Name:

YEAST: PRODUCTS AND DISCOVERY

Proceedings of the 4th Australian Conference on Yeast: Products and Discovery

**Charles Hawker Conference Centre,
The University of Adelaide, Waite Campus,
Urrbrae, South Australia, 2-4 December 2009.**

Editor: Vladimir Jiranek

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Organised by the Australasian Yeast Group

http://www.ayeastgroup.org/

Edited by Vladimir Jiranek

Cover: Phase contrast image of *Saccharomyces cerevisiae* schmoos (digitally modified). Courtesy of Jenny Bellon, Australian Wine Research Institute.

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Yeast: Products and Discovery 2009

Charles Hawker Conference Centre,
The University of Adelaide, Waite Campus, Urrbrae.

Program: 2–4 December 2009

Wednesday 2 December

9:00-9:50 **Registration**

9:50-10:00 **Welcome** by **Paul Chambers** (The Australian Wine Research Institute)

10:00-11:30 **Session 1. Molecular Cell Biology**

Chair: Trevor Lithgow

Trevor Lithgow, Monash University.
Yeast as *the* model for mitochondrial biogenesis.

Kate Howell, University of Melbourne.
Understanding the biological functions of ceramides and sphingolipids using *Saccharomyces cerevisiae*; structural requirements, stress signalling and protein trafficking.

Sylvie Callagari, University of South Australia.
Identifying pharmacogenetic candidates for statin-induced muscle toxicity using yeast.

Hongyuan Robert Yang, University of New South Wales. "Supersized" lipid droplets and phospholipids.

11:30-12:00 **Break**

12:00-1:30 **Session 2. Medically Important Yeast**

Chair: Wieland Meyer

Elaine Blignaut, University of Sydney.
Epidemiology and antifungal surveillance of oral yeasts: the situation in South Africa.

Wieland Meyer, University of Sydney.
Population genetics of the emerging human pathogenic yeasts *Candida glabrata* and *Candida krusei*.

Julie Djordjevic, Westmead Hospital, Sydney University.
Phospholipase C1: a key regulator of virulence-related signaling in the AIDS pathogen *Cryptococcus neoformans*.

James Fraser, University of Queesland.
GTP biosynthesis and drug resistance in the fungal pathogen *Cryptococcus neoformans.*

1:30-2:30 **Lunch**

2:30-4:00 **Session 3. 'Omics & Beyond**

Chair: Marc Wilkins

Justin O'Sullivan, Massey University New Zealand.
Re-constructing the yeast nucleus using post-genomic technologies.

David Bellows, Victoria University, New Zealand.
Sea cucumber makes a lethal finger sandwich: Using chemogenomics in yeast to explore mollisoside mode of action.

Cristian Varela/Simon Schmidt, Australian Wine Research Institute.
Systems Biology: a new approach to industrial yeast strain development.

Marc Wilkins, University of New South Wales.
The role of protein methylation in the interactome.

4:00–6:00 **Posters & Social gathering** (Charles Hawker Conference Centre Foyer)

Thursday 3 December

9:00-10:30 **Session 4. Molecular Biology: Signalling**

Chair: Jorg Heierhorst

Alan Munn, Griffith University.
3D structure of a yeast AAA-ATPase complex important for membrane protein sorting in endosomes and whose human ortholog plays a critical role in viral infection.

Nicolas Hoch, St Vincent's Institute of Medical Research.
The Rad53-SCD1 as a phospho-counting switch to fine-tune the checkpoint response to the strength of the DNA damage signal.

Brendon Monahan, CSIRO, Melbourne.
SWI/SNF chromatin remodeling complexes: new insights from fission yeast.

Evelyn Sattlegger, Massey University, New Zealand.
Starvation versus memory: What can yeast tell us about Yih1-mediated regulation of protein kinase Gcn2?

10:30-11:00 **Break**

11.00-12:30 **Session 5. Industrial Yeast I**

Chair: Vince Higgins

Richard Gardner, University of Auckland.
Temperature tolerance of growth and fermentation.

Philip Bell, Microbiogen.
Novel yeasts to enable a cellulosic food and fuel biorefinery.

Vince Higgins, University of Western Sydney.
Understanding the molecular mechanisms involved in zinc deficiency using *Saccharomyces cerevisiae* and microarray technology.

Anthony Borneman, Australian Wine Research Institute.
Genome sequencing and comparative genomics of industrial *Saccharomyces cerevisiae* strains.

12:30-1:00 **Lunch**

Remainder of sessions for Thursday held in McLaren Vale (return travel by coach)

1:00-4:00 **Winery Visits**

Wirra Wirra – Hosted by **Tim James.**

Chapel Hill – Hosted by **Mark Allgrove.**

4:30-6:00 **Session 6. Industrial Yeasts II (**Ben Chaffey Gallery, McLaren Vale)

Co-Chairs: Peter Rogers and Paul Henschke

Michelle Walker, University of Adelaide.
Progress towards improvement of industrial yeast for the wine industry.

Toni Cordente, Australian Wine Research Institute.
Identification and characterization of a novel flavour enhancing gene in *Saccharomyces cerevisiae*: *STR3*.

Alison Soden, Foster's Wine Estates.
Tutored tasting of wines made with novel yeast.

7:00-10:30 **Conference Dinner** (Cask Hall, Ben Chaffey Gallery, McLaren Vale)

10:30 Coaches depart for return to Adelaide.

Friday 4 December

9:00-10:30 **Session 7. Responses to Environmental Changes**

Chair: Grant Stanley

Brian Monk, University of Otago,
Mechanism of echinocandin resistance in *Candida glabrata*.

Tina Tran, Australian Wine Research Institute and Victoria University, Australia.
Identifying and characterising genes that confer the ethanol tolerance phenotype in *Saccharomyces cerevisiae*.

Xianning Lai, St Vincent's Institute of Medical Research.
Telomere and nonsense-mediated mRNA decay (NMD) independent DNA damage response functions of two newly identified yeast hEST1A/SMG6-like (ESL) proteins.

Rod Devenish, Monash University.
A late form of nucleophagy in *Saccharomyces cerevisiae*.

10:30-11:00 **Break**

11.00-12:30 **Session 8. Evolution & Ecology**

Chair: Mat Goddard

Jeremy Gray, University of Auckland.
Adaptation to new environments in the presence of migration: Is sex of benefit?

Chris Curtin, Australian Wine Research Institute.
Dekkera bruxellensis wine strains are genetically diverse and exhibit differences in tolerance to the common wine preservative sulphite.

Diana Leemon, Queensland Department of Primary Industries & Fisheries.
Have we a symbiotic yeast here?

Mat Goddard, University of Auckland, New Zealand.
The ecology of *S. cerevisiae*.

12:30-1:30 **Lunch**

1.30-3:00 **Session 9. Molecular Biology: Gene Expression**

Chair: Thomas Preiss

Traude Beilharz, Victor Chang Cardiac Research Institute.
What the length of the poly(A)-tail tells us about RNA metabolism.

Ana Traven, Monash University.
The Ccr4-Pop2 mRNA deadenylase regulates morphogenesis in the human fungal pathogen *Candida albicans*.

