibility to FLC, an FDA-approved squalene synthesis inhibitor (SSI), and a novel lipophilic derivative (SSI-L) using CLSI methods. Checkerboard assays were performed to assess drug interactions between FLC and the two inhibitors. SSI was most potent in *Candida glabrata,* with an MIC of 10 µg/mL, and worked equally well in highly FLC-resistant strains. Combinations of FLC and SSI showed broad synergy (FICI <0.5) in *Cryptococcus* sp. and *Ca. glabrata*. SSI-L was up to 256-fold more potent than SSI and exhibited potent fungicidal activity at 5-20 µg/mL, although it was less synergistic with FLC. When single-species and poly-microbial biofilms were treated with combined FLC and SSI-L they were significantly disrupted at only 2-4x the dosage used to inhibit planktonic cells. To test resistance acquisition, yeast cells were grown in sub-inhibitory concentrations of each drug separately and in combination, then repeatedly sub-cultured in increasing drug concentrations. The FLC+SSI-L combination retarded the development of antifungal resistance, which was rapidly acquired to FLC alone. In summary, combining FLC and SSI/SSI-L leads to increased fungicidal activity against both FLC-susceptible and -resistant isolates, inhibits fungal biofilms and retards resistance development. With further development, FLC and SSIs should provide promising novel antifungal combination strategies.

6-7

Wild Yeasts for Tasty Beer

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Yeast is essential for making beer, as it produces ethanol and contributes heavily to beer's flavour and sensory properties. Commercial breweries typically use *Saccharomyces cerevisiae* for production of ales, and *Saccharomyces pastorianus* for lagers. The most notable exceptions to this are sour, lambic, and American coolship ales, where wort is exposed to a mix of environmental yeast and bacteria, rather than commercially cultivated Saccharomyces strains. Spontaneous environmental inoculation for beer fermentation is becoming more common and rather trendy. However, such wild ferments have unpredictable rates, efficiencies, and end products. We propose instead to isolate clonal strains of wild yeast, to allow efficient and predictable production of beers with interesting and diverse flavour profiles. Here, we describe a method for isolating and characterising wild yeast for brewing unique and interesting beers. Yeast is collected from the wild and grown on solid selective media to isolate clonal strains. These clones are identified by internal transcribed region (ITS) PCR and Sanger sequencing. Each clone is grown in liquid wort to measure growth on oligomaltose, and to identify fermentation end products. Gas chromatography mass spectrometry (Headspace-GCMS) is then used to quantify ethanol and other fermentation end products from fermentation. Strains that efficiently grow and produce ethanol are then fermented at 10ºC and 20ºC, and flavour profiles are measured using Headspace-GCMS. Intermediate upscaling of strains with efficient growth, ethanol production, and pleasant or interesting smelling ferments is then performed in a food grade brewery to allow sensory evaluation. This workflow provides the tools needed go from environmental yeast isolation to producing brewery-quality beer. This workflow also has uses beyond brewing, allowing quick and robust identification of wild yeasts and identification of compounds related to flavours in diverse beverages or industrial applications.

6-8

Altering arginine catabolism highlights the importance of proline biosynthesis in wine-like fermentations

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Stuck and sluggish fermentations are a recurring problem for winemakers due to low assimilable nitrogen levels in grape must. To combat this, it has become common practise to supplement grape must with diammonium phosphate early in fermentation, which is not always reliable. The dependence on inorganic supplementation could be minimised by optimising the catabolism of already abundant endogenous nitrogen sources, which are not effectively utilised during fermentation. One such nitrogen source is arginine, a 4-nitrogen amino acid, where 3 of the nitrogens are efficiently catabolised to glutamate, with the final being incorporated into proline, which requires oxygen for catabolism in the mitochondrion [1]. Previously it has been shown that expression of a *PKG1* promoter-driven Put2p lacking a mitochondrial targeting sequence (*PUT2*(1-16)) in a Δ*pro3* mutant resulted in doubled biomass production and glutamate accumulation with arginine as the sole nitrogen source [2]. Put2p utilises Δ^1-pyrroline-5-carboxylate (P5C) formed from proline catabolism in the mitochondrion to create glutamate. By removing the mitochondrion targeting signal, Put2p can utilise cytoplasmic P5C, which, under normal circumstances, is utilised to biosynthesise proline from arginine catabolism. We introduced this plasmid, pOM, expressing *PUT2*(1-16) into a haploid derivative of Enoferm M2 (*ura3 pro3::KanMX4/PRO3*). When fermented in medium with arginine as the sole nitrogen source, strains containing pOM completed significantly faster than strains containing pJC1 (empty vector control) in both low (20 g L^{-1}) and high (200 g L^{-1}) sugar. However, in strains wildtype for *PRO3* in the presence or absence of pOM, fermentation was completed significantly faster and at identical times. Further analysis of the faster performing strains in a Chemically Defined Grape Juice Medium 'wine-like' fermentation showed that the strain containing pJC1 completed fermentation significantly faster (48 h) than the strain containing pOM; with increased cell viability throughout fermentation. Intracellular proline has been identified as important for response to stresses including hyperosmotic and ethanol stresses, which are experienced during fermentation. Altering proline biosynthesis may disrupt these stress responses and result in the slower performance and viability of the yeast observed. These results highlight the importance of proline biosynthesis from arginine catabolism under fermentation conditions.

