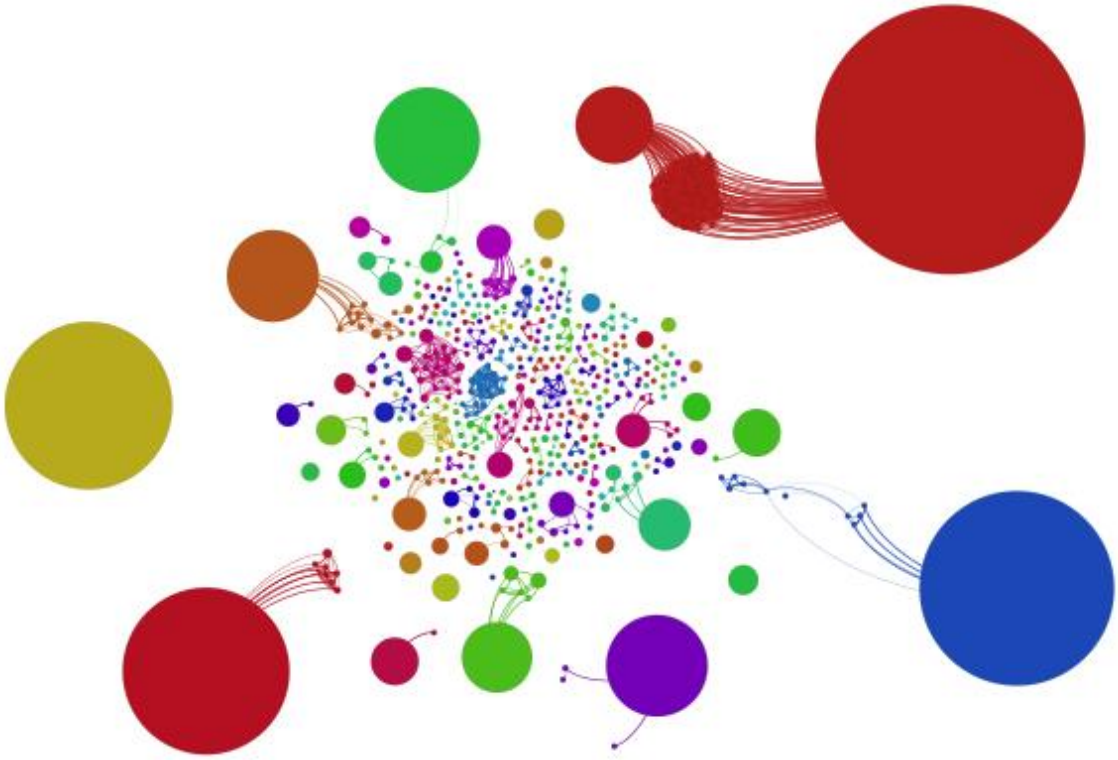
# YEAST: PRODUCTS AND DISCOVERY 2015

## A conference of the Australasian Yeast Group



**Proceedings of the 6th Australasian Conference on Yeast: Products and Discovery**

**Ingkarni Wardli Building, The University of Adelaide, North Terrace, Adelaide, 2 – 4 December 2015**

**Editors: Paul R Grbin & Paul Chambers**

## Organising Committee

Jenny Bellon
Australian Wine Research Institute
jenny.bellon@awri.com.au

Paul Chambers
Australian Wine Research Institute
paul.chambers@awri.com.au

Miguel de Barros Lopes
The University of South Australia
miguel.debarroslopes@unisa.edu.au

Paul Grbin
The University of Adelaide
paul.grbin@adelaide.edu.au

Paul Henschke
Australian Wine Research Institute
paul.henschke@awri.com.au

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**Cover art: 'Yeast Bubbles' by Anthony Borneman**. Anthony will explain all in his talk in Session 8

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YPD 2015**: SCIENTIFIC PROGRAM**

**Wednesday 2nd December**

9.00 am **Registration and Welcome**
9.45 am Opening Address:

**Ian Dawes** (University of New South Wales)
Redox homeostasis and cellular stress responses

**Chair: Ana Traven** (Monash University)

10.30 am **Session 1: Molecular Cell Biology**
**Chair: Julie Djordjevic** (Westmead Millennium Institute)

**Ben Schultz** (University of Queensland)
Beer Proteomics

**Trevor Lithgow** (Monash University)
Nanoscale mapping of the route for protein import into mitochondria

**Mark Prescott** (Monash University)
A genetic screen for genes that regulate mitophagy in *Saccharomyces cerevisiae*

**Jiyoti Verma-Gaur** (Monash University)
Integration of posttranscriptional gene networks into metabolic adaptation and biofilm maturation in *Candida albicans*

12.00 – 1.00 pm **Lunch**

1.00 pm **Session 2: Yeasts: Understanding and Treating Disease**

**Chair: Dee Carter** (University of Sydney)

**Traude Beilharz** (Monash University)
3' UTR dynamics predict mRNA fate

**Brian Monk** (University of Otago)
Insight for antifungal discovery from structures of full- length yeast lanosterol 14α-demethylase.

**Mark Bleakley** (LaTrobe University)
Plant innate immunity peptides as therapeutics for infection by *Candida albicans* and other fungal pathogens

**Dee Carter** (University of Sydney)
A transcriptomic analysis of drug synergy in yeast and the fungal pathogen *Cryptococcus*

2.30 – 3.00 pm **Break**

3.00 pm **Session 3. Systems and Synthetic Biology**

**Chair: Anthony Borneman** (Australian Wine Research Institute)

**Tom Williams** (Macquarie University)
Elucidating whole-genome design principles using synthetic genomics: The Yeast 2.0 project

**Danna Lee** (Australian Wine Research Institute)
Synthetic biology production of raspberry ketone in wine yeast.

**Cristian Varela** (Australian Wine Research Institute)
Systems Biology: a new approa*ch to* industrial yeast *st*rain development

**Jens Krömer** (University of Queensland)
Metabolic engineering of *Saccharomyces cerevisiae* for the production of aromatics

**Marc Wilkins** (University of New South Wales)
The Regulation of intracellular phosphate is affected by protein arginine methylation

4.30 – 6.00 pm **Social gathering with beer/wine & pizza**

**Thursday 3 December**

9.00 am **Session 4: Signalling**

**Chair: Evelyn Sattlegger** (Massey University)

**Janni Petersen** (Flinders University)
Target of rapamycin (TOR) control of cell growth and division in fission yeast

**Alan Munn** (Griffith University)
A mutational approach to locate the heart of WASP - a key regulator of actin dynamics from yeasts to beasts

**Jörg Heierhorst** (St. Vincent's Institute of Medical Research & The University of Melbourne)
Regulation of the yeast cell cycle checkpoint kinase Rad53

**Evelyn Sattlegger** (Massey University)
New insights into the regulation of protein kinase Gcn2.

10.30 – 11.00 am **Break**

11.00 am **Session 5. Yeast in Fermentation Industries**

**Chair: Paul Henschke** (Australian Wine Research Institute)

**Jenny Bellon** (Australian Wine Research Institute)
Breeding new life into the ancient art of winemaking

**Toni Cordente** (Australian Wine Research Institute)
Developing novel wine yeast as a tool to adjust wine flavour and aroma to market specifications

**Michelle Walker** (University of Adelaide)
Can further improvements be made in oenological traits of wine yeast?

**Ben Binder** (University of Adelaide)
How do we quantify the filamentous growth in a yeast colony?

12.30 – 1.00 pm **Lunch**

1.00 pm **Session 6: Student Presentations**
**Chair: Paul Grbin** (University of Adelaide)

Five minute presentations by students who are not presenting talks in main sessions

| | |
|---|---|
| **Jessica Chitty** | University of Queensland |
| **Namal Coorey** | Victoria University of Wellington |
| **Ana Hranilović** | University of Adelaide |
| **Chien-Wei (Max) Huang** | University of Adelaide |
| **Laurence Jennings** | Griffith University |
| **Heinrich Kroukamp** | University of Stellenboch |
| **Heike Mack** | Griffith University |
| **Seeseei Molimau-Samasoni** | Victoria University of Wellington |
| **Kathy Parisi** | La Trobe University |
| **Bingyin Peng** | University of Queensland |
| **Josephine Jasmine Peter** | University of Adelaide |
| **Ee Lin Tek** | University of Adelaide |
| **Federico Tondini** | University of Adelaide |
| **Greg Valentine** | University of Adelaide |
| **Ryan Zeppel** | The Australian Wine Research Institute |

**Questions**

2.30 pm **Trip to McLaren Vale and Conference Dinner**
Transport provided

Tour and wine tasting at Hardys Tintara OR Chapel Hill

Wine tasting and conference dinner at Wirra Wirra winery

Return to CBD approximately 10.00 pm

**Friday 4 December**

9.00 am **Session 7: Yeast/Environment Interactions**

**Chair: Mark Prescott** (Monash University)

**Simon Schmidt** (Australian Wine Research Institute)
Yeast competitive fitness in wine-like fermentation environments

**Ana Traven** (Monash University)
*Candida* in the host environment: mechanisms of immune evasion

**Alex Andrianopoulos** (University of Melbourne)
Sensing and responding to the environment - Specialised cell types of the dimorphic pathogen *Talaromyces marneffei*

**Alexander May** (Monash University)
What is the physiological role of autophagy in yeast?

10.30 – 11.00 am **Break**

11.00 am **Session 8: Evolution & Ecology**

**Chair: Chris Curtin** (Australian Wine Research Institute)

**Anthony Borneman** (Australian Wine Research Institute)
Metagenomic analysis of wild wine fermentations

**Wieland Meyer** (Westmead Millennium Institute)
Understanding the evolution and ecological spread of the *C. neoformans*/*C. gattii* species complex

**Samantha Arras** (University of Queensland)
Mutation of SGF29 in the *Cryptococcus neoformans* type strain H99 is responsible for altered virulence of the key laboratory version of this strain

**Chris Curtin** (Australian Wine Research Institute)
Genomic landscape of the industrial yeast species *Brettanomyces bruxellensis*

12.30 – 1.30 pm **Lunch**

1.30 pm **Session 9: Gene Expression**

**Chair: Traude Beilharz** (Monash University)

**Thomas Preiss** (Australian National University)
Dynamics of ribosome scanning and recycling revealed by steady-state translation complex profiling

**Julie Djordjevic** (Westmead Millennium Institute)
Impact of phosphate acquisition strategies on fungal virulence

**Gabriel Perrone** (University of Western Sydney)
Studying genetic interaction networks to better understand redox, DNA and lipid homeostasis on a cell- wide level

**Bernard Dichtl** (Deakin University)
Impact of Histone H3 lysine 4 methylation and RNA 3' end formation pathways on Benomyl toxicity

3.00 pm **Close**

## Opening Address

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

0–1

### Redox homeostasis and cellular stress responses

Ian Dawes

School of Biotechnology and Biomolecular Sciences, University of NSW, Sydney NSW 2052

While much has been published on the responses of yeast cells to oxidative stress, less is known about how cells maintain redox conditions under normal circumstances and in the face of different stresses. By using non-invasive probes for redox based on ro-GFP, and for pH (based on pHlourin) that can be targeted to specific organelles it is possible to study how organellar redox changes in response to different stresses. This approach has also been used to identify genes that are important for the maintenance of redox in different compartments, by screening mutants from the genome-wide deletion collections to identify those with altered redox. From somewhat initially less related research on responses of cells to addition of single amino acids it has become clear that cells have very highly interconnected transcriptional responses to a wide variety of stresses possibly as a result of the strong cross-regulation between the main transcription factors mediating responses to a variety of environmental cues.