Joyce Chiu, University of New South Wales.
Cell-cycle sensing of oxidative stress in *Saccharomyces cerevisiae* by oxidation of a specific cysteine residue in the transcription factor Swi6p.

Alex Andrianopoulos, University of Melbourne.
Title – to be advised.

3:00 **Close**

ORAL PRESENTATIONS

Session 1. Molecular Cell Biology
Chaired by **Trevor Lithgow** (Monash University)

1–1

Yeast as *the* model for mitochondrial biogenesis.

Trevor Lithgow

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Yeasts are eukaryotes. The model yeast *Saccharomyces cerevisiae* is often cited as a good experimental model for aspects of human biology, which it is, but it turns out that for fundamental aspects of biology yeast is a useful model for all eukaryotes. My laboratory has been studying the process of mitochondrial biogenesis and particularly how proteins are imported and assembled in mitochondria. Mitochondria evolved from an intracellular bacterium and some of the protein transport machinery of the bacterium has been retained through the course of evolution – some of it largely unchanged, some of it highly modified. We have worked towards understanding the mechanisms at play in this protein transport machinery, using yeast as our model of choice. The acquisition of mitochondria was a monophyletic event, *i.e.* it happened once in the first eukaryotes, so protein transport machines that produce mitochondria are common to all eukaryotes. Functional studies done in the model eukaryote (you know who) have enabled an understanding of the process of mitochondrial biogenesis as it occurs in all eukaryotes. Recent work on weird eukaryotes like Giardia, Trypanosomes and humans, as well as bacteria of the type that gave rise to mitochondria, have added additional colour to the picture first presented from experiments done with yeast.

1–2

Understanding the biological functions of ceramides and sphingolipids using *Saccharomyces cerevisiae*; structural requirements, stress signaling and protein trafficking.

Kate Howell[1, 2] and Howard Riezman[2]

[1]Current address: Department of Agriculture and Food Systems, University of Melbourne, Royal Parade Parkville 3010 Victoria, Australia. (03) 83440113, khowell@unimelb.edu.au
[2]Department of Biochemistry, University of Geneva, quai E. Ansermet 30, CH-1227 Geneva, Switzerland.

Ceramides are important intracellular second messengers involved in a number of regulatory processes which can impact upon cell growth, differentiation, death, GPI-anchored protein transport and remodeling of their lipid moiety. Ceramides also have a structural role, as the main component of sphingolipids in the lipid membrane. Ceramides are composed of a long chain base added via a C2-amide linkage to a long chain fatty acid. In yeast, this long chain fatty acid contains 26 carbons, while in mammalian cells the long chain fatty acyl chain can range from 14 to 24 carbons in length. Other species have other structural requirements, for example *Caenorhabditis elegans* contains ceramides with branched chain fatty acids. In yeast, the *de novo* synthesis of ceramide is mediated by the ceramide synthase complex, from the LAC1, LAG1 and LIP1 gene products. In mammalian cells, the Lag1 homologues are the Lass genes; Lass1 through to Lass6 have been cloned. No Lip1p homologue has been identified in mammalian cells. It appears that the specificity of chain length of fatty acids in the ceramide is controlled by the ceramide synthase, as expression of the Lass genes in yeast give a range of different ceramides (fatty acyl chain length C_{14} through to C_{24}). We have complemented the Δ*lag1*Δ*lac1* double deletion in yeast by the other genes encoding ceramide synthases and have measured the ceramide species produced. Recent work shows that ceramides with $C_{20\text{-}22}$ are involved in protecting *C. elegans* against anoxia.

1–3

Identifying pharmacogenetic candidates for statin-induced muscle toxicity using yeast

Sylvie Callegari[1], Jennifer Bellon[2], Ross McKinnon[1], Stuart Andrews[1], Miguel de Barros Lopes[1]

[1]Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia, GPO Box 2471, Adelaide, South Australia, 5001.
[2]The Australian Wine Research Institute, PO Box 197, Glen Osmond, South Australia, 5064.

Statins are among the most highly prescribed drugs in the world and their use has revolutionised the treatment of hypercholesterolemia. Despite their clinical effectiveness, as many as 10% of patients experience muscle pain or fatigue and in more serious cases, develop myalgia, rhabdomyolysis and liver toxicity. Currently, data on pharmacogenetic candidates relating to statin toxicity is lacking. This study exploits the simple unicellular eukaryote *Saccharomyces cerevisiae* to identify and characterise genes that influence statin sensitivity. *S. cerevisiae* demonstrates drastic morphological modification and growth inhibition in response to statins. Moreover, cells undergo a loss of mitochondrial function. Statins lower cholesterol by inhibiting HMG-CoA reductase, the rate-limiting enzyme of the sterol synthesis pathway. The addition of mevalonate, the product of HMG-CoA, suppresses this response, demonstrating that statin toxicity emanates from the sterol synthesis pathway. Supplementation with ergosterol was ineffective in rescuing these phenotypes, however, the addition of isoprenyl compounds restored cell viability, indicating disruption of protein isoprenylation is largely responsible for statin toxicity. Genome-wide screens, of *S. cerevisiae* deletion mutants exposed to statins have identified a number of genes whose inactivation increases cell sensitivity to statins. Further analysis of a selection of these genes, based on the presence of an associated human ortholog, led to the selection of the *MEF2* gene as a potential pharmacogenetic candidate. The yeast *MEF2* gene encodes a mitochondrial elongation factor, with significant homology to the human *EFG2* gene and furthermore, many of the Single Nucleotide Polymorphisms (SNPs) present in the human *EFG2* gene are conserved in the yeast gene. These polymorphisms in humans may influence patient susceptibility to statin side-effects. To assess whether the mutations increase cell sensitivity to statins, SNPs have been created in the yeast *MEF2* gene that correspond with the human ortholog. Results to date demonstrate that at least two SNPs have been shown to confer statin sensitivity, signifying that certain variations in the mitochondrial elongation factor can influence cell sensitivity to statins.

1–4

“Supersized” lipid droplets and phospholipids

Weihua Fei and Hongyuan Robert Yang

School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, 2052, Australia.
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Obesity is characterized by accumulation of adipocytes loaded with lipid droplets (LDs). By reverse genetic screening in yeast, we have recently identified a large number of gene products that regulate the size and number of cellular lipid droplets. In particular, we demonstrated that deletion of a previously uncharacterized gene, *FLD1*, resulted in the formation of “super-sized” lipid droplets (>50 times the volume of normal ones). We provide evidence that Fld1p plays a role in regulating the metabolism of fatty acids and phospholipids. We have recently identified additional yeast mutants that give rise to “supersized” lipid droplets and the function of those mutants in lipid metabolism is characterized. Our results suggest an important role for phospholipids, especially phosphatidic acid, in determining the size of cellular LDs.

Session 2. Medically Important Yeast
Chaired by **Wieland Meyer** (University of Sydney)

2-1

Epidemiology and antifungal surveillance of oral yeasts: the situation in South Africa.