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6-9

Deubiquitination as a modifier of Niemann-Pick type C disease

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Niemann-Pick type C (NP-C) disease is a fatal, paediatric neurodegenerative disease due to the lysosomal accumulation of free cholesterol and sphingolipids. There currently is no effective therapy to treat NP-C disease. Using the yeast model of NP-C disease (*ncr1*Δ), a genome-wide conditional synthetic lethality screen was conducted using myriocin to mimic the disruption of sphingolipid metabolism in human patients. We identified the conserved deubiquitinase UBP6 exacerbates the growth defect of *ncr1*Δ yeast model of NP-C disease. Specifically, the ubiquitin ligase RSP5 mediates the NCR1-UBP6 synthetic lethal interaction. Results investigating Usp14, the human orthologue of UBP6, will be presented to determine the conservation of the UBP6 modifier. Since the deletion of UBP6 exacerbates lethality of the yeast model of NP-C disease, we hypothesize that Usp14 activation will reverse the NP-C disease pathway and reduce disease severity in human patient cells.

6-10

Using Synergistic Drug Combinations to Inhibit Pathogenic *Aspergillus* Species

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Azoles act on the ergosterol biosynthesis pathway, which is dependent on squalene as a precursor. We hypothesised that by inhibiting squalene synthesis we might develop synergistic therapies that would also retard resistance development. We tested this in *Aspergillus*, an environmental saprotroph that is a frequent cause of opportunistic human and animal infections and is known to become azole resistant following drug exposure. Minimum inhibitory concentrations (MIC) were determined for medical azole drugs voriconazole (VRZ), itraconazole (ITZ), fluconazole (FLC) and agricultural triazole fungicides propiconazole (PRO), and tebuconazole (TEB) as well as a squalene synthesis inhibitor (SSI) and a lipophilic derivative designed to improve cell penetration (SSI-L), in *Aspergillus* species that have been implicated in human and animal infections, including *A. fumigatus, Aspergillus flavus, A. terreus, A. niger, A. parafelis, A. frankstonesis, A. felis, A. udagauae* and *A. nishimurae*. The SSI MICs were 640 µg/ml for most of the species and were and 160 µg/ml for *A. terreus* and *A. frankstonensis*. SSI-L MICs ranged from 0.3 – 5 µg/ml depending on the species, demonstrating up to a 256 fold increase in antifungal activity compared to the standard SSI. Checkerboard assays were performed on *A. flavus* and *A. fumigatus* to assess synergistic interactions between azoles and the SSI. Significantly synergistic results were obtained with FICI values from 0.17-0.46. Serial propagation in the presence of increasing drug concentrations led to high levels of resistance to the agricultural azoles TEB and PRO. Resistance was completely retarded, however, using azole-SSI combinations. From this it can be concluded that azole-SSI combinations increase antifungal activity, can interact synergistically and can retard resistance development. The use of squlalene synthesis inhibitors and their lipophilic derivatives may have potential in clinical treatment strategies in the future and could help to increase agricultural and food security.