# ORAL PRESENTATIONS

## Session 1. Molecular Cell Biology

Chaired by **Julie Djordjevic** (Westmead Millennium Institute)

1–1

### Beer Proteomics

Benjamin L Schultz

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

Beer production is a modern industrial process technology developed over millennia. However, many details of the biochemistry of the process remain unclear. Using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS), we have performed a global untargeted analysis of the proteomes of sweet wort, hopped wort, and fermented beer. This analysis identified over 200 unique proteins from barley, hops and yeast, emphasizing the complexity of the process and product. We then used data independent SWATH-MS to quantitatively compare the relative abundance of these proteins throughout the process, which measured large and significant changes in the proteome at each process step. These changes described enrichment of proteins by their biophysical properties, and identified the appearance of dominant yeast proteins during fermentation. Altered malting and milling parameters also quantitatively changed the proteomes throughout the process. Detailed inspection of the proteomic data revealed that many proteins were modified by protease digestion, glycation and oxidation during the processing steps. We have also performed SWATH-MS to compare the cell-wall and whole cell proteomes of selected diverse strains of brewing yeast. This showed large differences in the abundance of cell wall proteins, including floculins, that correlated with the strains' flocculation behaviour. Together, this demonstrates the opportunities offered by modern mass spectrometry proteomics in understanding the ancient process of beer production.

1–2

## Structure and function of the TOM complex in yeast mitochondria

Takuya Shiota and Trevor Lithgow

Biomedicine Discovery Institute & Department of Microbiology, Monash University, Melbourne VIC 3800

Eukaryotes are chimeras formed from a fusion of bacterial cells in the distant evolutionary past. We now have the vast majority of our genetic material located within the nuclear envelope, but an essential fraction of the genome is housed within our cell's mitochondria. This scenario provided the foundation on which multi-cellularity became possible, but also created fundamental problems that had to be solved in the primordial eukaryotes in order to establish coherent "systems biology" to integrate metabolic and cell-cycle control over what had been two independent organisms. Now, mitochondria are central to our capability to switch between aerobic and anaerobic metabolism, to regenerate ATP for use as energy currency, and as a ticking time-bomb that can be used to initiate suicidal programs of cell death to regulate tissue development and immunity from pathogens.

Mitochondria cannot be made *de novo*: in all eukaryotes, pre-existing mitochondria are used as templates to build more mitochondrial mass, ahead of cell division or in response to increased metabolic demand. Yeast has served as the premiere model organism for studies on how mitochondria grow and divide. In yeast, mitochondrial biogenesis requires ~1000 proteins to be imported into mitochondria, and the protein import pathways that these proteins travel rely on cellular nanomachines, such as the **t**ranslocase in the **o**uter **m**itochondrial membrane (TOM complex). Despite years of trial and tribulation with protein crystallography, no high-resolution structure of the TOM complex was available, and *how* it works to transport mitochondrial proteins remained under lively debate.

An exciting new technology was applied to address questions surrounding the architecture of the TOM complex and the structural basis of the translocation channel through which mitochondrial proteins are imported. Based on pioneering work from Peter Schultz (Scripps Institute, USA), a yeast system for incorporation of a photo-activatable amino acid, *p*-benzoylphenylalanine (BPA), at an amber codon engineered in the protein of interest can be used to investigate protein-protein contacts. Over one hundred appropriately engineered yeast strains were UV-irradiated to covalently link the BPA residue engineered in alleles of Tom40 to partner proteins (located within ~4-6 Å reach) in their native context.

We have comprehensively mapped the protein-protein interactions in the yeast TOM complex, yielding a detailed structural understanding and revealing two distinct transport paths through the Tom40 channel. For one of these paths, a series of acidic residues cut a swathe through the channel, thereby drawing through the positively-charged presequence found on many mitochondrial proteins. This "acid trail" leads to an acidic domain of a neighboring subunit of the TOM complex, Tom22. For the second pathway, an N-terminal segment of Tom40 passes from the cytosol through the channel to recruit chaperones from the mitochondrial intermembrane space: this enables unfolded membrane proteins to enter mitochondria and be correctly positioned before they assemble. The architecture of the TOM complex consists of three Tom40 beta-barrel channels sandwiched around a central alpha-helical Tom22 trimer cluster and external regulatory Tom proteins. The regions of protein-protein interaction explain several structural features in Tom20, Tom22 and Tom7 that have been tightly conserved through evolution.

Further layers of architectural complexity are now open for investigation. For example, regulation of the TOM complex has been suggested as a mechanism by which mitochondrial biogenesis can be shut-down rapidly in response to metabolic and cell-cycle changes, with implications for aspects of human biology including metabolic disease and cancer. Again, yeast is serving as an excellent model system for experiments to address these important aspects of cell biology.

1–3

**Regulation of mitophagy in *Saccharomyces cerevisiae***

Giuseppe Lucarelli, Karen Dawson, Alexander May, Hania Czerwinski, Kate Callow, Rod Devenish and Mark Prescott

Metabolic Disease and Obesity Program, Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton VIC 3800

Autophagy is an evolutionarily conserved pathway that involves the sequestration and delivery of material into the hydrolytic acidic environment of the vacuole (yeast) or lysosome (mammals) for degradation and recycling. Long thought to be a non-selective process for bulk turnover, it is now recognised that autophagy can in its different forms target cellular components in a selective fashion. The elimination of mitochondria by autophagy is termed mitophagy, and is involved in the quality control of mitochondria and the regulation of mitochondrial mass in the cell. We have used a genetic screen in yeast to identify genes that when overexpressed perturb mitophagy. Some outcomes of the screen will be presented and discussed.

1–4

**Integration of posttranscriptional gene networks into metabolic adaptation and biofilm maturation in *Candida albicans.***

Jiyoti Verma-Gaur[1], Yue Qu[2], Paul Harrison[3], Tricia L Lo[1], Traude Beilharz[1] and Ana Traven[1]

[1] Department of Biochemistry and Molecular Biology, Monash University, Clayton VIC 3800
[2] Department of Infectious Diseases, The Alfred Hospital and Monash University, Clayton VIC 3800
[3] Victorian Bioinformatics Consortium, Monash University, Clayton VIC 3800

*Candida albicans* is a fungal pathogen that normally lives as a harmless commensal in healthy individuals. The ability of *Candida* to efficiently thrive as pathogen or as commensal depends on its metabolic adaptation, and mitochondria are central organelles that orchestrate this process. We are studying pathways of mitochondrial biogenesis with a specific focus on a posttranscriptional mRNA regulon controlled by the RNA binding protein Puf3. Our bioinformatics analysis shows that Puf3 binds to a large network of mRNAs encoding proteins required for protein translation in the mitochondria, assembly of the respiratory complexes and mitochondrial protein import. These Puf3 binding sites are highly conserved in both *S. cerevisiae and C. albicans.* Puf3 regulates these RNAs by affecting their stability, particularly in response to different carbon sources. Furthermore, genes related to mitochondrial function are differentially regulated in the biofilms of *Candida* species, where accessibility of nutrients and oxygen is very different to growth in suspension cultures. Approximately one quarter of these mitochondria-related genes belong to the Puf3 dependent mRNA network. To regulate its mRNA targets, Puf3 recruits a mRNA deadenylase, Ccr4 to shorten the poly(A) tail and control transcript stability. We show that biofilms made by the *ccr4* mutant have altered structure, with reduced hyphal cells and increased production of extracellular matrix material. Our work provides insight into the interaction between metabolic reprogramming and biofilm development. These results have relevance for identifying new metabolic regulation mechanisms that are necessary for biofilm maturation.

**Session 2. Yeasts: understanding and treating disease**
Chaired by **Dee Carter** (University of Sydney)

2–1

## 3' UTR Dynamics Predict mRNA Fate

Stuart Archer[1], Melissa Curtis[2], Paul Harrison[1], Angavai Swaminathan[2], Andrew Pattison[2], David Powell[1], Ana Traven[2], and Traude Beilharz[2]

[1] Monash Bioinformatics Platform, Monash University, Clayton VIC 3800
[2] Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton VIC 3800

Changes to RNA metabolism are more and more implicated in in human health and disease. An example is the recent discovery of a wholesale switch to shorter 3'UTRs in many cancers. This results in mRNA with reduced scope for post-transcriptional regulation. For cancer patients, this means a worse prognosis, as oncogenes become deregulated (Xia et al, 2014). In addition to the changes to the site of polyadenylation; a second dynamic feature of 3'UTRs is the length-control of the Poly(A)-tail. Nascent mRNA undergo a poly(A) length-check as part of quality control prior to nuclear export. However, once in the cytoplasm, long poly(A)-tails can be rapidly trimmed to dial down protein translation, or to initiate RNA decay. Our research capitalises on a new RNA-seq approach developed in our lab called Poly(A)-Test-seq (PAT-seq)(Harrison et al, 2015). The PAT-seq approach records i) mRNA abundance, ii) polyadenylation site and iii) poly(A)-length distribution in a single statistically robust assay. We have applied this approach to better understand how encrypted information in the 3'UTR of mRNA directs *when*, *where* and *how-often* mRNA is translated. We will present unpublished data addressing questions fundamental to understanding the control of post-transcriptional gene expression. For example, we will show how changes to poly(A)-tail length-distribution can identify the substrates of specific RNA binding proteins. Specifically, showing for the first time that the binding of the pumilio domain protein Puf3 to its target mRNA specifically 'marks' these with changes to their adenylation-state in *Saccharomyces cerevisiae* and alters their association with ribosomes.

Harrison PF, Powell DR, Clancy JL, Preiss T, Boag PR, Traven A, Seemann T, Beilharz TH (2015) PAT-seq: a method to study the integration of 3'-UTR dynamics with gene expression in the eukaryotic transcriptome. *RNA* [Epub ahead of print; Jun 17]

Xia Z, Donehower LA, Cooper TA, Neilson JR, Wheeler DA, Wagner EJ, Li W (2014) Dynamic analyses of alternative polyadenylation from RNA-seq reveal a 3'-UTR landscape across seven tumour types. *Nature communications* **5:** 5274

2–2

## Insight for antifungal discovery from structures of full-length yeast lanosterol 14α-demethylase

Brian C Monk[1], Thomas M Tomasiak[2], Richard D Cannon[1], Alia Sagitova[1], Mikhail V Keniya[1], Rajni K Wilson[1], Franziska U Huschmann[1,3], Joel DA Tyndall[3], Joseph D O'Connell III[2], Janet Finer-Moore[2], Andrew Rodriguez[2], Jeffrey G McDonald[4], and Robert M Stroud[2]

[1] Sir John Walsh Research Institute and Department of Oral Sciences, Faculty of Dentistry, University of Otago, Dunedin, New Zealand.
[2] Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94158, USA.
[3] School of Pharmacy, University of Otago, Dunedin, New Zealand.
[4] Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

Lanosterol 14□-demethylase (Erg11p) in fungi is a membrane monospanning cytochrome P450 protein in the CYP51 class targeted by the triazole drugs and agrochemicals. It is therefore important to understand how Erg11p interacts with the membrane, binds its substrate, product and potential inhibitors, and how the structure and function of the enzyme is affected by mutations that confer resistance to triazole drugs. To date, homology models of fungal Erg11ps have been based on the crystal structures of CYP51s without the N-terminal transmembrane domain. We have obtained high-resolution X-ray crystal structures of full-length, C-terminal hexahistidine-tagged, *Saccharomyces cerevisiae* Erg11p (ScErg11p) with, and without, substrates or inhibitors bound, and compared these structures with those for Erg11p containing single mutations reported to confer triazole resistance. The empty structure of ScErg11p and five ligand complexes (with the substrate lanosterol, the pseudosubstrate estriol, and the triazole inhibitors itraconazole, fluconazole and voriconazole) were determined at resolutions ranging from 1.9 to 2.8 Å. The structures revealed two N-terminal helices oriented at ~60° to each other which orient the partially embedded enzyme relative to the bilayer. The ScErg11p structures showed lanosterol in the active site, a substrate channel linked to the lipid bilayer, and a proposed product exit channel. The structures indicate how triazole antifungals block catalysis and identified possible interactions that confer triazole resistance. The Y132F mutation in *Candida albicans* Erg11p confers azole resistance on clinical isolates. Yeast cells with the corresponding mutation (Y140F) introduced into ScErg11p became resistant to short-tailed, but not long-tailed, triazoles. The crystal structure of ScErg11p Y140F showed modified binding of fluconazole and normal binding of itraconazole. Crystal structures of wild type and mutant ScErg11p and crystal structures recently obtained for the lanosterol 14α-demethylases of leading fungal pathogens are enabling the development of new molecular models to facilitate drug design that will target fungal CYP51s and minimize off-target effects.