Elaine Blignaut

Faculty of Dentistry, University of Sydney, Level 6, Sydney Dental Hospital, 2 Chalmers Street, Surry Hills NSW 2010, Australia.
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Candida spp exist as normal flora on the skin and mucous membranes of humans and animals, displaying a high degree of plasticity in readily transforming to a pathogenic state when a change in the host environment occurs. The association of oral candidiasis with immunosuppression, and notably HIV/AIDS, is well known. Together with the modified immune status, there is the prolonged use of antifungal agents, resulting in frequently reported antifungal resistance of candida in AIDS patients. Despite this adaptability, and the increased and prolonged use of therapy, there is surprisingly no evidence to date of an emergence of a stable antifungal resistance or that of hypervirulent strains of *Candida albicans*. While no other reservoir of *C. albicans* in nature has been established, a number of genetic subtypes of the organism, with distinct geographic specificity, have been described. Phenotypic characteristics associated with some of the genetic subtypes, or clades, are emerging, and in particular antifungal resistance and cross-resistance to different antifungal agents. The findings on the predominant *C. albicans* clades and *Candida* species among healthy individuals and HIV/AIDs patients in South Africa will be presented, highlighting the surprisingly high prevalence of a ‘natural resistance’ to amphotericin B, and a likely explanation for this. The data obtained from surveillance studies on oral yeasts among HIV/AIDS orphans in orphanages will also be presented, revealing the colonisation of the mouth and toothbrushes by uncommon species.

2-2

Population genetics of the emerging human pathogenic yeasts *Candida glabrata* and *Candida krusei*

Wieland Meyer, Fabian Carriconde, Lusia Leal, Carolina Serena, The Australian Candidemia Study Group

Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Westmead Millennium Institute, Sydney Medical School – Western, University of Sydney at Westmead Hospital, Westmead, NSW, Australia.

Candida species are well-known opportunistic human pathogens. Their invasive infections, especially Candidemia, results in longer hospitalization and high mortality rates. Infections due to non-*Candida albicans* species are more and more frequently reported. *C. glabrata* and *C. krusei* are among the most important non-*albicans* species isolated. In addition, both species are known to be resistant to antifungal drugs. However, there is insufficient understanding regarding the various aspects of molecular epidemiology of these species. The current study aimed to assess (i) the level of genetic variability, and (ii) the geographic distribution of genetic diversity of these species in Australia. One hundred forty five *C. glabrata* isolates and 42 *C. krusei* isolates, collected between August 2001 and July 2004 from major hospital centres in Australia were genotyped by M13 fingerprinting. A subset of 96 *C. glabrata* isolates and the 42 *C. krusei* isolates were genotyped by multilocus sequence typing (MLST). Overall, numerous genotypes were delineated. Indeed, 113 genotypes and 32 sequence types (STs) were defined within *C. glabrata* by M13 fingerprinting and MLST typing, respectively. Within *C. krusei* 40 genotypes and 31 diploid sequence types (DSTs) were recognized. Several STs and DSTs were newly identified and seemed to be endemic to Australia. These results reveal the presence of a high genetic variability within both species. Investigation of the spatial population structure globally indicated genetic differentiation among the states and among the medical institutions, thus revealing some extent of endemic diversity. Ongoing studies are aimed to correlate the prevalence of drug-resistance and genotype. Comparison to the world-wide dataset (www.mlst.net) will also lead to a better understanding of the origin of these species and the impact of biological processes (e.g. local adaptations, dispersal abilities) on the genetic diversity and the structure of this human pathogen.

2-3

Phospholipase C1: a key regulator of virulence-related signaling in the AIDS pathogen *Cryptococcus neoformans*

Chayakulkeeree, M.[1], Crossett, B.[2], Sorrell, T.C.[1], Wilson, C.F.[1], Gerega, S.[3], Gandhi, K.S.[1], Diefenbach, E.[4], Yang, G.[3], Djordjevic, J.T.[1]

[1]Centre for Infectious Diseases & Microbiology, Westmead Millennium Institute (WMI), Westmead Hospital, NSW, Australia.
[2]School of Molecular and Microbial Biosciences, University of Sydney, NSW, Australia.
[3]Sydney Bioinformatics, University of Sydney.
[4] Westmead Millennium Institute Protein Laboratory.

Cryptococcus neoformans is an established model used to elucidate the role of phosphatidylinositol-specific phospholipase C (PIPLC/Plc) in the virulence of pathogenic fungi. Deletion of cryptococcal *PLC1* abolishes multiple virulence phenotypes, including survival at host temperature, secretion of phospholipase B1(Plb1), melanin production, and maintenance of cell wall integrity under stress. To determine how Plc1 exerts its pleiotropic effect, we characterized recombinant *Cn*Plc1, performed comparative transcriptomics (microarray) and proteomics using two-Dimensional Fluorescence Difference Gel Electrophoresis (DIGE), and investigated additional phenotypes of the *PLC1* deletion mutant (Δ*plc1*). Recombinant *Cn*Plc1 generated DAG and IP_3, via preferential hydrolysis of phosphorylated PI, consistent with its proposed role in signaling. Genes/proteins with putative roles in secretion and cell wall homeostasis were differentially expressed/abundant and concordant with the above Δ*plc1* phenotypes. Components of three virulence-related signaling pathways, the PKC/Mpk1 MAPK, cAMP/PKA and Ca^{2+}/calcineurin pathways, all potential targets of *Cn*Plc1 and/or its hydrolysis products, were differentially expressed/abundant. Δ*plc1* phenotypes consistent with activation of the G-protein-regulated cAMP/PKA pathway via Plc1, included reduced cAMP, defective mating and altered expression of genes and proteins regulating Golgi PI/GPI homeostasis. Upregulation of a novel negative regulator of G protein signaling (RGS) protein in Δ*plc1* and decreased abundance of the G-protein b-subunit (Gib2), further support a role for Plc1 in the cAMP/PKA pathway. We have established a model that positions *Cn*Plc1 as a pivotal regulator of three signaling pathways and early secretory events required for expression of the major cryptococcal virulence determinants.

2-4

GTP biosynthesis and drug resistance in the fungal pathogen *Cryptococcus neoformans*

Carl A. Morrow, Ulrike Kappler, Anna Stamp, Eugene Valkov, Justine M. Hill, Bostjan Kobe and James A. Fraser.

School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland, 4072 Australia.

Novel therapeutics and synergistic approaches to treatment are required for opportunistic fungal infections due to the increasing incidence of relapse and resistance among the AIDS population combined with the lack of effective antifungal agents. We have investigated the GTP biosynthesis and salvage pathways in the human pathogenic fungus *Cryptococcus neoformans*, a common cause of fatal fungal meningoencephalitis in primarily immunocompromised patients worldwide. We have identified and characterised homologues of *IMD1*, encoding the rate-limiting enzyme inosine 5'-monophosphate dehydrogenase and *HPT1*, encoding the purine salvage enzyme hypoxanthine xanthine guanine phosphoribosyltransferase, via targeted gene disruptions and phenotypic assays. Additionally, we have examined the basis for naturally occurring susceptibility and resistance to the IMP dehydrogenase inhibitor mycophenolic acid through the creation of mutant and chimaeric *IMD1* alleles. Detailed structural and functional analysis of two *Cryptococcus* IMP dehydrogenase alleles reveals further insight into the mechanism of this potential antifungal drug target.