6-11

A novel high-throughput confocal microscopy assay for drug discovery

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Drug discovery is a multi-disciplinary field incorporating both chemistry and biology to create novel pharmaceuticals. The bioactivity of a novel focussed compound library was explored using a phenotypic screen measuring growth inhibition. A compound, S13, was determined to be the most potent in the library, therefore genome-wide screening was performed using S13. High-throughput confocal microscopy of 4,100 strains, each with a different GFP-tagged protein and a defective pleiotropic drug response, was utilized to determine proteins that increased in abundance or changed localization in response to perturbation with S13. Following treatment with S13, the yeast vacuole increased in size due to an aggregation of proteins in the vacuolar lumen. The increase in vacuole size was coincident with a decrease in vacuolar acidity, potentially disrupted autophagy and the upregulation of several proteins involved in ergosterol biosynthesis. Together, these results reveal a novel compound that increased vacuole size and pH through an epistatic mechanism involving ergosterol biosynthesis.

6-12

SWItching it up: Purification of fungal SWI/SNF complexes reveals compositional differences from their yeast counterparts

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Talaromyces marneffei is a pathogenic fungus, endemic to South-East Asia, capable of causing lethal systemic infection in immunocompromised humans. In response to temperature changes, *T. marneffei* alternates between hyphal and pathogenic yeast growth forms: a process known as dimorphic switching. As a potential avenue to design novel anti-fungal therapies, we are interested in the molecular mechanism of dimorphic switching and how it is regulated at the chromatin level. SWI/SNF chromatin-remodelling complexes are evolutionarily conserved, multi-subunit protein complexes, which act as DNA translocases to alter nucleosome position. These complexes regulate transcription by remodelling nucleosomes in the promoter regions of genes, facilitating access to transcriptional machinery. Tandem-Affinity Purification (TAP) coupled with Mass Spectrometry (MS) identifies the subunit compositions of the *T. marneffei* SWI/SNF complexes; SWI/SNF and RSC. These purifications reveal compositional differences between the *T. marneffei* SWI/SNF complexes and those purified from yeast, including the identification of four novel proteins conserved across the filamentous fungi. Purification of SWI/SNF and RSC from the model filamentous fungus *Aspergillus nidulans* suggests these compositional differences are conserved in other filamentous fungi, and confirms the presence of three of these novel proteins in the homologous *A. nidulans* complexes. These findings highlight similarities and differences between the compositions of fungal SWI/SNF complexes and those previously published. Going forward, we have identified clear targets for interrogation of the role of SWI/SNF complexes in dimorphic switching, as well as key fungal processes.

6-13

The effect of hybrid histidine kinase response regulator SRRA on growth of the dimorphic pathogen fungus *Talaromyces marneffei*

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Fungi occupy a range of niches with changing environmental conditions. Thus, adaptation is essential for survival and growth. Signalling systems play a key role in allowing fungi to detect and respond to environmental stimuli. One of the signalling systems utilised by fungi is the two-component histidine kinase signalling system. It allows the activation of MAPK pathway, which results in the regulation of gene expression for stress responses, such as osmotic, oxidative, and cell wall stress. The fungal two-component histidine kinase system consists of sensory hybrid histidine kinase (HHK), an intermediate phosphotransmitter protein (HPt), and response regulator proteins (RR). In both pathogenic and non-pathogenic fungi, this system is involved in a range of essential cellular processes, from hyphal morphology and conidiation, to spore viability and stress adaptation. Furthermore, it is crucial for establishing virulence attributes in pathogenic fungi, such as dimorphism and adaptation within a host. As this system plays a major role in fungi during disease manifestation, it is important to understand how it contributes to pathogenicity. Talaromyces marneffei is an opportunistic pathogenic fungus, which exhibits temperature dependent dimorphic switching. At 25°C, it grows as filamentous hyphae, which undergo asexual development to produce infectious conidia, while at 37°C, T. marneffei grows as a pathogenic yeast, which proliferates by fission. Inhaled T. marneffei conidia are engulfed by the pulmonary alveolar macrophages, which will attempt to destroy them through fusion with the phagolysosomal system. T. marneffei needs to adapt to survive destruction by these macrophages, and use them as a host for growth and infection establishment. In T. marneffei, three of the HHKs and one of the RRs were found to be important for several aspects of cellular development and pathogenicity. This study investigates the role of a second RR, SrrA, on T. marneffei growth. Deletion of the srrA gene shows effect on intracellular growth and response to oxidative stress and fungicide. Furthermore, preliminary data shows that SrrA, which may act as a transcription factor, can act as a link to MAPK pathway for cell stress responses.