2–3

## Plant innate immunity peptides as therapeutics for infection by *Candida albicans* and other fungal pathogens

Mark Bleackley

La Trobe Institute for Molecular Science, La Trobe University, Bundoora VIC 3083

*Candida albicans* is a fungal pathogen that causes infections ranging from thrush on mucous membranes to life threatening systemic infections. Treatment of Candida and other fungal infections is difficult because like humans, fungi are eukaryotes. Thus the differences between host and pathogen that are available for targeting effective therapeutics are limited. Current antifungal molecules mostly target the fungal specific membrane sterol ergosterol or the biosynthetic machinery for the fungal cell wall. However, issues with host toxicity and fungal resistance are decreasing the efficacy of available antifungals. This has made the development of novel antifungals paramount to continued control of fungal disease. One source of promising new antifungals is the plant innate immune system. Plant defensins are small, cationic proteins, many of which have potent antifungal activity. The plant defenins NaD1 from *Nicotiana alata* is among the most active of plant defensins against *C. albicans* and other pathogenic fungi. Investigation of the mechanism of NaD1 has revealed specific interactions with fungal cell wall components, production of reactive oxygen species and membrane permeabilization as components of the antifungal activity. NaD1 and other plant defensins have shown an improved lifetime with respect to fungal resistance compared to antifungals currently used in the clinic. Genetic screens in both *S. cerevisiae* and *C. albicans* have identified a link between fungal susceptibility to defensins and the fungal cell wall. Additionally, treatment of *C. albicans* with NaD1 in combinations with the beta-glucan synthesis inhibitor caspofungin revealed a synergistic antifungal activity that furthers the link between the fungal cell wall and defensins.

2–4

## A transcriptomic analysis of drug synergy in yeast and the fungal pathogen *Cryptococcus*

Dee Carter[1] Yu-Wen Lai[1] Leona Campbell[1] Sharon Chen[3] Chi Nam Ignatius Pang [2] and Marc R. Wilkins[2]

[1] School of Molecular Bioscience, University of Sydney, Darlington NSW 2008
[2] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Kensington NSW 2033
[3] Institute for Clinical Pathology and Medical Research, Westmead Hospital, Westmead NSW

Unlike antibiotics, there has never been a "golden age" of antifungals. Drugs for fungal infections have always been limited in number and spectrum, and treatment is compromised by issues with toxicity, poor bioavailability and the induction of resistance. Developing new antifungals is expensive, high risk and protracted; for example echincandins, the latest class of antifungal agents to reach the market, took more than 30 years to go from bench to bedside. For these reasons there is increasing interest in potentiating existing antifungal drugs with synergising agents. Combining antifungals with iron chelating agents has been used as a last resort therapy for severe invasive fungal infections, however this can be unpredictable as some chelators promote the growth of certain fungal pathogens, and chelation could be contraindicated in debilitated patients. We therefore set out to determine the mechanistic basis of chelator-antifungal synergy, using the pathogenic yeast species *Cryptococcus neoformans* and *Cryptococcus gattii*, and including *Saccharomyces cerevisiae* to enable subsequent systems biology analysis. We systematically tested a range of chelators, including FDA-approved clinical chelators deferoxamine (DFO), deferiprone (DFP), deferasirox (DSX) and ciclopirox olamine (CPO), the natural product lactoferrin (LF), and the common chemical chelator EDTA, in combination with commonly used antifungal agents that included the polyene amphotericin (AMB), azole drugs fluconazole (FLC), itraconazole (ITC), voriconazole (VRC) and the echinocandin caspofungin (CAS). Significant synergy was seen between AMB and LF, however this could not be rescued by the addition of iron, indicating that synergy was not due primarily to iron chelation but to other properties of LF that were greatly potentiated in the presence of AMB. RNA-Seq analysis of *S. cerevisiae* cells treated with AMB alone and combined with LF revealed up-regulation of genes responsive to iron following treatment with AMB, but paradoxically many of these became downregulated or not differentially expressed when cells were treated with AMB+LF. In addition, GO terms enriched for cellular homeostasis and stress responses were upregulated by AMB but downregulated by the presence of LF. Our data suggest that AMB induces stress and invokes an iron limitation response that is dysregulated by the addition of LF, provoking cell death. Transcription factors Aft1 and Zap1 were identified as central to this response, and knock-out mutants become hypersensitive to AMB. Targeting processes that dysregulate the ability of pathogens to sense and respond to the cellular stress induced by antifungals may provide a novel approach for synergistic drug therapy.

**Session 3. Systems and synthetic biology**
Chaired by **Anthony Borneman** (Australian Wine Research Institute)

3–1

## Elucidating whole-genome design principles using synthetic genomics: The Yeast 2.0 project

Thomas C. Williams, Elizabeth L. I. Wightman, Elizabeth L. Daniel, Heinrich Kroukamp, Helena K. M. Nevalainen, Ian T. Paulsen and Isak S. Pretorius

Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde NSW 2109

The emerging field of synthetic genomics involves the re-design and construction of entire genomes. This approach to synthetic biology will enable an unparalleled understanding of minimal biological modules and genome organisation, as well as the construction of superior industrial strains. As part of the global 'Yeast 2.0' consortium, the Australian team is building chromosomes XIV and XVI of the synthetic *Saccharomyces cerevisiae* genome. In addition to an overview of our progress on synthetic chromosome XIV, synthetic biology approaches for creating and screening novel versions of the synthetic yeast genome will be presented. A defining feature of the synthetic yeast genome is an inducible genome shuffling system that is facilitated by the flanking of every non-essential gene with Cre recombinase LoxP recognition sites. This Synthetic Chromosome Recombination and Modification by LoxP mediated Evolution (SCRaMbLE) system can facilitate deletion, inversion, duplication, and translocation events between LoxP sites upon Cre recombinase induction. After the synthetic yeast genome is complete, we will be able to generate millions of different versions that vary in genomic architecture and content using SCRaMbLE. The tools of systems biology can then be used to elucidate novel genome design principles that are common to SCRaMbLEd genomes with superior industrial properties. There are two major limitations to this system. The first is that in its current form SCRaMbLE is ultimately limited to variations of existing *S. cerevisiae* phenotypes. We have therefore developed methods to introduce heterologous DNA into the LoxP sites of the synthetic yeast genome with high efficiency. The second major limitation is that screening of SCRaMbLEd populations is restricted to phenotypes that are naturally coupled to cell survival such as stress tolerance and altered carbon source utilisation. If selection pressures can be created to screen for superior biofuel and chemical production phenotypes, then many of the limitations in the field of metabolic engineering would be rapidly overcome using the power of directed evolution and genome SCRaMbLEing. We have developed a novel *in vivo* biosensor that can be used to detect the intracellular concentration of valuable organic acid products such as para-hydroxybenzoic acid and propionic acid, and activate GFP expression in response. Fluorescence activated cell sorting can now be used to screen millions of cells with SCRaMbLEd genomes for higher production levels. These developments, combined with genome sequencing and metabolomics analysis will allow us to identify novel genome 'design principles' for metabolic productivity, catalysing the progression of rational engineering in synthetic biology.

3–2

**Synthetic biology production of raspberry ketone in wine yeast.**

Danna Lee and Anthony Borneman

Australian Wine Research Institute, The Waite Research Precinct, Urrbrae SA 5064

Raspberry ketone (RK) is the primary aroma compound found in raspberries. With the identification of genes and enzymes responsible for the bioconversion of amino acid precursors into RK, it is possible to synthetically engineer the production of RK in microbial systems. A set of genes from both plants and oleaginous yeast were selected to construct the full RK biosynthesis pathway in *Saccharomyces cerevisiae*. We have now successfully created a series of wine yeast strains that are able to produce RK during fermentation. The highest RK concentration achieved growing in minimal media exceeded 7.5mg/L when strains were fed with 3mM p-coumaric acid; or 2.8mg/L for complete de novo synthesis.

3–3

**Systems Biology: a new approach to industrial yeast strain development**

Cristian Varela[1], Anthony R. Borneman[1], Simon Schmidt[1], Paul J Chambers[1] and The Australian Wine Yeast Systems Biology Consortium[2]

[1] Australian Wine Research Institute, The Waite Research Precinct, Urrbrae SA 5064
[2] Genomics Australia, Proteomics Australia, Metabolomics Australia, The New South Wales Systems Biology Initiative and The Australian Wine Research Institute.

The ability to interrogate genome-wide biological datasets as part of a Systems Biology framework is poised to revolutionize the development of industrial microorganisms such as the yeast *S. cerevisiae*. Over recent years, laboratory strains of *S. cerevisiae* have been applied at the cutting edge of Systems Biology research. However, relative to laboratory strains, industrial *S. cerevisiae* strains display very distinct phenotypes, such as increased stress tolerance and the production of key secondary metabolites that are critical for industrial applications. Given the intellectual and economic benefits that fundamental understanding of industrial yeasts will provide, we have undertaken a collaborative Systems Biology investigation of industrial wine yeast fermentation. Particularly, we focused on understanding the production of metabolites that can negatively affect wine aroma in strains engineered for the production of low-alcohol wine.

We performed triplicate model batch wine fermentations using a wine yeast parental strain and mutants thereof that were engineered to produce wine with reduced alcohol concentration. Fermentations were sampled at five wine-relevant key time points, and for each of these, transcript profiles were determined using RNAseq, proteomic analysis was performed using iTRAQ, and targeted approaches were used for metabolomic profiling. We were able to identify genes and metabolites responsible for the production of sensorially undesirable volatile compounds in engineered 'low-alcohol' strains. Based on this information further modifications were made to improve on the first round of strain development.

3–4

**Metabolic Engineering of *Saccharomyces cerevisiae* for the production of aromatics**

Jens Krömer

Centre for Microbial Electrosynthesis, University of Queensland, Brisbane QLD 4072

The shikimate pathway in yeast is a natural source of aromatics that can be used as bio-replacements for petro-chemicals. In order to turn *S. cerevisiae* into a cell factory for such aromatics, we have used a combination of *in-silico* modelling and metabolic engineering based on systems biology and synthetic biology. The deployment of new regulatory circuits to control key pathways along with modelling supported knock-out strategies and medium design led to the highest reported titres of the aromatics p-hydroxy benzoic acid and p-amino benzoic acid in yeast fermentation.

3–5

**The Regulation of Intracellular Phosphate is affected by Protein Arginine Methylation**

Samantha Chia, Melissa Erce, Gene Hart-Smith, Zhiliang Chen and Marc Wilkins

Systems Biology Initiative, University of New South Wales, NSW 2052

Hmt1 is the predominant arginine methyltransferase in yeast. It methylates proteins involved in processes such as transcription, transcriptional regulation, nucleocytoplasmic transport of proteins and mRNAs and RNA splicing. Hmt1 is also known to mediate protein-protein and protein-RNA interactions. Despite its well-studied role in regulatory processes, the effect of Hmt1 knockout on the transcriptome and proteome has not been reported. RNA-seq and SILAC-based proteomic analyses were undertaken on the Hmt1 knockout. In mid-log phase growth, we found acid phosphatases (*PHO5*, *PHO11* and *PHO12*), phosphate transporters (*PHO84* and *PHO89*) and the vacuolar transporter chaperone *VTC3* were amongst a small number of genes and proteins significantly downregulated on knockout. Concomitant decreases were observed in intracellular polyphosphate levels and extracellular phosphatase activity. Network-based analyses revealed no obvious connection between the known substrates of Hmt1 methyltransferase and the phenotype of dysregulated phosphate. However, we determined that transcription factor Pho4, responsible for the activation of many phosphate regulatory proteins, could be methylated by Hmt1 *in vitro* at Arg-241. Genomic mutation of Arg-241 to Lys in wild type reproduced the same phenotype as *hmt1Δ*. The methylation of Arg-241 is likely to affect Pho4 dimerisation, and is thus essential for the correct function of Pho4 in the cell.