Session 3. 'Omics & Beyond
Chaired by **Marc Wilkins** (University of New South Wales)

3-1

Re-constructing the yeast nucleus using post-genomic technologies.

Gerd Grünert[1,2], Chris Rodley[1], Justin M. O'Sullivan[1]

[1]Institute of Natural Sciences, Massey University, Auckland, New Zealand.
[2]Division Biophysics of Macromolecules, German Cancer Research Center, Heidelberg, Germany.

Here we present a probabilistic three-dimensional reconstruction of the yeast genome. This reconstruction was formed using a global map of the chromosomal interactions occurring within exponentially growing yeast cells. The chromosomal interactions themselves were simultaneously identified using Genome conformation capture (GCC), a novel 3C derivative that uses post-genomic high through-put sequencing. The occurrence of yeast long-range chromosomal interactions will be discussed from a systems biology viewpoint integrating these connections into the biology of the yeast genome. GCC and the associated three-dimensional reconstructions of the yeast nucleus provide us with a means by which we can extend the formulation and testing of hypotheses related to the structural and functional segregation of the eukaryotic nucleus. As such, these findings have major implications for our understanding of the yeast phenotype and eukaryotic genome organization.

3-2

Sea cucumber makes a lethal finger sandwich: Using chemogenomics in yeast to explore mollisoside mode of action.

David Bellows[1], Ploi Yibmantasiri[1], S. Andreas Angermayr[1], Alice Sorgo[1], Kristina Boeger[1], Dora Leahy[1], Jonathan Singh[2] and Peter Northcote[2]

[1]School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand.
[2]School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand.

Mollisoside is a potently fungicidal novel triterpene glycoside (TG) isolated from the sea cucumber *Australostichopus mollis*. To investigate mollisoside mode of action, we employed a chemo-genomic approach using the baker's yeast, *Saccharomyces cerevisiae*. Triterpene glycosides have been demonstrated to interfere with membrane integrity through a variety of proposed mechanisms; however, experiments to test the three known mechanisms of TG activity were not revealing. We then employed a microarray-based genome-wide chemical genetic interaction profiling assay, confirming yeast membranes as a probable site of activity. To further explore the mechanism of action of this molecule, we generated a mollisoside-resistant ($moll^R$) mutant strain. We adapted a genetic whole-genome linkage mapping assay (Jorgensen *et al.*, Genetics 2002) for use with a small molecule and identified *NCP1* as the locus of resistance. *NCP1* is a cytochrome P450 reductase that transfers electrons to *ERG11* and is essential for sterol biosynthesis. Biochemical assays revealed that $moll^R$ mutants have reduced ergosterol content compared to the parental strain, implicating sterols as a potential target. To rule out mollisoside metabolism as the mechanism of resistance, we employed a chemical genetic strategy using sterol synthesis inhibitors upstream of *ERG11* to phenocopy mollisoside resistance in wild type cells. We then showed that exogenous ergosterol rescued growth inhibition in cells treated with mollisoside, but not ketoconazole, verifying ergosterol as the presumptive mollisoside target. This example demonstrates that an integrated platform of genomic technologies based on a simple genetic model organism is a potent tool for rapid identification of small molecule mode of action.

3-3

Systems Biology: a new approach to industrial yeast strain development

Paul Chambers[1], Simon Schmidt[1], Cristian Varela[1], Anthony Borneman[1], Jeremy Hack[1,4], Meagan Mercurio[1,4], Daniel Cozzolino[1], Maurizio Ugliano[1], Chris Curtin[1], Annette McGrath[2], Alamgir Khan[3], Peter Hoffman[3], Mark Baker[3], Jens Kroemer[4], Lars Keld-Nielsen[4], Roberto Barrero[5], Paula Moolhuijzen[5], Matthew Bellgard[5], Ute Roessner[4], Tony Bacic[4], David Adelson[6], Simone Li[7], Marc Wilkins[7], Andrew Gilbert[8]

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Yeast strain development for industrial applications has benefited enormously from technological advances. 'Omics approaches, combined with the capacity to store and interrogate massive data sets, is poised to drive the next major shift in microbial strain development, particularly if done in a Systems Biology framework. Over recent years laboratory strains of the yeast *Saccharomyces cerevisiae* have, for many scientists, been the organisms of choice to establish methodologies for Systems Biology. Thus, substantial groundwork is in place for research and development of industrial *S. cerevisiae* used in winemaking, brewing, baking, bioethanol production and the pharmaceutical industry.

Whilst industrial yeasts are the same species as the laboratory strains used in pioneering Systems Biology work they are unique in many features, including robustness and 'productivity'; traits that are critical for industrial applications. There are also differences in the generation of secondary metabolites, which are essential for the sensory properties of wines and beers. Consistent with this variation in phenotypes, a recent comparative genomics study (Borneman et al., 2008, FEMS Yeast Research 8:1185-1195) found that there is considerable intraspecific genetic variation between a wine and a laboratory strain of *S. cerevisiae*. From an industrial perspective, therefore, it is important in research and development, including strain development programs, to utilise industrial yeast strains.

The Australian Wine Research Institute in collaboration with Genomics Australia, Proteomics Australia, Metabolomics Australia, and Bioinformatics Australia, has embarked on a Systems Biology project that focuses on wine yeast fermentation. The aim of this project is to harness the expertise, infrastructure and technologies available at each of these platforms to develop systems-based mathematical models of yeast metabolism. These models will, in turn, be used to develop predictive models to inform design of new strains with improved, wine relevant, traits.

3-4

The role of protein methylation in the interactome

M.R. Wilkins, C.N.I. Pang, S. Li, E. Ho, Huang K.-Y., T.A. Couttas, J. Low

NSW Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

Interactome research has shown that proteins participate in a wide number and variety of interactions. Together, these form networks of great complexity. However, the dynamics of these networks remain virtually unknown. Post-translational modifications, such as protein methylation and phosphorylation, are one means of regulating protein-protein interactions. Whilst the predominance of phosphorylation in the proteome, especially for the model organism S. cerevisiae, is well understood, the post-translational modification of protein methylation is poorly understood both in its presence in the proteome and its possible role in controlling protein-protein interactions.

We have developed two approaches to help detect protein methylation in the proteome. One approach is immonium ion scanning using a wide range of collision energies (1). This has been applied to the study of the S. cerevisiae proteome. We have also analysed ~26,000 peptide mass spectra of purified S. cerevisiae proteins for the presence of methylation, using the FindMod tool (http://au.expasy.org/tools/findmod/).

To understand the role that protein methylation plays in the control of protein-protein interactions, we have studied the effect of lysine- and arginine- methyltransferase knockout mutants on the formation of protein complexes. Blue native PAGE, and a prefractionation technique of continuous elution blue-native PAGE (2) have been used for this purpose.

The above results have been interpreted in the context of the yeast interactome. To do this, we have developed the GEOMI software platform to co-analyse protein-protein interactions in the context of the interactome (3).

We have undertaken some of the first proteome-wide analyses for protein methylation. We have found that the methylation, notably on lysine and arginine residues, is much more widespread than previously thought. It is enriched in proteins of specific function, such as those associated with protein translation, and is predominantly found in proteins of the cytoplasm, nucleus and nucleolus. It is found associated with particular sequence motifs.