Session 7. ECR presentations

Chaired by **Rod Devenish** (Monash University)

7-1

Expanding the yeast toolbox to study the secretory pathway

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The secretory pathway is responsible for shaping 1/3rd of the eukaryotic proteome. Most of this secretory proteome has the key role of allowing cells to interact with and respond to a changing environment. In the case of yeasts, secretory proteins also contribute to the bouquet of some of our favorite drinks and are industrial bioproducts. Vital post-translational modifications like disulfide bond formation and N-linked glycosylation occur inside the secretory pathway. Complex molecular machinery is involved in controlling the quality of secretory protein folding and in targeting unfolded proteins to degradation. Major homeostatic responses are regulated at the membrane surfaces of this pathway. Thus, understanding the biological mechanisms at play in the secretory pathway is important to better understand cell biology and to better understand how to manipulate the secretory machinery for biotechnological purposes. We have designed genetic and mass spectrometry proteomic tools that allow us to dissect diverse functional aspects of the secretory pathway. Firstly, we designed a new automated genome-wide genetic screen to identify genetic modifiers of protein levels in the Endoplasmic Reticulum. This screen allows us to identify genes/processes acting on the folding, degradation, and secretion of specific proteins of interest. Using this screen we identified >300 genetic modifiers of the levels of a secretory protein variant involved in the neurological disease Dystonia. Secondly, we combined biochemical methods with SWATH-mass spectrometry (glyco)proteomics to obtain a quantitative measurement of how perturbations to Endoplasmic Reticulum homeostasis impact protein levels and Nglycosylation. Our methods considerably expand the capabilities of yeast as a model organism, allow us to identify novel players involved in the secretory pathway, and inform on the function of known secretory pathway proteins and processes.

7-2

Investigating the evolution of complex novel traits using whole genome sequencing and molecular palaeontology

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Understanding how new biochemical pathways evolve in a sexually reproducing population is a complex and largely unanswered question. We are using PacBio whole genome sequencing and deep population resequencing to explore the evolution of a novel biochemical pathway in yeast over several thousand generations. Growth of wild *Saccharomyces cerevisiae* (Baker's yeast) strains on the pentose sugar xylose is barely perceptible. A massmated starting population was evolved under selection on Xylose Minimal Media (XMM) with forced sexual mating every two months for four years. This produced a population that could grow on and utilise xylose as its sole carbon source.

We are now using a novel "molecular palaeontology" approach to trace the evolutionary process and identify functionally significant loci under selection. Populations at seven key time points during the course of evolution have been sequenced using Illumina paired-end sequencing. In addition, all the parental strains from the founding population have been subject to PacBio de novo whole genome sequencing and assembly. By constructing reliable full genomes of the ancestors of our populations, we can the trace evolution of these populations over time. We can therefore track the trajectory of allele frequencies through time, identifying the contributions of different founding strains and novel mutations. We are using these data to estimate the proportions and regions of the genome that have evolved neutrally (due to genetic drift), under purifying selection, or adaptively in response to xylose selection. To date, much of our understanding of evolutionary processes is derived from theoretical models, and/or by reconstructing theoretical ancestors of extant individuals. Our unique array of both extant and past, but not extinct, populations allows us to put these theories to the test.

7-3

Plant Defensins Rapidly Kill *Candida* Based Biofilms

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The plant defensins are a family of innate immunity peptides. Selected members of this family kill or retard the growth of a wide variety of fungal species and have been reported to be active against *C. albicans* biofilms (Vriens et al 2015). We have been investigating the potential of several members of the plant defensin family for the treatment of *C. albicans* based biofilms and have been characterizing the ways these defensins kill fungal biofilms. Systemic Candida infections often arise from biofilms that form on cannulas, catheters and surgical implants. Systemic candida infections are a major problem in the immunocompromised where they are associated with high morbidity and mortality. The global incidence of systemic Candida infections is estimated to be approximately 400,000 cases each year with mortality rates ranging from 46-75% (Brown et al., 2012). *Candida* based biofilms are recalcitrant to standard antifungal therapies, only responding to two kinds of agents, echinocandins (e.g. Caspofungin) and polyenes (e.g. amphotericin B). These agents are susceptible to the development of resistance in the pathogen and have associated toxicity effects respectively. To investigate the potential of several plant defensins as a therapeutic treatment we have been using a modified microplate model to generate *C. albicans* biofilms based on the procedure of Pierce et al (2008). When a 24-hour old biofilm is treated with either the defensin HXP124 or caspofungin the IC100s were about 20μM and 0.5μM respectively. In this system we observed total cell killing by HXP124 within the first hour after application. The plant defensins tested began killing the *C. albicans* based biofilms within the first five minutes after application, whereas caspofungin took longer than one and half hours to induce cell death. To date, we have investigated several recombinantly produced plant defensins for their ability to kill established *C. albicans* biofilms. Recently we have begun to assess the synergy between these plant defensins and caspofungin on biofilms.