**Session 4. Signalling**
Chaired by **Evelyn Sattlegger** (Massey University)

4–1

## Target of rapamycin (TOR) control of cell growth and division in fission yeast

Janni Petersen
Division of Anatomy & Histology, Flinders University, SA

Abstract not provided

4–2

**A mutational approach to locate the heart of WASP - a key regulator of actin dynamics from yeasts to beasts**

Michael A. Hahn, Anh Van Trinh, Heike Mack, and Alan L. Munn

School of Medical Science and Molecular Basis of Disease Program, Menzies Health Institute Queensland, Griffith University (Gold Coast campus), Southport QLD 4222

Wiskott-Aldrich Syndrome Protein (WASP) and WASP-Interacting Protein (WIP) are human proline-rich signalling proteins that associate to form a complex that plays an important role in the actin cytoskeletal rearrangements required for cellular functions such as cell motility, cell-cell adhesion, endocytosis and cytokinesis. *Saccharomyces cerevisiae* has functional homologues of both WASP (Las17p) and WIP (Vrp1p/verprolin). Yeast *vrp1Δ* mutant cells are viable at 24°C but not at 37°C (i.e. they are temperature-sensitive for growth) and have defects in endocytosis and cytokinesis. We have previously shown that high-copy *LAS17* (resulting in Las17p over-expression) suppresses the growth and endocytosis defects of *vrp1* mutant cells. The aim of this study is to determine the mechanism by which this high-copy suppression occurs.

Multiple fragments of Las17p were cloned downstream of the *LAS17* promoter and in-frame with a Pk (V5) epitope tag in a yeast high-copy-number (2μ) vector. Each of these constructs was then introduced into *vrp1Δ* cells and tested for its ability to suppress the temperature-sensitive growth defect.

Expression of a Pk-tagged N-terminal fragment of Las17p (residues 1-150) known to bind Vrp1p, a large proline-rich fragment (residues 151-530) known to bind Src Homology 3 (SH3) domains, or a C-terminal fragment (residues 531-633) containing a WASP Homology 2 (WH2) domain known to bind actin monomers could not individually suppress the *vrp1Δ* growth defect. In contrast, expression of a Pk-tagged fragment containing both the large proline-rich central region and C-terminal region could suppress the growth defect. Further experiments have shown that within the proline-rich central region residues 302-370 are critical for suppression. The C-terminal region contains two sequences that are both essential for efficient suppression of the growth defect: the WH2 actin-binding domain and a second sequence of as yet unknown function.

Both the proline-rich and actin-binding domains of Las17p are required for high-copy suppression of the *vrp1Δ* temperature-sensitive growth defect. Further experiments are aimed at identifying which of the numerous proteins known to interact with the Las17p proline-rich region (residues 302-370) are important for high-copy suppression of *vrp1Δ*. Deficiencies in WASP in humans result in various diseases including immunodeficiency, blood clotting defects, auto-immune disease, and cancer. Identification of the evolutionarily conserved function of WASP/Las17p may provide insight into the molecular basis of these conditions and inform the development of novel molecular therapies.

The authors kindly acknowledge the financial support of an ARC Discovery Project grant (DP110100389).

4–3

## Regulation of the yeast cell cycle checkpoint kinase Rad53

Nicolas Hoch[1], Erich Chen[2], Ming-Daw Tsai[2] and Jörg Heierhorst[1]

[1] St. Vincent's Institute of Medical Research & Dept of Medicine SVH, The University of Melbourne, Melbourne VIC 3010
[2] Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

The Rad53 kinase plays central roles during the coordination of normal DNA replication and in response to all types of DNA damage in budding yeast (*Saccharomyces cerevisiae*). Rad53 is widely studied as a model to understand the regulation of the human DNA damage checkpoint kinase Chk2. Rad53 is comprised of five main structural domains, and therefore also used as an experimental system to understand structure-function relationships of protein modularity. Rad53 contains an N-terminal SQ/TQ cluster domain (SCD1), containing four phosphorylation sites for its upstream activating kinases Mec1/Tel1, followed by a forkhead-associated domain (FHA1), the protein serine/threonine kinase catalytic domain, a second SQ/TQ cluster (SCD2) and a second FHA domain (FHA2) towards its C-terminus. We have previously shown that the two FHA domains have some overlapping functions in the activation of Rad53, as well as distinctive functions, including linking Rad53 to downstream effectors (1). Even though the two FHA domains have partially redundant functions, they act in a strictly location-specific manner; i.e. they cannot be transposed without loss of Rad53 functionality (2). The general paradigm for Chk2-like kinases is that their activation involves an intermolecular auto-phosphorylation step of two kinase molecules that are dimerized by a pThr-specific interaction of the N-terminal FHA domain with a phosphorylated TQ motif in the N-terminal SCD. However, DNA damage response phenotypes and kinase activation kinetics differ substantially between SCD1-mutated and FHA-mutated *rad53* alleles (3-5). These data indicate that the increased domain complexity in Rad53 compared to other Chk2-like kinase, which only contain single SCD and FHA domains, results in more complex regulatory mechanisms for the budding yeast kinase.

(1) Pike et al *J. Biol. Chem.* **278**, 30421-30424 (2003); (2) Tam et al *Biochemistry* **47**, 3912-3916 (2008); (3) Lee et al *Mol. Cell* **30**, 767-778 (2008); (4) Hoch et al *Mol. Cell. Biol.* **33**, 3202-3213 (2013); (5) Chen et al, *Mol. Cell. Proteomics* **13**, 551-565 (2014).

4–4

**New insights into the regulation of protein kinase Gcn2**

Evelyn Sattlegger

Institute of Natural & Mathematical Sciences, Massey University, Auckland NZ

Abstract not provided

**Session 5: Yeast in Fermentation Industries**
Chaired by **Paul Henschke** (Australian Wine Research Institute)

5–1

## Breeding new life into the ancient art of winemaking

Jenny Bellon[1], Paul Chambers[1], Dimitra Capone[1], Barbara Dunn[2], Gavin Sherlock[2] and Chris Curtin[1]

[1] Australian Wine Research Institute, Urrbrae SA 5064
[2] Department of Genetics, Stanford University, Stanford, CA 94305-5120, USA

Archaeology provides evidence of winemaking dating back more than 7000 years and, until very recently, winemakers have relied on spontaneous fermentations to convert grape juice into an alcoholic beverage. Spontaneous (uninoculated) fermentations contain a multitude of different yeast and bacterial species that add complexity to the sensory properties of wine. However, relying on indigenous microbial communities can lead to stuck or sluggish fermentations, while spoilage yeast may render the wine unusable. In today's large-scale commercial wine production rapid, reliable and consistent fermentations are essential and this has led to the modern practice of inoculating grape juice with a single industrial *S. cerevisiae* wine yeast strain that is capable of efficiently completing fermentation. Whilst this approach delivers on the economic front, the wines produced may lack the sensory complexity obtained from spontaneous fermentations where metabolites from many different genetic backgrounds contribute to the final wine. To address this limitation of single inocula fermentations, a new breed of wine yeast has been developed. *Saccharomyces* interspecific hybrids have been generated between robust, commercial *S. cerevisiae* wine yeast strains and all other *Saccharomyces* species, including recently discovered *S. arboricolus* and *S. eubayanus*. The introduction of different genetic material from a closely-related but DNA sequence-divergent species has resulted in new wine yeast strains that generate alternative wine styles through the formation of novel and wider-ranging yeast volatile fermentation metabolite profiles. The new hybrid yeasts have the potential to produce complex wines comparable to spontaneous fermentations while giving winemakers the safeguard of an inoculated ferment.

5–2

**Generation of novel wine yeast that overproduce 'rose' aroma compounds 2-phenylethanol and 2-phenylethyl acetate**

Antonio G. Cordente, Jenny Bellon, Caroline Abrahamse, Mark Solomon, Peter Sternes and Chris Curtin

Australian Wine Research Institute, Urrbrae SA 5064

It is well established that the choice of yeast used to perform wine fermentation impacts significantly on sensory attributes of wines; different yeast species and strains impart different profiles of esters, volatile fatty acids, higher alcohols and volatile sulfur compounds. Indeed, this remains one of the simplest means by which winemakers can modulate the sensory attributes of wine. As a consequence, there are more than 100 commercially available *Saccharomyces cerevisiae* wine yeast strains available, mostly derived by isolation from vineyards and successful fermentations. Nevertheless, some desirable characteristics are not present amongst existing strains.

The fusel alcohol 2-phenylethanol (2-PE) and its acetate ester, 2-phenylethyl acetate (2-PEA), are derived from the aromatic amino acid phenylalanine and confer desirable 'rose' and 'floral' aromas in wine, respectively. These metabolites are known to be produced at relatively high concentrations by *Saccharomyces uvarum* and to a lesser degree by some *S. cerevisiae* strains. *Saccharomyces* interspecific hybrids have been generated that produce higher concentrations of 2-PE and 2-PEA relative to widely used *S. cerevisiae* wine strain PDM (2-fold). Natural and chemically mutagenised populations of another popular *S. cerevisiae* wine strain, AWRI 796, were subsequently exposed to toxic analogues of phenylalanine. Resistant colonies were found to overproduce 2-PE and 2-PEA by up to 30-fold. Genome sequencing of these newly developed strains alongside existing wine strains revealed mutations in some of the genes in the biosynthetic pathway of aromatic amino acids, and several others that appear to mediate natural variation across *S. cerevisiae* for this oenologically important characteristic.

5–3

**Can further improvements be made in oenological traits of wine yeast?**

Michelle E. Walker, Tommaso Watson, Danfeng Long, Tom Lang, Jin Zhang, Jennie M. Gardner and Vladimir Jiranek

School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae SA 5064 and Wine Innovation Cluster, SA

Australia's wine industry increasingly faces shortened, hot vintages and diminished water supply as a result of climate warming and weather extremes. Together, with the winemakers' push towards more full-bodied wine, fruit can have excessively high sugar content, which leads to increased ethanol concentrations (>15%) or problematic fermentation due to microbial failure. Therefore, predictable and reliable alcoholic fermentation is a primary concern for the winemaker and has a direct impact on wine quality and value.
Our group is tackling this issue 'head-on', using non-recombinant strategies to further improve existing commercial strains, such that they can reliably complete fermentation without negatively impacting upon wine quality. We report on approaches being used to further increase the genetic diversity of existing commercial wine *Saccharomyces cerevisiae* strains. Our goals are to improve fermentation outcomes through more efficient nutrient usage (sugar and nitrogen) and tolerance to stressors in the grape must (sugar, ethanol, chemical inhibitors). The successful outcomes of this research will be the availability of 'industry ready' wine yeast better suited to Australian winemaking.

5–4

**How do we quantify the filamentous growth in a yeast colony?**

Ben Binder[1], Joanna Sundstrom[2], Jennie Gardner[2], Vladimir Jiranek[2] and Steve Oliver[3]

[1] School of Mathematical Sciences, University of Adelaide, Adelaide SA 5005
[2] School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae SA 5064
[3] Cambridge Systems Biology Centre and Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.

In nutrient-depleted environments, it is commonly observed that strains of the yeast *Saccharomyces cerevisiae* forage by the mechanism of filamentous growth. How do we quantify this spatial patterning of outward growth from a yeast colony? Previous studies have primarily relied on measuring the amount of filamentous growth, but do not take into account the spatial distribution of this highly non-uniform process. In this talk, we fill this void by providing a statistical approach that enables the quantification of this important spatial information, enabling a more detailed mathematical analysis of the filamentous growth process.

**Session 6. Student Presentations**
Chaired by **Paul Grbin** (University of Adelaide)

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

6–1

## Halting *Cryptococcus neoformans* Infections via inhibition of ADS Lyase

Jessica Chitty[1,2], Kirsten Blake, Simon Williams[1,2], Andre Koh, Ulkrike Kappler[1], Bostjan Kobe[1,2] and James Fraser[1]

[1] Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072
[2] Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072

*Cryptococcus neoformans* is the leading cause of fungal meningoencephalitis and one of the major causes of death in immunocompromised individuals[1]. Current treatment is limited to three antifungals that each has its shortcomings. It is therefore essential that we develop additional classes of antifungal drugs, particularly ones that are more effective than those currently available. But due to the shared eukaryotic physiology of fungi and humans, gross differences that can be exploited as drug targets are limited.