The analysis of methyltransferase knockouts has revealed that these enzymes display redundancy. Double and triple knockouts, at least for yeast, are viable. However, BN-PAGE analysis of mutants has revealed a number of protein complexes which fail to form in the absence of methylation. Whilst further confirmatory analyses are required, this suggests that methylation can play a role in the control of protein-protein interactions.

(1) Couttas TA et al. (2008) Immonium ion scanning for the discovery of post-translational modifications and its application to histones. Journal of Proteome Research 7, 2632–2641.
(2) Huang K-Y et al. (2009) Micropreparative fractionation of the complexome by blue native continuous elution electrophoresis. Proteomics, 9, 2494-2502.
(3) Ho E et al. (2008) Interactive three-dimensional visualization and contextual analysis of protein interaction networks. Journal of Proteome Research, 7, 104-112

Session 4. Molecular Biology: Signalling
Chaired by **Jorg Heierhorst** (St Vincent's Institute of Medical Research)

4-1

3D structure of a yeast AAA-ATPase complex important for membrane protein sorting in endosomes and whose human ortholog plays a critical role in viral infection

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Vps4 is an AAA-family ATPase highly conserved between yeast and humans. In both yeast and humans, Vps4 mediates endosomal membrane protein sorting. In humans, Vps4 is also a host factor hijacked by a diverse set of clinically important enveloped viruses, including HIV, Hepatitis B Virus, Hepatitis C Virus, Epstein-Barr Virus, Paramyxoviruses, and Ebola Virus, to facilitate their egress. Knockdown of Vps4 in mice has been reported to protect against mortality caused by Ebola Virus infection without apparent adverse effects. Vps4 is therefore a potential target for development of novel broad-spectrum antivirals. We previously showed that mutation of a novel Vps4-specific β domain blocks assembly of yeast Vps4 monomers into active oligomers. However, it remained unclear whether the β domain is directly involved in Vps4 assembly or if these mutations have general effects on protein folding. We have used negative stain electron microscopy and single particle analysis as well as a range of biophysical methods to determine the subunit stoichiometry and 3D structure of the yeast Vps4 oligomer. Vps4 has 11-12 subunits arranged in two hexameric rings stacked in a tail-to-tail orientation. The Vps4 β domain is well placed to mediate contact between rings. The β domain is likely to play a direct role in Vps4 assembly into an active oligomer. Because the β domain is critical for assembly but found only in Vps4 it may be a target for specific Vps4 inhibitors.

4-2

The Rad53-SCD1 as a phospho-counting switch to fine-tune the checkpoint response to the strength of the DNA damage signal

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Maintenance of genome integrity is critical for cellular growth and survival. In budding yeast, the kinase Rad53 is the major effector of the checkpoint response, a tightly regulated pathway that senses DNA damage and activates mechanisms to both repair this damage and to arrest the cell cycle while repair is underway.

Rad53/Chk2-like kinases are characterized by regulatory forkhead-associated (FHA) and SQ/TQ cluster (SCD) domains. While simultaneous phosphorylation of the two adjacent threonines T5 and T8 in the Rad53-SCD1 is required for activation of the downstream kinase Dun1, it is widely assumed that phosphorylation of any one of the four TQ motifs in the N-terminal SCD is sufficient for activation of all other Rad53 functions.

Here, we show that the *rad53-4AQ* mutant, where all four threonines in the SCD1 are changed to alanine, shows signs of spontaneous damage and checkpoint activation during S-phase and is synthetic lethal/severely sick with deletions of either of the checkpoint mediators Rad9 or Mrc1. Interestingly, these phenotypes are prevented in the *T8-3AQ* mutant, where the only phosphorylatable residue is threonine 8, but still present in any of the other three threonine add-back mutants, indicating a prominent role of T8 phosphorylation in Rad53 activity.

Strikingly, *rad53-T8-3AQ* and even the Dun1-activation competent *rad53-T5-T8-2AQ* mutant showed a dramatic increase in MMS sensitivity when combined with a Rad9 deletion. This indicates that multi-site phosphorylation of the SCD1 is required when other buffering pathways are absent.

Taken together, our data indicate that an increasing number of RAD53 SCD1 phosphorylation is required to cope with escalating DNA damage, with T8 possibly being the first and most important residue to be modified.

4-3

SWI/SNF chromatin remodeling complexes: new insights from fission yeast

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The SWI/SNF chromatin remodelling complexes play critical roles in transcription and other chromatin-related processes. Much of our understanding of the roles of these complexes *in vivo* has come from the analysis of the two members of this class in *Saccharomyces cerevisiae,* SWI/SNF and RSC. To understand the *in vivo* functions of SWI/SNF and RSC in an evolutionarily distant organism, we have characterized these complexes in the fission yeast, *Schizosaccharomyces pombe*. While core components are conserved between the two yeasts, the compositions of *S. pombe* SWI/SNF and RSC differ significantly from their *S. cerevisiae* counterparts and in some ways are more similar to metazoan complexes. Furthermore, several of the conserved proteins, including actin-like proteins, are strikingly different between the two yeasts with respect to their requirement for viability. Finally, phenotypic and microarray analyses have identified widespread requirements for SWI/SNF and RSC on transcription including strong evidence that SWI/SNF directly represses iron transport genes.

4-4

Starvation versus memory: What can yeast tell us about Yih1-mediated regulation of protein kinase Gcn2?

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How do cells endure/overcome environmental or intracellular stresses? In all eukaryotes, one major mechanism is to adjust protein synthesis via phosphorylating the α subunit of translation initiation factor 2 (eIF2α). In all eukaryotes the eIF2α kinase Gcn2 is essential for overcoming amino acid hunger. For sensing starvation, the Gcn2 RWD domain must directly bind to its effector protein Gcn1. Curiously, Gcn2 is also required for long term memory formation. The mammalian protein IMPACT, highly expressed in neurons, and its yeast ortholog Yih1, consist of an Ancient Domain and an RWD domain with Gcn1 binding activity. Yih1/IMPACT competes with Gcn2 for Gcn1 binding thereby diminishing Gcn2 activity. Yih1 also binds monomeric G-actin, a component of the cytoskeleton. Our studies suggest that Yih1/IMPACT inhibits Gcn2 when released from G-actin, and only under certain physiological conditions or in specific cellular compartments.

Our goal is to further elucidate the cellular roles of Yih1/IMPACT. We found that Yih1-actin interaction is independent of Gcn1, that Yih1-Gcn1 interaction does not require actin. The Yih1 RDW domain does not bind actin, while the Yih1 central region is sufficient for actin or Gcn1 binding. We compared the Gcn1/actin binding efficiency of various Yih1 fragments with their ability to affect Gcn2 activity, and our findings suggest that apart from Gcn2 sequestration additional factors play a role in Gcn2 inhibition, and possible mechanisms are discussed. Structural exercises identified amino acids in Yih1 putatively involved in Gcn1 binding. Furthermore, we uncovered a conserved putative interaction surface characteristic for Ancient Domains that harbor determinants characteristic to either eukaryotes or prokaryotes, suggesting that, despite of evolutionary divergence, it is of fundamental cellular importance. These results reinforce our model for a universal role of Yih1/IMPACT in a cross-talk between the cytoskeleton and translation, and likely relevant in neurons given that actin and mammalian Gcn2 are also involved in memory formation.