Session 8. Gene Expression and Signalling

Chaired by **James Fraser** (The University of Queensland)

8-1

AMPK and Target of Rapamycin (TOR) integrate environmental signals to control cell growth and division

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Target of rapamycin (TOR) signalling co-ordinates cell growth and cell proliferation with the specific cellular context and environment. Several distinct conserved signaling pathways mediate nutrient sensing and of these, the TOR pathway plays a central role. It is widely accepted that AMPK is a major energy sensor in cells and its effect on cell metabolism is in part through its inhibition of TOR Complex 1 (TORC1) signalling. We have recently identified a third, conserved, amino-acid independent mode of nitrogen sensing in fission yeast. Nitrogen stress changed TORC1 activity and this nitrogen control of TORC1 and cell proliferation is driven by AMPK. Our future aim is to uncover the principles of nitrogen control of AMPK and TORC1 in both fission yeast and human cell lines. We have exploited proteome-wide SILAC and mass spectrometric based approaches to identify changes in phosphorylation events that arise from perturbation of TOR signalling, via either nitrogen stress or Torin1 inhibition of TOR. This has identified significant changes in phosphorylation of more than 300 conserved proteins. GO term analyses of the 117 proteins specifically regulated by nitrogen stress indicated that 50% are known regulators of nitrogen metabolic processes. Thus novel AMPK and TORC1 regulators may be identified amongst these 117 proteins regulated by nitrogen stress.

8-2

Molecular genetic analyses of the yeast Set1C histone methyltransferase

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Post-translational modifications present on the amino-terminal tails of histone proteins are important regulators of gene expression and other chromatin associated processes. Mono-, di- and tri-methylation of histone H3 lysine 4 (H3K4) is a hallmark of actively transcribed chromatin, which *is facilitated* exclusively by the Set1C/COMPASS histone methyltransferase in *Saccharomyces cerevisiae*. Set1C is a multisubunit complex composed of eight subunits: Set1, Swd1, Swd2, Swd3, Spp1, Bre2, Sdc1 and Shg1. The Set1 protein carries the catalytic SET domain and requires the presence of other complex components for stability and activity. While much effort has been spent in elucidating how distinct H3K4 methylation states correlate with gene expression patterns, less is known about associated biological functions that are regulated via this chromatin modification. In order to enhance our functional understanding of H3K4 methylation and Set1C components, respectively, we performed global yeast two hybrid screening with individual Set1C subunits. The obtained cellular Set1C interaction network revealed numerous novel links to cellular processes including meiotic recombination, cell-cycle dependent gene regulation and the SUMOylation network. We will present molecular genetic analyses for some of the identified interactors.

8-3

RNA-DNA hybrids and RNase H activity are required for efficient DSB repair

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RNA-DNA hybrids are a major internal cause of DNA damage within cells, and their degradation by RNase H enzymes is important for maintaining genomic stability. We have identified an unexpected role for RNA-DNA hybrids and RNase H enzymes in DNA repair. Using a site-specific DNA double-stranded break (DSB) system in *Schizosaccharomyces pombe*, we showed that RNA-DNA hybrids form as part of the homologous recombination (HR)-mediated DSB repair process and RNase H enzymes are essential for their degradation and efficient completion of DNA repair. Deleting RNase H stabilizes RNA-DNA hybrids around DSB sites and strongly impairs recruitment of the ssDNA-binding RPA complex. In contrast, overexpressing RNase H1 destabilizes these hybrids, leading to excessive strand resection and RPA recruitment, and to severe loss of repeat regions around DSBs. Our findings challenge the existing model of HR-mediated DSB repair, and reveal a surprising role for RNA-DNA hybrids in maintaining genomic stability.