An alternative approach is to exploit subtle differences in otherwise conserved pathways. This rational drug design approach was pioneered in the purine biosynthetic pathway leading to the anticancer drug selenazofurin[2, 3]. This drug, as with other purine biosynthesis inhibitors, is successful in the inhibition of rapidly proliferating cells due to the increased demand for purines. Recent analysis of inosine monophosphate dehydrogenase (IMPDH), the first committed enzyme in the *de novo* GTP biosynthesis pathway, found it was essential for *C. neoformans* virulence in a murine model[4]. Although it shares a high sequence identity with that of the human enzyme, the tertiary structure of *C. neoformans* has revealed differences that may be exploitable for drug design.

To continue this rational drug design program other enzymes of the pathway have been characterised and structures solved to find other exploitable differences. One such example is adenylosuccinate lyase, a bifunctional enzyme required for both the production of ATP and GTP. ADS lyase performs two non-sequential steps in *de novo* purine biosynthesis, firstly conversion of SAICAR to AICAR and secondly ADS to AMP, both reactions are catalysed by β-elimination of fumarate. This enzyme is crucial in the production of key virulence factors in *C. neoformans* and virulence in a murine model. Structure determination to 2.1 Å has identified a key residue change in the active site pocket.

1. Perfect, J.R., *Cryptococcosis: a model for the understanding of infectious diseases.* J Clin Invest, 2014. **124**: 1893-5.
2. Christopherson, R.I., S.D. Lyons, and P.K. Wilson, *Inhibitors of de novo nucleotide biosynthesis as drugs.* Acc Chem Res, 2002. **35**: 961-71.
3. Elion, G.B., *Nobel Lecture. The purine path to chemotherapy.* Biosci Rep, 1989. **9**: 509-29.
4. Morrow, C.A., et al., *De novo GTP biosynthesis is critical for virulence of the fungal pathogen Cryptococcus neoformans.* PLOS Pathog, 2012. **8**: e1002957.

6–2

**From baker's yeast to carcinomas: determining the anti-cancer mechanisms of nucleoside analogue MTDIA**

Namal Coorey[1], Vern Schramm[2], Gary Evans[3], Peter Tyler[3], Richard Furneaux[3], Andrew Munkacsi[1] and Paul Atkinson[1]

[1]Centre for Biodiscovery, Victoria University of Wellington, Wellington, New Zealand.
[2] Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461, United States.
[3] Ferrier Research Institute, Victoria University of Wellington, Wellington, New Zealand

Methylthio-DADMe-Immucilin-A (MTDIA) is a transition state analogue of methylthio adenosine involved in polyamine biosynthesis, therefore MTDIA is specifically active in cancer cells with upregulated polyamine biosynthesis. Although MTDIA has demonstrated *in vivo* efficacy in mouse xenograft models for human lung, prostate, colon, cervical, head and neck, and triple negative breast cancer as well as efficacy in various human cancer cell lines, the cellular mechanisms that link the drug target with cell death are not known. Our novel genome-wide approach to elucidate the therapeutic mechanism of MTDIA using *Saccharomyces cerevisiae* has identified disruptions to autophagy, transcription, translation initiation, $NAD^+$ biosynthesis and lipid biosynthesis which can be used to further understand the results in the respective cancer cell lines and pending clinical trials.

6–3

## Non-*Saccharomyces* yeast to manage ethanol and sensory compounds in wine

Ana Hranilović [1], Tertius van der Westhuizen[2], Paul Grbin[1] and Vladimir Jiranek[1]

[1] School of Agriculture, Food & Wine, The University of Adelaide, Waite Campus, Urrbrae SA 5064
[2] Laffort Australia, Woodville North, SA 5012,

A globally observed increase in wine ethanol content is related to various negative technological and financial implications, as well as adverse health and sensory aspects of the final product. As such, it is deemed a major challenge for the wine industry; the Australian winemaking sector not being the exception. Various methods for ethanol reduction are therefore implemented, however, their application is often costly and detrimentally affects wine quality. The use of non-*Saccharomyces* yeast to conduct the fermentation, on the other hand, represents an easy-to-adopt strategy with the potential to achieve lower alcohol wines with concomitant quality improvement. The term 'non-*Saccharomyces*' refers to yeasts indigenous to the wine-related environment, excluding *Saccharomyces cerevisiae,* that contribute either positively or negatively to the fermentation. The aim of this study was to select non-*Saccharomyces* strains capable of diverting sugar away from ethanol production. For this purpose, non-*Saccharomyces* isolates were identified through PCR amplification of 5.8R-ITS region, amplicon sequencing and comparison with the NCBI database. Isolates were then screened for their fermentation performance and metabolite production in a synthetic grape juice-like medium containing 230 g/L sugar and 350 mg/L nitrogen. The fermentation kinetics were monitored regularly, and concentrations of main metabolites were determined at the end of the fermentation. Observed differences in the ethanol yield per sugar consumed and other parameters analysed suggest the potential applicability of non-*Saccharomyces* yeasts to manage wine ethanol and sensory compound levels. Subsequent work is focusing on grape juice multi-starter fermentation trials with selected non-*Saccharomyces* strains and sequentially inoculated *Saccharomyces cerevisiae*.

6–4

**Identification of yeast genes responsible for the production of hydrogen sulfide and volatile thiols in wine.**

Max Huang[1], Richard Gardner[2], Bruno Fedrizzi[3], Michelle Walker[1] and Vladimir Jiranek[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae SA 5064
[2] Wine Science Group, School of Biological Sciences, University of Auckland, New Zealand
[3] Wine Science Group, School of Chemical Sciences, University of Auckland, New Zealand

The undesirable, rotten-egg odour of hydrogen sulfide ($H_2S$) produced by yeast is known to reduce the sensory quality of wines. Hydrogen sulfide produced shortly after yeast inoculation of grape musts however, might be an important source of desirable varietal thiols, which contribute to the tropical aromas in varieties such as Sauvignon Blanc.

In this project, the addition of bismuth (Bi) to plates made from chemically-defined grape juice medium (CDGJM) has been developed as a rapid screen for $H_2S$ production in fermentation-like conditions. Yeast deletion libraries have been screened on (CDGJM+ Bi+ Cysteine) plates and candidate genes have been identified. In addition, two types of $H_2S$ production during fermentation from cysteine have been observed. Type I: most yeast strains can produce this early peak of $H_2S$; Type II: some yeast strains produce an additional more delayed burst of $H_2S$ from cysteine.

The yeast genes responsible for the formation of Type I $H_2S$ from cysteine identified here could potentially lead to development of better yeast strains and winemaking practices that the wine industry could apply to create the fruit-driven styles of wines that many consumers prefer.

6–5

**Using yeast prions as a mechanism for screening for novel anti-prion compounds**

Laurence K. Jennings, Alan L. Munn and Anthony R. Carroll

School of Medical Science and Molecular Basis of Disease Program, Menzies Health Institute Queensland, Griffith University (Gold Coast campus), Southport QLD 4222
School of Environment, Environmental Futures Research Institute, Griffith University (Gold Coast campus), Southport QLD 4222

Prion diseases are a rare group of infectious neurological disorders in animals that are caused by modified isoform prion proteins. These prions aggregate and form amyloids, causing the disease in mammalian cells. Prion diseases include Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, Fatal Familial insomnia, and kuru in humans. These amyloid formations caused by prions are also potentially associated with over 30 other human diseases including Alzheimer's, Parkinson's, and Huntington's diseases. There have been several amyloid-based prion proteins discovered in the yeast Saccharomyces cerevisiae. It has been shown that many of the mechanisms are very similar between mammalian and yeast prions. Because of these similarities these prions in yeast represent a good model for research into the formation and spread of amyloids and thereby to increase our knowledge of prions and prion mechanisms. A new high-throughput screening method to test for antiprion activity using the yeast strains [PSI+] and [URE3] was developed. This assay was developed specifically to screen natural extracts for novel compounds with antiprion activity. Natural products have previously been shown to have a high chemical diversity and many natural products have been used as drugs. This method was used to screen a collection of 57 marine invertebrate extracts. A sponge extract was found to show activity in the bioassay indicating that it contains compound(s) with antiprion activity. The active compound(s) in the extract will be isolated and identified. This compound(s) could be used as a tool to increase knowledge of prions and possibly aid in the development of a treatment.

6–6

**Protein *N*-glycosylation chain length impacts protein secretion capacity and cell wall retention of recombinant *Saccharomyces cerevisiae***

Heinrich Kroukamp[1], Riaan den Haan[2] and Willem H. van Zyl[1]

[1] Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.
[2] Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville, 7535, South Africa

Protein *N*-glycosylation plays an important role in protein folding, solubility, recognition and cell wall integrity. The removal or addition of glycosylation sites on proteins and mutations in genes of enzymes involved in glycosylation have been shown to effect heterologous protein secretion in *Saccharomyces cerevisiae*. Like many other native gene alterations that were previously shown to influence protein secretion in yeast, the manner by which *N*-glycosylation mutants engender higher protein yields is poorly understood. In this study we investigated the effect of sequential Golgi-resident mannosyltransferase gene deletions on cellulase secretion and cell wall retention of different reporter protein glycoforms, in order to elucidate some of the underlying mechanisms responsible for this high secretion phenotype. We found that strains harboring mutations preventing outer chain *N*-glycan extension (*och1Δ*, *mnn9Δ*, *mnn10Δ* and *mnn11Δ*), differed in their sensitivity to cell wall inhibitors and heterologous protein glycosylation patterns, compared to the other evaluated mannosyltransferase mutants. The outer chain mutants had up to 2, 2.4 and 1.3-fold higher total volumetric enzymatic activities for three different β-glucosidases, respectively containing 0, 5 and 14 putative *N*-glycosylation motifs. In addition to higher enzyme activities per dry cell weight, these outer chain mutants also had reduced cell wall retention of β-glucosidases. Although this enhanced secretion phenotype of the outer chain mutants was not observed for recombinant endoglucanases, the expression of either endoglucanse or β-glucosidase containing no glycosylation sites, imparted a greater metabolic burden on the production host than their glycosylated counterparts. These results shed some light on the intricate and unique relationships of diverse heterologous proteins with various components of the expression host secretion machinery that inevitably lead to protein specific secretion improvements during strain engineering strategies.

6–7

**Human WIP interacts with yeast Hof1p, a key regulator of the yeast cell cycle**

Heike B. Mack and Alan L. Munn

School of Medical Science and Molecular Basis of Disease Program, Menzies Health Institute Queensland, Griffith University, (Gold Coast campus), Southport QLD 4222,

Wiskott-Aldrich Syndrome Protein-Interacting Protein (WIP) interacts with Wiskott-Aldrich Syndrome Protein (WASP) to activate the Arp2/3 complex and promote the nucleation of branched actin filaments. Dysfunction of WIP and WASP in humans is implicated in a range of diseases such as the inherited immunodeficiency disease WAS (Wiskott-Aldrich Syndrome) and cancer.
In *Saccharomyces cerevisiae* (baker's yeast), the Src Homology 3 (SH3) domain of Hof1p, a key regulator of the yeast cell cycle, binds proline-rich motifs in yeast WIP (verprolin/Vrp1p). In the absence of yeast WIP, the Hof1p SH3 domain has a toxic effect and appears to inhibit actin-dependent processes such as cytokinesis and endocytosis. Yeast cells lacking yeast WIP can be rescued by the expression of human WIP.
If interactions between the SH3 domains of yeast Hof1p and its human equivalents and proline-rich motifs in human and yeast WIP have been conserved and mediate similar functions in actin-dependent processes, then the results of this study could aid in finding new treatments for WAS and elucidating the roles of WASP and WIP in tumor-suppression.
The yeast two-hybrid system and *in vitro* pull-down assays using purified recombinant proteins were employed to test if the yeast Hof1p SH3 domain binds human WIP. We found that the yeast Hof1p SH3 domain interacts with two proline-rich motifs in the C-terminus of human WIP one of which is already reported to be important for human WIP function in yeast. This result indicates that these protein interactions have been conserved from yeasts to humans.