Session 5. Industrial Yeast I
Chaired by **Vince Higgins** (University of Western Sydney)

5-1

Temperature tolerance of growth and fermentation

Justin Goh, Rebecca Deed and Richard Gardner

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The ability to ferment well at extremes of temperature is critical for industrial yeasts: cold tolerance for baking, winemaking and beer, and heat tolerance for commercial bioethanol production. Relatively little is known about genes that confer good growth and fermentation at extremes of temperature, in contrast to our detailed knowledge of the temperature stress response. Our lab is investigating the genetics of temperature tolerance using batch fermentation, which requires that cells have a reasonable rate of initial growth, attain a high final cell titre, and subsequently maintain viability and achieve a good rate of fermentation per cell throughout the course of fermentation.

A set of 39 genetically diverse isolates of yeast were grown and fermented at temperatures from optimum to cold in different media, including grape juice. None of the growth rate datasets correlated with fermentation rate between strains, except in media with high osmotic content. The results suggest that the ability to grow in high osmotic stress is a critical component of yeasts ability to ferment. There was no general correlation in the strains' responses to cold temperature in individual media, suggesting that there is no common genetic programme for "cold adaptation" between yeasts.

Two industrial strains with good capacity for high temperature fermentation were crossed with the lab strain S288C and progeny analysed for high temperature growth (Htg). In both crosses, a 65-kb region of chromosome 14 containing *MKT1* and *NCS2* from the industrial strains was linked to the Htg phenotype, as previously found for a clinical yeast isolate (Sinha et al 2008). This region may represent an 'Achilles heel' of the S288C strain. The Htg phenotype segregated with the ability for high temperature fermentation, but not with fermentation rate per se, consistent with the idea that growth is necessary, but not sufficient, for good fermentation. We are currently mapping additional loci affecting Htg.

5-2

Novel yeasts to enable a cellulosic food and fuel biorefinery

Philip Bell

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The global challenges of liquid fuel supply and global warming have led to a renewed interest in the production of biofuels that can be used to substitute for fossil based petroleum fuels. Ethanol is already by far the dominant biofuel, with almost the entire industry based on the conversion of sugars into ethanol by the yeast *Saccharomyces cerevisiae.* The US is one of the major producers of ethanol, where over 9 billion gallons (~35 billion litres) of ethanol is produced using sugars derived from corn. To achieve this scale of production, the US consumes approximately 30% of its corn crop, and yet this relatively large amount of production represents only a few percent of its petroleum fuel requirements. It is therefore clear that complete substitution of petroleum in the US is impossible without a very significant impact on its ability to produce food. One solution to this 'food and fuel' dilemma is to utilize non-food sources, such as plant biomass, for the production of ethanol. Unlike food crops, the sugars in these biomass sources are not in a form that can readily be fermented by yeast, and there are a range of other issues that make their conversion into ethanol more difficult than the currently established 'first generation' (food based) ethanol industry. One key problem is that the sugars present in plant biomass include both C6 and C5 sugars, and *Saccharomyces* is only efficient at fermenting and otherwise utilising a fairly narrow range of C6 based sugars such as glucose, fructose, sucrose and maltose. For over 20 years research groups have used genetic engineering approaches to enable *Saccharomyces* to utilize xylose, one of the key C5 sugars in biomass. Using a novel evolution based approach, Microbiogen has developed non-genetically engineered strains of *Saccharomyces* that can efficiently utilize xylose and enable a unique 'food and fuel' biorefinery concept to be implemented.

5-3

Understanding the molecular mechanisms involved in zinc deficiency using *Saccharomyces cerevisiae* and microarray technology

Vincent J. Higgins

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Deficiency of the essential nutrient zinc is a major contributor to retarded yeast fermentation in the brewing and wine making processes. An understanding of the molecular mechanisms involved in the response of *Saccharomyces cerevisiae* to zinc deficiency can give insights into how this condition affects metabolism and may provide opportunities to overcome this challenge. An initial microarray study on yeast in zinc deficient medium confirmed the Zap1 transcriptional activator induced the transcription of a regulon of 46 genes that contained the Zap1 motif in their promoter. Many of these genes have since been assigned functions that contribute to zinc homeostasis in the cell. Also genetic markers have been identified useful in the early detection of zinc deficiency in industrial processes. The induction of approximately half of the genes differentially expressed in response to zinc deficiency was found to be independent of the Zap1 protein. Promoter sequence analysis identified that many of these genes contained the stress response element (STRE) motif, recognised by Msn2/4p. Genome-wide expression analysis of a msn2/4p mutant revealed a distinct regulon that was regulated in response to zinc deficiency. This Msn2/4p regulon was not identified in zinc deficient studies in continuously fed chemostats where zinc was added in a high enough level to sustain growth. The zinc availability in the batch method did not support long-term growth thus, better reflecting the conditions found in industrial 'stuck' fermentations and also indicates that the induction of the Msn2/4p regulon is a stress response as a result of zinc starvation rather than a cellular mechanism to overcome zinc deficiency. Glucose was used in these experiments even though maltose is the most abundant sugar available to yeast in fermentations. This presentation looks at the significance of global gene expression results using maltose as the carbon source instead of glucose.

5-4

Genome sequencing and comparative genomics of industrial *Saccharomyces cerevisiae* strains

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Industrial strains of *S. cerevisiae*, such as those used in biofuel production, brewing and winemaking, comprise a diverse collection of yeast strains that have been selected for their ability to efficiently convert sugars into ethanol despite exposure to osmotic, nutrient and ethanol stress. This high-stress environment is very different to the nutrient rich, hospitable environment generally encountered by laboratory strains and as a consequence, industrial strains have evolved common phenotypes, such as increased ethanol tolerance, when compared to their laboratory relatives. However, despite the common environmental stresses encountered by industrial strains, they also possess a diverse array of strain-specific characteristics, such as flavour production or nutrient utilization profiles, which reflect their diverse histories and match particular strains to specific industrial roles.

Initial comparative genomic studies performed by our group using a wine (industrial) yeast and a common laboratory strain uncovered numerous differences in genomic content including over 60,000 SNPs (1 per 160bp) and 37 wine-specific ORFs. The presence of these strain-specific ORFs complicate the development of systems-level analysis of industrial strains as they may contain genetic components that cannot be accounted for during the data collection phase of a systems biology investigation.

In order to develop a solid platform for the systems biology analysis of industrial yeasts we have sequenced the genomes of several industrial yeast strains (comprising both commercial wine and ale yeasts), at high coverage using the latest GS-FLX Titanium (Roche-454) sequencing platform. Whole-genome comparisons have been used to identify the genomic diversity (SNPs, large-scale rearrangements, novel ORFs) present across these industrial isolates that can then be integrated into a larger systems biology investigation to associate genomic signatures with important industrial phenotypes.