8-4

Deciphering the interaction between Gcn1 and Gcn2, proteins involved in many biological functions

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In all Eukaryotes, the General Amino Acid Control signalling pathway is essential for coping with amino acid starvation. In this pathway, the protein kinase Gcn2 detects amino acid shortage and subsequently phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α). This in turn alters the translation and transcription profile, to trigger the necessary cellular responses. In order to detect starvation, Gcn2 must directly bind to its effector protein Gcn1, via its RWD domain. In addition to amino acid starvation, Gcn2 - and likely also Gcn1-Gcn2 interaction - is implicated in many additional biological functions, as well as in diseases and disorders, such as memory formation, neuronal differentiation, immune system regulation, insulin and lipid homeostasis, cell cycle, cancer. Here, we aim to shed more light on this important Gcn1-Gcn2 protein-protein interaction, using yeast (*Saccharomyces cerevisiae*) as model.

Session 9. Systems and Synthetic Biology

Chaired by **Gabriel Perrone** (Western Sydney University)

9-1

Building transcriptional landscapes using t-SNE based dimensionality reduction of public data

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Every RNA-seq or microarray expression analysis holds two sets of information: i) the specific response to altered experimental conditions intended by the researcher; and ii) the general output of a native repertoire of transcriptional wiring options. We reason that large public transcriptomic databases now hold such quantities of gene expression data, collected under such a variety of experimental conditions, that we might be able to dissect the fine-detail of transcriptional and post-transcription connectivity in an unbiased manner. That is, the expression of some genes are likely always co-regulated with a set of functionally related genes, because they share either transcription factors, post-transcriptional regulatory elements, or both. Our approach has therefore been to use the t-SNE algorithm to project genes in high-dimensional 'gene-expression' space down to a two-dimensional layout to understand native wiring from across 1000's of public expression datasets. Our first attempts using ~7000 experiments from the highly curated SPELL data for baker's yeast, reveals discretely ordered and biologically meaningful islands of co-regulated genes. Importantly, guilt by association might ascribe function to many unannotated genes. These visual landscapes are particularly useful when used as a framework for overlay and interpretation of new data. To this end, we built Shiny apps in R-studio to overlay, search, explore and annotate the yeast expression landscape. We will present our unpublished work in progress using t-SNE for visualisation of the native wiring of gene expression in eukaryotic cells.

Key Words
Transcriptomics, exploratory data analysis, data visualisation, t-SNE

9-2

A synthetic biology pipeline for understanding antibiotic resistance and producing new antibiotics

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The emergence of “superbugs”, bacteria that resist all current antibiotics, poses a significant risk to public health. However, the development of new antibiotics has dwindled since most of the pharmaceutical giants have abandoned or severely curtailed their antibiotic discovery programs. Therefore the need for new approaches to address this problem is immediate. Reassembling the genes for antibiotic production in well-characterized microbes that can be readily grown at industrial scales provides a promising avenue to address these issues. Our vision is to harness the tremendous potential of synthetic biology to build yeast that can make valuable new antibiotics. We have developed a variety of genetic selection systems that allow the re-engineering of macromolecules via life/death selections in yeast and we have applied these systems to enable the determinants of antibiotic resistance to be systematically mapped. Furthermore, we are now adapting these approaches to link the survival of yeast engineered to contain genetic pathways for antibiotic biosynthesis to the production of new antibiotic derivatives.

9-3

Chemical genetic analyses of antifungal compounds in feijoa fruit

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The feijoa plant, *Acca sellowiana*, is classified in the family Myrtaceae, native to South America, and currently grown worldwide to produce feijoa fruit. Antifungal compounds have been isolated from feijoa; however, the diversity of these compounds is not known nor is the mechanism of action of any of these compounds. We hypothesize that understanding the chemical diversity of antifungal compounds across feijoa cultivars and determining the antifungal mechanism of action of feijoa compounds will provide insight into the pharmaceutical potential of these compounds. First, GC-MS analyses were used to obtain an unbiased profile of 151 compounds from 16 cultivars of feijoa, of which six were experimental cultivars. Multivariate analysis distinguished 18 compounds that were significantly correlated to antifungal activity based on growth inhibition of *Saccharomyces cerevisiae*, of which seven had not previously been described from feijoa. Two novel cultivars were identified as the most bioactive cultivars, and the compound 4-cyclopentene-1,3-dione found in a couple of cultivars was potently antifungal against human pathogenic isolates of four *Candida* species. Chemical genetic analyses were used to investigate the bioactivity of 4-cyclopentene-1,3-dione and additional compounds. The molecular mechanisms underlying the value of feijoa as a source of antifungal drugs will be discussed.