6–8

## *Psychotria insularum*, a Samoan medicinal plant, is therapeutic *via* disruption of iron availability

Seeseei Molimau-Samasoni[1,2,3], Vimal Patel[1,2], Dan Sinclair[4], Anne La Flamme[1,2], Paul H Atkinson[1,2] and Andrew Munkacsi[1,2]

[1] Centre of Biodiscovery, Victoria University of Wellington, Wellington, New Zealand
[2] School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand
[3] Scientific Research Organization of Samoa, Apia, Samoa
[4] School of Geography, Environment and Earth Sciences, Victoria University of Wellington, Wellington, New Zealand

*Psychtoria insularum* is widely used in Samoan traditional medicine for the treatment of various ailments, such as inflammation, wounds, abdominal distress and *maternity difficulties*. Additionally, the plant is used for the treatment of non-physical malaise attributed to supernatural causes. However, despite its extensive use, the underlying mechanism of its activity are unknown. Given its multifaceted range of applications, we predicted the plant will have a mechanism of action affecting a fundamental cellular function which impacts a wide array of molecular processes. We report herein our genome-wide screen conducted in *Saccharomyces cerevisiae*, using the aqueous extract of *P. insularum* leaves. This screen implicated the involvement of iron transport in the extract mechanism of action, and supplementation with exogenous iron restored wild type growth in treated cells. Paradoxically, quantification of total intracellular iron showed an increase in cells grown in the presence of *P. insularum*. Further investigation showed *P. insularum* treatment up-regulated iron transporters, although total quantified heme was significantly reduced. These results indicate *P. insularum* reduces bioavailable iron, leading to activation of the low-iron response. Interestingly, we also determined a reduction in heme coincides with the anti-inflammatory response in *P. insularum* treated macrophages. Thus, we conclude reduced iron bioavailability is responsible for the anti-inflammatory effects of *P. insularum*, correlating the elucidated mechanism of action and Samoan medicinal application.

6–9

**Barcode sequencing of the yeast deletion collection to define the antifungal mechanisms of plant defensins**

Kathy Parisi, Mark Bleackley, Nicole van der Weerden and Marilyn Anderson

La Trobe University, Bundoora VIC 3083

Plants produce a number of different molecules to protect them against damaging pathogens as part of their innate immune system. One such class of molecules is the defensins. Many plant defensins are potent antifungal molecules. Defensins are similar in structure but vary significantly in their sequence. This variation in sequence is thought to account for the different mechanisms of action observed for plant defensins. My hypotheses are
1) that sequence variability between defensins correlates with different mechanisms of action and 2) that defensins with different mechanisms of action can be used in combination to provide more effective disease resistance.
Currently a number of defensins have been identified yet they are not all characterised. We are screening the *Saccharomyces cerevisiae* non-essential gene deletion yeast library with the aid of next generation sequencing to determine whether a group of selected defensins have different mechanisms of action and what these mechanisms are. The yeast deletion set contains 5000 unique knockout strains that represent each of the 5000 non-essential genes. Each of these strains can be identified by individual barcodes that have been incorporated into the gene as part of the deletion procedure. The pool of this library was treated in triplicate with four different defensins in conjunction with the untreated library. NGS allowed us to identify strains that are resistant or sensitive to a particular defensin giving us insight into the mechanism of action and identified pathways that are targeted in the yeast genome. A clustering program was used to determine the similarity of fitness profiles for each strain in each of the defensin treatments. Using this process we identified several strains as sensitive or resistant to all four defensins indicating that elements of the activity of plant defensins are conserved. These strains may represent general stress response genes. However, the clustering revealed elements that are unique to each defensin and suggests at least three different mechanisms of action. Two of the defensins we tested appear to have a conserved mechanism of action which correlates with their degree of sequence identity. Understanding how plant defensins exert their antifungal activity will assist in selection of defensins with different antifungal mechanisms which can be used in transgenic plants to create broad spectrum resistance to fungi. Combinations of defensins with different mechanisms of action can be used to produce robust transgenic plants that are protected from fungal infection and will prevent resistance developing in the fungus.

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6–10

**Engineered squalene synthase degradation to redirect metabolic flux to sesquiterpene bio-products in *Saccharomyces cerevisiae***

Bingyin Peng, Lars K. Nielsen and Claudia Vickers

Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, St. Lucia QLD 4072

Sesquiterpenes commonly function as active molecules in fragrances, flavours and traditional herbs, and because of the high-energy content, sesquiterpenes can also be applied as future alternative aviation fuels. Microbial fermentation is being examined as a competitive approach for bulk production of these compounds. In the current study, *S. cerevisiae* was used as the platform organism for producing an acyclic sequeterpene alcohol, trans-nerolidol. Nerolidol production was firstly improved by enhancing the upstream mevalonate pathway for the synthesis of the precursor farnesyl pyrophosphate (FPP). However, excess FPP produced was instead directed towards squalene by squalene synthase (Erg9p), resulting in squalene accumulation to 1 % biomass. It was shown that Erg9p is located on endoplasmic reticulum (ER) membrane through a C-terminal ER-targeted transmembrane peptide. An artificial ER-associated protein degradation mechanism was developed to decrease cellular levels of Erg9p. This improved nerolidol titre by 86% to ~100 mg $L^{-1}$. To further improve titres, the strong inducible *CUP1* promoter was applied for over-expression of the MVA pathway and nerolidol synthase. This resulted in a nerolidol titre of 250 mg $L^{-1}$ in the pathway-enhanced, Erg9p-destabilized strain. In this strain, squalene levels were similar to the wild type control strain, and there was no negative effect on cell growth. These data demonstrate that the novel ER-associated protein degradation mechanism can be a useful strategy to balance competition between the endogenous sterol pathway and introduced bio-product pathways at the FPP node for metabolic engineering applications.

6–11

**Screening for wine yeast mutants, whereby gene deletion results in faster fermentation under limited nitrogen conditions**

Josephine Jasmine Peter, Tommaso Watson, Michelle Walker, Jennie Gardner and Vladimir Jiranek

School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae SA 5064

Nitrogen in grape juice promotes yeast biomass formation and shortens fermentation duration. Insufficient nitrogen often causes stuck/sluggish fermentation and can result in wine spoilage. These problems can be rectified by optimizing vineyard fertilization or more commonly supplementing the fermentation with ammonium salts. An alternative to supplementation is to use wine yeast that can efficiently assimilate nitrogen and complete fermentation in low nitrogen juices. In order to generate such "nitrogen efficient" strains, it is important to investigate gene functions related to nitrogen assimilation under fermentative conditions.
Several genome wide studies in the past have used yeast deletion libraries (collections of strains; each with a single gene deleted) to identify gene functions. However, these libraries contain auxotrophic markers (requiring amino acid supplementation) which make quantitative physiological studies harder, especially under limited-nitrogen conditions. Therefore, to reduce the bias caused by auxotrophic markers we have used a wine yeast deletion collection which is naturally prototrophic to identify genes related to nitrogen assimilation.
The approach was firstly to screen a wine yeast library in AWRI 1631 for growth and sugar consumption under limited-nitrogen concentrations, to identify candidate genes. Secondly, the study sought to characterise the function of these genes in relation to fermentation; specifically their activity when cells are subject to nitrogen limitation. The project's outcomes will help to understand the genetic basis behind nitrogen efficiency in these strains. Further, this knowledge could be used to formulate new strategies to generate improved strains exhibiting high nitrogen efficiency to benefit the wine making process.

6–12

**Wine yeast biofilms and quorum sensing**

Ee Lin Tek[1], Jennie Gardner[1], Joanna Sundstrom[1], Stephen G Oliver[2] and Vladimir Jiranek[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae SA 5064
[2]University of Cambridge, Dept. Biochemistry & Cambridge Systems Biology Centre, University of Cambridge, Cambridge CB2 1GA, United Kingdom.

Wine yeast exhibit an extraordinary ability to survive in the harsh environment of wine. The ability to form biofilms may have a role as it enables yeast to colonise and persist in various stressful ecological niches. This study aims to investigate biofilm formation and regulation of wine yeast and whether the yeast quorum-sensing molecules (tryptophol and 2-phenylethanol) influence this process. The ability of three commercial wine yeasts and the laboratory strain Σ1278b to form biofilms (mats) on rich or nitrogen-limiting low-agar media were compared. Cell morphologies were examined and compared between different parts of the mats, as well as with and without the addition of tryptophol, 2-phenylethanol, or ethanol. Each wine yeast strain formed mats with unique structures. Within the biofilm, cells from the mat rim showed a uniform actively growing population whereas those from the mat body showed a variety of morphologies. Under nitrogen-limitation, a subset of cells switched from surface growth to filamentous and invasive growth. Ethanol enhanced this filamentous invasive growth for a wine yeast, yet the effect was suppressed by the aromatic alcohols. The morphological complexity of wine yeast mats, make them suitable models to study cellular differentiation and organisation. Nutrient availability and the presence of signaling molecules could influence strategies for colonisation and survival.

6–13

**Dealing with indigenous wine fermentation: wild yeast behaviour**

Federico Tondini[1], Ana Hranilovic[1], Paul Grbin[1], Markus Herderich[1,2], Tertius van der Westhuizen[3] and Vladimir Jiranek[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae SA 5064
[2] Australian Wine Research Institute, Urrbrae SA 5064,
[3] Laffort Australia Pty Ltd, Woodville North SA 5012

'Indigenous' fermentations in which the micro-flora present on the grapes/in the winery are responsible for the alcoholic fermentation are favoured for their added complexity even though they can be less tolerant of conditions in juices of even average sugar content. The research into the behaviour of non-*Saccharomyces* yeast is not as extensive as that for *Saccharomyces*. In particular the molecular basis of oenological properties of these so-called wild yeast needs to be defined under the range of conditions and oenological practice seen between wineries.
An in-house collection of wild yeast isolates has been created and the identification of non-*Saccharomyces* and *Saccharomyces* species undertaken. The fitness of these yeast species was measured individually in environments mimicking increasing osmotic and ethanol stress, found in the wine fermentation. Differences in growth rate and fermentative metabolism were defined.
Understanding what metabolic changes that yeast cells undergo in order to deal with high sugar environments will be the focus in the subsequent stage of this study. Genetic expression and fermentation behaviour are going to be monitored to determine side effects of different genetic expression patters on functionality and final products. This information will give winemakers guidelines on how to avoid risky fermentations and also how to modulate yeast growth and sensory contribution.

6–14

**The impact of brief temperature shifts on wine fermentation**

Greg Valentine, Michelle Walker, Jennie Gardner, Frank Schmid and Vladimir Jiranek

School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae SA 5064

A number of practices during winemaking involve exposure of the fermenting yeast to a rapid change in temperature. While it is commonly thought that the potential negative effects of these temperature shifts are negligible, the actual impact of these exposures has not yet been studied. To examine this, a bench scale heat exchanger system was developed to apply a precisely controlled temperature shift for 20 seconds to specific volumes of a 20°C or 30°C fermenting culture. The temperatures applied ranged from 0°C to 60°C. The treated sample was then allowed to complete fermentation, with fermentation progression being monitored by weight loss. A catastrophic change in fermentation progress was seen when the temperature shift exceeded 50°C (55-60°C), even with the limited exposure period. At the lower culture temperature (20°C), a temporary decrease in growth rate was seen when treated at 50°C, indicating that the initial culture temperature influences tolerance to these short exposures.
The effects on cells of such brief temperature exposures are currently being further examined. Viability loss is being evaluated by flow cytometry and the genetic response by utilising quantitative PCR and RNA (deep) sequencing.

6–15

## Variable nitrate assimilation potential amongst *Brettanomyces bruxellensis* isolates

Ryan Zeppel[1,2], Chris Curtin[1], Anthony Borneman[1,2]

[1] Australian Wine Research Institute, Urrbrae SA 5064
[2] Department of Genetics & Evolution, The University of Adelaide, Adelaide SA 5005

*Brettanomyces bruxellensis* is a yeast species that is well-adapted to fermentative ecosystems, thanks to characteristics such as ethanol accumulation and tolerance, acid tolerance and the assimilation of limited or alternative nutrients. Despite its industrial importance, the understanding of the biology of *B. bruxellensis* lags well behind that of other industrial fungal species, particularly the major industrial yeast, *Saccharomyces cerevisiae*. However, the recent falling costs of next-generation DNA sequencing have facilitated an acceleration in the understanding of *B. bruxellensis* biology through comparative genomics.