Session 6. Industrial Yeasts II
Chaired by **Peter Rogers** (Fosters) and **Paul Henschke** (Australian Wine Research Institute)

6-1

Progress towards improvement of industrial yeast for the wine industry

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To maintain profitability and a competitive advantage in a dynamic global market the Australian wine industry needs to give consideration to wine quality and consumers' demand, irrespective of associated inherent vinification problems. Understanding of the impact of wine microbiology, in particular yeast, during industrial wine fermentations is quintessential to tailoring wine quality and style. We currently focus our research efforts on yeast strain improvement, using a number of different approaches in order to gain an in depth knowledge of the biochemical complexities associated with industrially significant parameters. Through the screening of yeast libraries under oenological conditions, we have, for instance identified genes (or gene modifications) with the potential to influence wine colour, nitrogen efficiency, or fermentation duration. Also, adaptive evolution strategies have yielded improved strains, with shortened fermentation duration under conditions of increased stress. A systematic approach combining data from genomic, transcriptomic and metabolomic analyses is being used to better understand the broader impact that these modifications may confer or, in the case of evolved stains, the precise genetic basis for their observed phenotypes.

6-2

Identification and characterization of a novel flavour enhancing gene in *Saccharomyces cerevisiae*: *STR3*

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To better understand the metabolism of sulphur-containing compounds in the budding yeast *Saccharomyces cerevisiae,* we characterised the product of the yeast *STR3* gene, which encodes a protein (Str3p) with carbon sulphur β-lyase activity. Str3p is involved in the methionine biosynthetic process, and catalyses the conversion of cystathionine into homocysteine, which in a later step is converted to methionine. In this study, Str3p was expressed in *Escherichia coli* and purified by a one-step nickel affinity method to near homogeneity. Recombinant Str3p was found to form a stable tetramer under the expression conditions, and to be active towards its physiological substrate L-cystathionine, L-cystine and the non-protein amino acid L-djenkolate, similar to previously characterised cystathionine β-lyase enzymes. Str3p was strongly inhibited by certain divalent cations, particularly Cu^{2+}, and formed insoluble aggregates with the enzyme in a stoichiometric (1:1.5, Str3p : Cu^{2+}) relation, as observed by the IC_{50} of 0.26 μM Cu^{2+}.

Our main focus was the potential role of Str3p in the release of aromatic compounds during wine fermentation. Previously, it was demonstrated that the product of the *E. coli tnaA* gene, a tryptophanase with strong carbon-sulphur β-lyase activity, was able to release a large amount of the polyfunctional thiols 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexan-1-ol (3MH) from their cysteine precursors in wine when overexpressed in a commercial wine yeast. Polyfunctional thiols are potent aroma compounds that impart characters such as '*passionfruit'*, '*grapefruit'* and '*box hedge'* in Sauvignon Blanc wines.

Purified Str3p exhibited a modest activity *in-vitro* towards the cysteine-S-conjugated forms of 4MMP and 3MH, with 1.5% of the *E. coli* tryptophanase activity towards 2 mM cysteine-S-conjugated 4MMP. Importantly, overexpression of *STR3* in a commercial wine yeast during fermentation of Sauvignon Blanc juice resulted in the release of 30% more 3MH. This is the first direct evidence for an *S. cerevisiae* gene encoding an enzyme involved in polyfunctional thiol release.

6-3

Tutored tasting of wines made with novel yeast

Alison Soden

Foster’s Wine Estates, Australia.

Session 7. Responses to Environmental Changes
Chaired by **Grant Stanley** (Victoria University, Australia)

7-1

Mechanism of echinocandin resistance in *Candida glabrata*.

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Echinocandins such as micafungin and caspofungin inhibit the synthesis of β-1,3-glucan, an essential component of fungal cell walls. The incidence of echinocandin-resistant fungal pathogens is rare but has been associated with mutations in the echinocandin resistance region (the 'hot spot') of glucan synthase subunits encoded by *FKS* genes. Single nucleotide changes were detected in both *FKS* genes in a micafungin-resistant clinical isolate of the haploid pathogen *Candida glabrata*. One mutation was predicted to cause an amino acid change in the hot spot of *CgFKS1* and the other a premature stop codon in *CgFKS2*. Relationships between the observed mutations and candin resistance were assessed experimentally by reproducing the nucleotide changes in the *FKS* genes of a candin-susceptible *C. glabrata* strain using site-directed mutagenesis. Introduction of the hot spot mutation into the *CgFKS1* gene alone conferred intermediate resistance whereas the introduction of a premature stop codon in *CgFKS2* alone had no effect on susceptibility. However, the insertion of both mutations conferred high level resistance equivalent to that of the clinical isolate, and cross-resistance to caspofungin. The phenotypes and candin susceptibilities of Δ*fks1* and Δ*fks2* strains indicate that the two *CgFKS* genes are functionally redundant, with each encoding a β-1,3 glucan synthase catalytic subunit. The deletion of both *CgFKS1* and *CgFKS2* was found to be lethal. Despite differential expression of *CgFKS1* and *CgFKS2*, clinically significant micafungin resistance in *C. glabrata* appears to be rare because mutations in both *FKS* genes are required for the acquisition of high level echinocandin resistance.

7-2

Identifying and characterising genes that confer the ethanol tolerance phenotype in *Saccharomyces cerevisiae*

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Compared to other organisms, the yeast *Saccharomyces cerevisiae* is relatively tolerant to ethanol stress however; the extent of tolerance is limited. This is of particular importance to the wine industry because the inhibitory effects of ethanol on yeast viability are a major cause of stuck or sluggish fermentations. The aim of this project is to identify and characterise genes in *S. cerevisiae* that can confer improved ethanol tolerance. To this end, we are analysing spontaneous and chemically-induced adaptively evolved mutants of *S. cerevisiae* that have increased ethanol tolerance compared to the parent they were derived from. Serially backcrossing and sporulation was used to determine if the approximate number of genes involved in conferring the improved ethanol tolerance phenotype. In the chemically-induced mutant, segregation patterns suggested that a single region in the genome was responsible for conferring the ethanol tolerance phenotype. Following several backcrosses, the genome of an ethanol tolerant spore was sequenced. Single nucleotide polymorphisms (SNPs) were identified and these SNPs were systematically introduced into the parent. The mutation conferring ethanol tolerance phenotype in the chemical-mutant was identified.

7-3

Telomere and nonsense-mediated mRNA decay (NMD) independent DNA damage response functions of two newly identified yeast hEST1A/SMG6-like (ESL) proteins

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Human EST1A-C/SMG5-7 proteins play important roles in telomere maintenance and nonsense-mediated mRNA decay (NMD). Here, we describe two closely related, uncharacterised yeast proteins, termed Esl1 and Esl2 (hEST1A/B-SMG6/5-like), which contain 14-3-3-like Est-one-homology (EOH) and PINc nuclease domains similar to hEST1A. In contrast to their human orthologs, *ESL1/2* do not participate in telomere maintenance or NMD. *esl1Δ, esl2Δ* and *esl1Δesl2Δ* double mutants have telomere lengths similar to wild type in the presence of telomerase, and display normal senescence and survivor formation via alternative lengthening of telomeres (ALT) similar to telomerase-negative *est2Δ* mutants. NMD substrates such as *ade2-1* or *CYH2* did not accumulate in the mutants. Epistasis analyses showed that the deletion and nuclease-dead versions of *ESL1* and/or *ESL2* mutants altered sensitivity to various drugs, including a range of DNA damaging drugs. Deletion mutants also displayed >5 fold increase in mitochondrial genome instability. In a genome-wide synthetic lethality screen, *esl1Δesl2Δ* was found to be synthetic sick with *trf4Δ*, a protein recently shown to contribute to genome stability by regulating histone mRNA levels and also a component of the exosome-activating TRAMP complex that is required for the degradation of cryptic transcripts. Interestingly, consistent with an overlapping function with Trf4, the most significantly deregulated transcripts identified in a genome-wide expression array of *esl1Δesl2Δ* have neighbouring cryptic transcripts. These results suggest that Esl1 and Esl2 are important for the maintenance of genome stability, possibly via a role in RNA surveillance by regulating the expression of cryptic transcripts.