9-4

Yeast 2.0: Construction Update and Progress Towards Industrial Application

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The construction and study of synthetic genomes are crucial for understanding the complexities of genome structure, organisation and plasticity. The Synthetic Yeast 2.0 project introduced a novel concept of forced inducible evolution and massive genomic rearrangement. This is facilitated by the incorporation of loxPsym recombination sites downstream of all nonessential genes and the subsequent recombination at these sites by an inducible Cre-recombinase; induction of this LoxP/Cre recombination is termed SCRaMbLE. This system has been used to generate libraries of yeast strains with diverse genome rearrangements. SCRaMbLE-ed populations are generally enriched for gene duplications and inversions. Longer recombination induction periods result in an increased frequency of recombination events, increases the post-induction population diversity, but also severely impair cell fitness due to the loss of essential genes or gene combinations. While these genomic rearrangements will facilitate a greater understanding of yeast genetics, it is currently only available in specific designer laboratory strains and are unlikely to convey industrially relevant phenotypes at a high frequency. Several studies have previously shown that subtle changes in native gene copy number can result in significant industrially relevant phenotype improvements. It has also been reported that increased ploidy may stabilise toxic euploidy effects that are generated during SCRaMbLE. In this study we set out to construct synthetic yeast 2.0 strains with increasing ploidy states (n, 2n, 3n, 4n). We aim to determine the effect of ploidy on the relative population fitness after extended periods of SCRaMbLE and to improve the concomitant enhancement in foreign DNA incorporation through loxPsym-associated recombination. In addition, we will investigate various gene copy number effects (including that of essential genes and epistatic--like effects) on industrially relevant phenotypes. In this initial part of the study, we demonstrated an increased post-SCRaMbLE population viability with an increase in strain ploidy. The higher ploidy states and concomitant increased loxPsym recombination sites allowed significantly higher URA3 marker-cassette integration. These preliminary findings suggest that the synthetic lab strains could serve as stable, tunable gene dosage ‘add-ons’ to established industrial strains for further optimisation and serve as conduits for enhanced heterologous gene integration.

Miscellaneous Abstracts (Poster presentation)

Compounds from marine invertebrates that cure yeast of prions

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A number of neurodegenerative disorders are caused by the accumulation of aberrant misfolded amyloid forms of normal proteins as aggregates in the brain. In some cases the conversion of the normal protein to the insoluble amyloid form is attributable to an infectious process. Prions (an infectious protein fold) are the etiological agent and represent the smallest known infectious agent. The neurodegenerative diseases caused by prions are invariably fatal and there is a lack of curative treatments. Therefore, there is an urgent need to identify small molecules that have the ability to enter cells and cure amyloid prions. Screens using animals or cell lines infected by prions are expensive and time-consuming. However, the discovery that yeast (*Saccharomyces cerevisiae*) also harbours a range of different amyloid prions enables the use of yeast as a tool to facilitate the screen for anti-prion compounds. We have used two amyloid yeast prions [*PSI*$^{+}$] and [*URE3*] to optimise previous high-throughput methods for using yeast in anti-prion screens to enable us to for the first time apply such screens to natural extracts. Our aim to identify natural yeast prion inhibitors that can be used in the future to develop more effective treatment strategies for human neurodegenerative disorders. We screened >500 marine invertebrate extracts from temperate waters in northern NSW and identified 24 that cure yeast cells of one yeast prion ([*PSI*$^{+}$]. Of these, 6 extracts also cure the unrelated yeast prion [*URE3*]. Bioassay-driven chemical investigation of one of these 6 bioactive sponge extracts showed that a group of bromotyrosine derivatives were active. Active molecules in the other 5 extracts have now also been identified. This study outlines the importance of screening natural products and the use of yeast strains infected with amyloid prions as a first stage screen for the identification of new chemically-diverse bioactive compounds to treat neurodegenerative diseases.

List of YPD 2017 delegates

Alex Andrianopoulos	The University of Melbourne
Traude Beilharz	Monash University
Mark Bleackley	La Trobe University
Anthony Borneman	The Australian Wine Research Institute
Kylie Boyce	The University of Melbourne
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