A potentially important factor in the adaptation of *B. bruxellensis* to fermentative ecosystems is its ability to metabolise alternative nitrogen sources, such as nitrate, that *S. cerevisiae* is unable to consume. Interestingly, it is the only fungal species that is commonly found in wine that is known to assimilate nitrate. However, nitrate assimilation is not a global characteristic of the species as only around half of *B. bruxellensis* show this nitrate assimilatory phenotype.

In order to determine the basis for the variable ability in nitrate assimilatory potential, forty five *B. bruxellensis* isolates were phenotyped and their assimilatory status correlated with whole genome sequence information. Surprisingly, a large number of nitrate-negative isolates were found to possess a common homozygous complement of the cluster of genes responsible for the assimilation of nitrate. However, it appears that a subset of a clonal culture of such a strain can switch to nitrate-positive. Research is now directed towards understanding the genetic mechanism behind this phenotype switch.

**Session 7. Yeast/Environment Interactions**
Chaired by **Mark Prescott** (Monash University)

7–1

## Yeast competitive fitness in wine-like fermentation environments

Simon A. Schmidt, Radka Kolochova, Angus Forgan, Anthony Borneman, Paul Henschke, Paul Chambers

Australian Wine Research Institute, Urrbrae, SA, 5064

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 and grape juice composition is the subject of this presentation. In order to survey the largest possible diversity of wine yeast in the widest array of environmental conditions we have used competition experiments to evaluate differential yeast fitness in response to environmental challenges. This has been achieved through the DNA barcoding of 90 wine yeasts strains of commercial and environmental origin, enabling the parallel determination of fitness profiles in a range of industrially relevant medium formulations. We have made use of highly multiplexed sequencing to estimate population frequencies in these mixed culture experiments. Environmental variables commonly associated with poor fermentation performance, such as sugar concentration and temperature, were not determining factors of yeast strain fitness. Copper 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.

7–2

## *Candida* in the host environment: mechanisms of immune evasion

Timothy Tucey, Nathalie Uwamahoro, Jiyoti Verma-Gaur, Tricia Lo, Julie Nguyen, Joshua Nickson, Traude Beilharz, Thomas Naderer and Ana Traven

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

*Candida albicans* is a human commensal yeast, which can cause serious infections in susceptible individuals. The developmental switch form yeast to hyphal morphology controls the commensal-to-pathogen transition, and the interactions with innate immunity. The hyphal form is recognized by macrophages as "pathogenic" and triggers inflammation, but, paradoxically, the ability to switch to hyphal growth is also required for *Candida* to evade innate immune responses by killing macrophages and escaping after phagocytosis. It is therefore poorly understood how the *Candida*-macrophage interaction and inflammation are controlled in health and disease.

We have devised live-cell imaging assays to monitor the *Candida*-macrophage interaction at unprecedented resolution. With this, we have shown that *Candida* hyphae kill macrophages by triggering programmed host cell death that depends on caspase 1 and the NLRP3 inflammasome. This discovery changed a long-standing view that hyphal filaments kill macrophages "simply" by causing physical damage. Our data further demonstrated that hyphal morphogenesis is not sufficient for triggering caspase 1-inflammasome activation, bur rather hyphal cell wall architecture is critical. The transcriptional regulator Mediator orchestrates morphogenesis and cell surface restructuring in macrophages that trigger inflammasome activation. In current work, we are probing the mechanism of inflammasome activation by *Candida* using imaging, genetic and pharmacological approaches, for a holistic understanding of how the interaction of *Candida* with the NLRP3 inflammasome is fine-tuned to control inflammatory responses.

7–3

## Sensing and responding to the environment - Specialised cell types of the dimorphic pathogen *Talaromyces marneffei*

Alex Andrianopoulos

Genetics, Genomics and Development, School of BioSciences, University of Melbourne, Melbourne VIC 3010

*Talaromyces marneffei* (*Penicillium marneffei*) is an important fungal pathogen of humans, in particular those who are immunocompromised. *T. marneffei* has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching. The strongest extrinsic trigger for dimorphic switching is in response to temperature. *T. marneffei* grows in the hyphal form at 25°C and in the yeast form at 37°C. The hyphal form produces conidia, which are likely to be the infectious agent, and believed to establish infection after inhalation. The yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host.

*T. marneffei* is the only true pathogen in a genus comprising a large number of species and is also the only dimorphic fungus in this group. Yet there are a number of other fungi in more distantly related orders, which also exhibit the capacity to alternate between hyphal and yeast growth forms. Many of these are also pathogens of animal or plants. As an intracellular pathogen, *T. marneffei* must be able to utilise the available nutrient sources in order to grow while evading or tolerating the host's defence systems. The results from a number of lines of investigation into the molecular control of the dimorphic switch and the events which establish and maintain the morphological states in *T. marneffei*, both of which are central to understanding pathogenicity, will be presented.

7–4

**What is the physiological role of autophagy in yeast?**

Alexander I. May, Rodney J. Devenish, and Mark Prescott

Department of Biochemistry and Molecular Biology, Monash University, Clayton Campus, Clayton VIC 3800

Autophagy, which was originally identified in yeast, is a fundamental catabolic pathway that facilitates the isolation and transport of cytoplasmic material to the vacuole for degradation. Products of this degradation can then be returned to the cytosol for subsequent biosynthesis. Although autophagy is highly conserved throughout virtually all eukaryotes and the core mechanism of this pathway is well characterised, the physiological significance of autophagy remains poorly understood. As yeast often face conditions of nutrient limitation, it is generally assumed that autophagy acts as a recycling mechanism, supplying anabolic pathways during unfavourable environmental conditions, but little evidence supports this notion. In this talk, the physiological role of autophagy in *S. cerevisiae* is discussed with a particular focus on the growth phenotypes of strains defective for autophagy. We have found that autophagy mutant strains on media supplemented with respiratory carbon sources experience a prolonged lag phase. This phenotype is limited to strains lacking core components of the autophagy machinery and not selective forms of autophagy, such as mitophagy. Further interrogation of this phenotype revealed that autophagy most likely plays a role in the provision of one-carbon metabolites to the mitochondrion, supporting mitochondrial protein translation. These results suggest that rather than a role in bulk synthetic processes, such as general protein synthesis, autophagy may be important in the provision of essential precursor metabolites to selected metabolic pathways under specific conditions. Such intricacy is likely a reflection of the deep integration of this fundamental eukaryotic phenomenon into the core metabolism of the cell.

**Session 8. Evolution & Ecology**
Chaired by **Chris Curtin** (Australian Wine Research Institute)

8–1

## Metagenomic analysis of wild wine fermentations

Anthony R. Borneman, Peter Sternes, Danna Lee and Darek Kutyna

Australian Wine Research Institute, Urrbrae SA 5064

Wine is a complex beverage that is comprised of thousands of metabolites that are produced through the action of yeasts and bacteria in fermenting grape must. To ensure a robust and reliable fermentation, most commercial wines are produced by inoculating crushed grapes with large amounts of the major wine yeast, *Saccharomyces cerevisiae*. However, there is a growing trend towards the use of classical, uninoculated or "wild" fermentations, in which the yeasts and bacteria that are naturally associated with the vineyard or winery perform the fermentation. This results in a far more complex progression of fungal species, with *S. cerevisiae* only becoming dominant late in the fermentation process. The varied metabolic contributions of these non-*Saccharomyces* species impart desirable taste and aroma attributes to wild ferments when compared to their inoculated counterparts.
In order the map the microflora of spontaneous fermentation, metagenomic techniques were used to monitor the progression of fungal species in several wild fermentations. Both amplicon-based ITS phylotyping and shotgun metagenomics were used to assess community structure, with the isolation, sequencing and *de novo* assembly of individual strains of the dominant wine-associated species also being performed.
Initial results support the view that uninoculated ferments begin with a diverse ecosystem of fungal species, but converge on the wine yeast *S. cerevisiae* as the ferment progresses. Notable differences between regions, vineyards and wineries were also apparent and these can be broadly defined by their microbial composition.

8–2

**Understanding the evolution and ecological spread of the *C. neoformans/C. gattii* species complex**

Wieland Meyer

Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School – Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Millennium Institute, Sydney, Australia

The sibling species *Cryptococcus neoformans* and *C. gattii* are the cause of cryptococcosis, a life-threatening invasive infection that compromises the respiratory and/or central nervous systems. When caused by *C. neoformans*, cryptococcosis appears as an opportunistic infection mainly affecting HIV-positive patients, whereas cryptococcosis caused by *C. gattii* occurs more frequently in immunocompetent hosts. Seven haploid monophyletic lineages/major molecular types have been identified based on PCR fingerprinting, AFLP, MLMT and MLST (ISHAM consensus scheme: *CAP59, GPD1,* IGS1*, LAC1, PLB1, SOD1* & *URA5*) (accessible at http://mlst.mycologylab.org/): *C. neoformans* (VNI [VNB], VNII and VNIV) and *C. gattii* (VGI, VGII, VGIII, VGIV) and a number of hybrids, indicating a continuing speciation process. Applying the molecular clock *C. gattii* diverged from *C. neoformans* 71, *C. neoformans* var. *grubii* (VNI/VNII) separated from *C. neoformans* var. *neoformans* (VNIV) 59, VNI and VNII 51 and VNB 42, and the *C. gattii* major molecular types VGIII and VGI 47, VGIV 52 and VGII 62 million years ago. The genetic variation found between the haploid monophyletic lineages indicates that they all warrant variety or species status. These suggested "species" can be identified by a number of alternative techniques including e.g. *URA5*-RFLP analysis, Hyberbranched Rolling Circle PCR and MALDI-TOF. The recently proposed taxonomy will be discussed in the light of these findings. Population genetic analysis indicating the highest genetic variation and the presence of recombination identified Brazil as the origin of the VGII population and the outbreak strains on Vancouver Island, Australia and other parts of the world. Whole population comparative genomics analysis highlight multiple dispersal events to North America and elsewhere, originating from South America, especially form Brazil and verified that Australia and other parts of the world are stepping stones in the global spread. The identification of novel genes among and between the US Pacific Northwest populations provides evidence of genomic evolution that allowed for the recent expansion of its habitat.

8–3

**Mutation of *SGF29* in the *Cryptococcus neoformans* type strain H99 is responsible for altered virulence of the key laboratory version of this strain**

Samantha Arras[1,2], Kate Ormerod[1,2], Alex Carpenter[1,2], Monica Espinosa[1,2], Ben Schulz[1,2] and James Fraser[1,2].

[1] Australian Infectious Diseases Research Centre,
[2] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

The choice of a reference strain for molecular studies is recognised as critical, but is often restricted by long-established use of a particular strain by a research community. This makes it essential to understand the idiosyncrasies of the chosen strain that may make it behave differently from other examples of the species. Here we have resolved the microevolutionary history of the type strain of the fungal pathogen *Cryptococcus neoformans*. Strain H99 was originally isolated from a patient in 1978 and multiple lineages have emerged from it since that time. Our earlier investigations had revealed an indel associated with decreased mating proficiency and virulence in one group of evolved strains. We have now characterised a deletion within the diverged H99 strain that is used in the vast majority of molecular studies of *C. neoformans*. This deletion affects a gene encoding a putative component of the SAGA complex, *SGF29*. Deletion of this gene in the closest remaining representative of the original clinical H99 strain results in increased melanisation at 37°C, which is characteristic of the diverged strains used in the laboratory. The discovery of a mutation in a component of a key regulatory complex may also require the reinterpretation of previous results.

8–4

**Genomic landscape of the industrial yeast species *Brettanomyces bruxellensis***

Chris Curtin, Ryan Zeppel, Caroline Abrahamse, Cristian Varela and Anthony Borneman

Australian Wine Research Institute, Urrbrae SA 5064

*Brettanomyces bruxellensis*, like its wine yeast counterpart *Saccharomyces cerevisiae*, is intrinsically linked with industrial fermentations. In wine, *B. bruxellensis* has what are generally considered negative influences on wine quality, whereas for some styles of beer it is an essential contributor. B*. bruxellensis* also plays a role in bioethanol fermentation – sometimes beneficial, but in other systems detracting from production efficiency by outcompeting *S. cerevisiae*. We previously investigated the level of inter-strain variation that is present within this economically important species, by comparing the genomes of four diverse *B. bruxellensis* isolates. Two of these genomes were predicted to be triploid, comprising a core diploid set of chromosomes and a third divergent haploid set, reminiscent of allotriploids within *Saccharomyces*. Re-sequencing of a further 38 isolates from around the world revealed that triploidy is relatively common for *B. bruxellensis*. Our data suggest at least five independent 'hybridisation' events have occurred to generate these triploid lineages that now populate brewing, winemaking and soft-drink related niches.

**Session 9. Gene Expression**
Chaired by **Traude Beilharz** (Monash University)

9–1

## Dynamics of ribosome scanning and recycling revealed by steady-state translation complex profiling

Nikolay Shirokikh[1], Stuart Archer[1,2] , Traude Beilharz[3] , Thomas Preiss[1,4]

[1] EMBL–Australia Collaborating Group, Department of Genome Sciences, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT,
[2] Monash Bioinformatics Platform, Monash University, Melbourne, VIC
[3] Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC
[4] Victor Chang Cardiac Research Institute, Sydney, NSW

Regulation of mRNA translation is central to eukaryotic gene expression control. Regulatory inputs are specified by the mRNA untranslated regions (UTRs) and often target translation initiation. Initiation involves binding of the 40S ribosomal small subunit (SSU) and associated translation initiation factors (eIFs) near the mRNA 5' cap, then “scanning” in the 3' direction until it detects the start codon, and is joined by the 60S ribosomal large subunit (LSU) to form the 80S ribosome (RS). Scanning of mRNA and other dynamic aspects of the eukaryotic initiation model remain conjecture as methods to trap early intermediates are lacking. Here we record the dynamics of all stages of translation in live yeast cells using translation complex profile sequencing, a new method developed from the ribosome profiling approach. We document ribosomal scanning of mRNA by observing SSU footprints along 5' UTRs. Scanning SSUs have extended 5' footprints (up to about 70 nt), indicative of the addition of tensile interactions to mRNA emerging from the exit channel, enforcing forward movement. We visualise changes in initiation complex conformation as SSU footprints coalesce into three major sizes at start codons (19, 29 and 37 nt). These share the same 5' start but differ at the 3' end, reflecting successive changes at the SSU entry channel from an open to a closed state. We also observe SSU “lingering” at stop codons after LSU departure. These results underpin mechanistic models of translation initiation and termination, built on decades of biochemical and structural investigation, with direct genome-wide *in vivo* evidence. Our approach captures ribosomal complexes at all stages of translation and will aid in studying translation dynamics in diverse cellular contexts. Dysregulation of translation is common in disease and, for example, SSU scanning is a major target of anti-cancer drug development. Our method will prove useful in discerning differences in mRNA-specific initiation in pathologies and their response to treatment.

9–2

## Phosphate acquisition strategies in fungal pathogens

Sophie Lev[1], Desmarini Desmarini[1], Yong-Sun Bahn[2] and Julie Djordjevic[1]

[1] Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research and the Sydney Medical School, University of Sydney at Westmead Hospital, Westmead NSW 2145
[2] Dept. of Biotechnology College of Life Science and Biotechnology, Yonsei University, Seoul 120-749 Republic of Korea.

Opportunistic fungal pathogens are a major cause of morbidity and mortality worldwide and resistance is emerging. With only a limited repertoire of antifungal agents available, many of which are toxic and in some cases inefficacious as pan-fungal treatments, new avenues for drug development must be explored. Fungal nutrient acquisition pathways, including those involved in phosphate uptake, may represent a new target for drug development to complement existing antifungal agents. Phosphate (PHO) homeostasis in fungi is governed by the PHO regulatory pathway but is understudied in fungal pathogens. We identified a transcription factor (PHO4) in the model fungal pathogen, *Cryptococcus neoformans,* and demonstrated its requirement for brain infectivity and pathogenicity in a mouse dissemination model. PHO4 induces expression of a large number of PHO-responsive genes during phosphate deprivation, including *BTA1,* which is absent in *Saccharomyces cerevisiae*. *BTA1* is predicted to encode a diacylglyceryl-N,N,N-trimethylhomoserine (DGTS) synthase which catalyses production of DGTS: a phosphate-free lipid with structural similarity to phosphatidylcholine (PC). We show that phosphate can be mobilized from PC in *C. neoformans* when phosphate is scarce, and that this mobilization strategy is PHO4-dependent. In summary, we have identified 2 key elements of the PHO regulatory pathway in *C. neoformans,* a major transcription factor and a novel phosphate conservation strategy. We show that PHO4 is essential for disseminated infection in the human host.

9–3

**Studying genetic interaction networks to better understand redox, DNA and lipid homeostasis on a cell-wide level**

Alex Phrakaysone, Kristen Brenner, Jordan Lewis-Conway, Ming Wu and Gabriel Perrone

School of Science and Health, Western Sydney University, Campbelltown Campus, Campbelltown NSW 2560

Maintenance of appropriate cellular redox homeostasis is critical for the function of numerous cellular processes and cell viability. Two vital redox systems in cells are the glutathione and thioredoxin systems that function in processes including: detoxification of reactive oxygen species (ROS) and xenobiotics; protection, regulation, maintenance and/or folding of proteins; iron-sulfur metabolism; and; synthesis of deoxyribonucleotide triphosphates. While many specific aspects are known regarding these redox systems, how they are regulated and impacted on a cellular level, and whether this is impacted by changes in environmental conditions including nutrient availability is not clearly understood on a global level. How aberrant glutathione or thioredoxin impact cellular functions on a cell-wide level is also not thoroughly understood. This talk will present recent work that has exploited in vivo redox probes, high-through flow cytometry and genetic-genetic interaction analyses (synthetic fitness and lethality or exploitation of viable genome-wide double deletion or gene-over-expression strain collections) to study glutathione redox homeostasis. Furthermore how an aspect of this study, specifically how changes in glutathione impacts chromosomal and/or mitochondrial DNA homeostasis or survival/repair of DNA damage will presented. The broader implications of using analogous approaches to study other areas of interest in the group including the thioredoxin redox system, ROS production, and neutral lipid homeostasis will also be discussed.

9–4

**Impact of histone H3 lysine 4 methylation and RNA 3' end formation pathways on Benomyl toxicity**

Ming Kalanon[1], Melissa Curtis[1], Thanasis Margaritis[2], Frank Holstege[2], Vinenct Geli[3], Traude Beilharz[4] and Bernhard Dichtl[1]

[1] Centre for Cellular and Molecular Biology, Deakin University, Melbourne VIC
[2] Molecular Cancer Research, University Medical Center Utrecht, Utrecht, Netherlands.
[3] Centre de Recherche en Canderologie de Marseille, CNRS, Marseille, France
[4] Department of Biochemistry and Molecular Biology, Monash University, Melbourne VIC

Faithful segregation of chromosomes involves proper attachment of microtubles on the kinetochore. The Set1C histone H3 lysine 4 (H3K4) methyltransferase has previously been involved in the regulation of this process via methylation of the non-histone substrate Dam1, a component of the kinetochore. We used the microtuble destabilizing drug benomyl to probe a role of H3K4 methylation during mitosis and delineated a novel pathway that determines benomyl toxicity. Benomyl sensitive growth of wild-type cells requires mono- and di-methylation of H3K4 and Pho23, a Rpd3L deacetylase component that is known to bind to methylated H3K4. Moreover, *Δset1* and *Δpho23* mutations suppress defects associated with temperature sensitive *ipl1-2*/aurora kinase alleles and *tub2-423* and *Δtub3* mutations suppress benomyl resistance of *Δset1*. Alpha factor synchronised *Δset1* cells displayed coordinated de-repression of all four tubulin genes, up-regulation of certain kinetochore subunits and miss-regulation of other gene products involved in chromosome segregation. Interestingly, the absence of the 3'-5' exonuclease Rrp6 also suppresses benomyl resistant growth of *Δset1* suggesting the involvement of non-coding RNA. In addition, we show that poly(A) dependent and independent RNA 3'end formation pathways balance benomyl toxicity. Our results support the view that chromatin modification and RNA processing activities are important components of a regulatory system contributing to the robust implementation of chromosome segregation.

**Miscellaneous Abstracts**

# The SMRT Way to Sequence a Yeast Genome

Åsa Pérez-Bercoff[1], Tonia L Russell[2], Zhiliang Chen[1,3], Marc R Wilkins[1,3], Paul V Attfield[4], Philip JL Bell[4], Richard J Edwards[1,3]

1 School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney NSW, Australia.
2 Ramaciotti Centre for Genomics, University of New South Wales, Sydney NSW, Australia.
3 NSW Systems Biology Initiative, University of New South Wales, Sydney NSW, Australia.
4 Microbiogen Pty Ltd, Sydney NSW, Australia.

PacBio Single Molecule Real Time (SMRT) sequencing is rapidly becoming the technology of choice for *de novo* whole genome sequencing. The long read lengths and random error of PacBio data make genome assembly considerably easier and more accurate than short read data. Here, we report on *de novo* genome sequencing and assembly of three *Saccharomyces cerevisiae* genomes using the PacBio RSII at the UNSW Ramaciotti Centre for Genomics. A haploid reference yeast genome strain, S288C, and two novel diploid strains were sequenced as part of a larger functional genomics project. For each strain, 20kb SMRT Bell library preps were performed and sequenced on two SMRT Cells using the P6-C4 chemistry with read lengths of up to 53.3 kb. Whole genome *de novo* assemblies are then generated through the PacBio SMRT Portal.
We are using the S288C data to explore performance in comparison to the published genome as a reference. An initial assembly of S288C yielded over 99.97% genome coverage at 99.99% accuracy on only 26 contigs, with 16/17 reference chromosomes (16 nuclear chromosomes plus mitochondrion) essentially returned as a single, complete contig. The long reads enable accurate reconstruction of tandemly repeated genes (except >900kb of rRNA repeats), transposition and chromosomal translocations. We are now using the S288C data to optimise the assembly process and derive assembly settings for two novel strains. To this end, we have developed a new pipeline for the comparative assessment of high quality whole genomes against a reference.

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| Bleackley, Dr Mark | La Trobe Institute for Molecular Science |
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| Chambers, Dr Paul | Australian Wine Research Institute |
| Chitty, Miss Jessica | The University of Queensland |
| Coorey, Mr Namal | Victoria University of Wellington |
| Costello, Mr Peter | Australian Wine Research Institute |
| Curach, Dr Natalie | Macquarie University |
| Curtin, Dr Chris | Australian Wine Research Institute |
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| de Barros Lopes, Dr Miguel | University of South Australia |
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| Dillon, Mr Simon | Australian Wine Research Institute |
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| Edwards, Dr Richard | University of New South Wales |
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| Garcia Cordente, Dr Antonio | Australian Wine Research Institute |
| Gardner, Dr Jennie | The University of Adelaide |
| Goold, Dr Hugh | NSW Department of Primary Industries/ Macquarie University |
| Grbin, Assoc Prof Paul | The University of Adelaide |
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| Hayes, Dr Brigitte | La Trobe Institute for Molecular Science |
| Heierhorst, Assoc Prof Jörg | St. Vincent's Institute of Medical Research |

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| Mack, Ms Heike | Griffith University |
| May, Mr Alexander | Monash University |
| Meyer, Prof Wieland | University of Sydney/<br>Westmead Institute for Medical Research |
| Molimau-Samasoni, Ms Seeseei | Victoria University of Wellington |
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| Sattlegger, Dr Evelyn | Massey University |
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| Sternes, Dr Peter | Australian Wine Research Institute |
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| Sundstrom, Dr Joanna | The University of Adelaide |
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| Tondini, Mr Federico | The University of Adelaide |

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| Tran, Dr Tina | AB Mauri |
| Traven, Dr Ana | Monash University |
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| Van Holst Pellekaan, Mr Nick | University of Adelaide Wine microbiology |
| Varela, Dr Cristian | Australian Wine Research Institute |
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