7-4

A late form of nucleophagy in *Saccharomyces cerevisiae*

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Yeasts are capable of rapid adaptation to changing environments. Autophagy is a catabolic process that occurs in response to dramatic changes in the nutrients available to yeast cells, for example during starvation. In the predominant form of this process, macroautophagy, cytoplasmic material (including organelles) is sequestered in double-membrane vesicles called autophagosomes (APs) which are subsequently delivered to the vacuole. Here, the APs and their cargo are degraded by lytic enzymes and the resultant products recycled.

The yeast nucleus is subject to a form of autophagic degradation by a process called Piecemeal Microautophagy of the Nucleus (PMN). Thus, following short periods of nitrogen starvation (up to 3 h), interactions between the outer nuclear envelope membrane protein, Nvj1p, and the vacuolar membrane protein, Vac8p, contribute to the formation of nuclear-vacuole junctions and ultimately of membrane vesicles containing nuclear material that are subsequently released into the vacuole.

Using a nucleoplasm-targeted fluorescent reporter, NAB35-Rosella, we observed nucleophagy (designated LN for late nucleophagy) only after longer periods of nitrogen starvation (20+ h). Dual labeling of cells with a nuclear membrane reporter, Nvj1-EYFP, and a nucleoplasm reporter, NAB35-DsRed.T3, confirms that initiation of the delivery of the two respective nuclear reporters to the vacuole is temporally separated. Furthermore, our data suggest that LN occurs by a mechanistically different process to PMN because it can occur in *nvj1* or *vac8* null cells. Nevertheless, several components of the macroautophagic machinery are required for LN as it is efficiently inhibited in a spectrum of autophagy null mutant cells. Moreover, the inhibition of LN in these mutants is accompanied by alterations in nuclear morphology. Collectively, these results suggest that different compartments of the nucleus are selected for delivery to the vacuole by mechanistically distinct forms of autophagy.

Session 8. Evolution & Ecology
Chaired by **Mat Goddard** (University of Auckland)

8-1

Adaptation to new environments in the presence of migration: Is sex of benefit?

Jeremy Gray and Mat Goddard

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Experimental evolution of *Saccharomyces cerevisiae* provides an excellent study system for empirically testing theories related to the predicted benefits and disadvantages of sexual reproduction. Previous work using *S. cerevisiae* has shown that sex increases the rate of adaptation to a new environment. The system consisted of sexual and asexual strains of *S. cerevisiae*, isogenic except for two gene knockouts which result in asexual spores. Here we attempt to extend this system to investigate adaptation to a new environment under the presence of gene flow, a more complex and realistic scenario.

While sex is predicted to be of benefit in a simple unstructured environment, sex in a more complex environment with disruptive selection is likely to be detrimental due to the formation of maladapted hybrids. Theory predicts that the asexual lines will adapt more quickly than the sexual lines in the presence of migration. Here we set up a system to test this hypothesis: Replicate sexual and asexual populations are evolved in two environments – heat with Carbon stress, and salt with Nitrogen stress, which have trade offs between them. After 25 generations, populations are subject to migration from the alternate environments, in order to simulate gene flow between the niches. The resulting populations are then put through sexual or asexual reproduction, and continued on through the cycle until a total of 300 generations are reached. The fitness of populations is then measured by head-to-head competing with a marked ancestor.

These data do not support theory. In contrast, the sexual strains show a significantly greater gain in fitness in both environments: with a gain of 23% fitness in the Carbon stress niche and 8% in Nitrogen stress, compared with an asexual 12% and 2%. Work is underway to attempt to explain the deviation from theory.

8-2

Dekkera bruxellensis wine strains are genetically diverse and exhibit differences in tolerance to the common wine preservative sulphite

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Yeasts of the genus *Dekkera* and its anamorph *Brettanomyces* are highly adapted to growth in wine, and are generally considered the foremost microbial spoilage issue facing the global wine industry. Despite this, there is limited knowledge of genetic diversity and strain distribution within wine and winery-related environments, and how intra-specific differences might influence success of winery control strategies – such as use of the common wine preservative sulphite. In this study, amplified fragment length polymorphism (AFLP) analysis was used to genotype 244 *Dekkera bruxellensis* isolates from red wine made in 31 winemaking regions of Australia. The results indicated there were eight genotypes amongst the isolates, with the degree of divergence exceeding that previously known for the *D. bruxellensis* species. Distribution of the major genotype (Genotype-I, 85% of isolates) across 29 of the regions studied suggested, however, that the highly selective wine environment has favored certain adaptations over others. Screening identified tolerance to sulphite as an adaptation that may contribute to this skewed distribution, as isolates from the Genotype-I group were able to tolerate significantly higher sulphite concentrations than isolates from the other major genotype groups. Whole genome sequencing has been undertaken to gain insight into the genetic determinants of this, and other, important adaptations to the wine environment.

8-3

Have we a symbiotic yeast here?

Diana Leemon

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Aethina tumida, the Small Hive Beetle (SHB) is a native scavenger of bee hives in South Africa where it is regarded as a minor pest. Larvae of these beetles cause extensive damage to honey frames, stored honey, pollen and brood when they feed and leave wastes behind. Resulting fermented honey is rejected by honey bees and cannot be marketed by the beekeeper. Heavy infestations may also result in hive death, queens ceasing to lay eggs or bees abandoning their hives. The small hive beetle was found in Australia in 2002, and a recent survey found that it is now causing major damage to Queensland bee hives. Current control options are limited; however QPIF is investigating different biological control strategies to target both the larval and adult stages. Overseas researchers have isolated a yeast (*Kodamaea ohmeri*) associated with the beetle that produces volatiles highly attractive to the beetle. This same yeast has been identified from at least one of the isolates taken from a beetle collected in Queensland. Isolations from many other beetles, the mucilage surrounding the beetle eggs and the “fermented slime” produced by the developing larvae in both the lab and hives have yielded a yeast of similar morphology. Further investigations have suggested that we may have a yeast that is crucial to the development of the larvae and is possibly a beetle symbiont. Future research is looking to show whether the yeast is present in all stages of the beetle, the identification of the yeast and whether it is a symbiont, with the view to looking for opportunities to use this information for novel control methods.

8-4

The ecology of *S. cerevisiae*

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Saccharomyces sp yeasts and humans have been in close association since the dawn of civilization as we have unwittingly employed these single celled fungi to make bread, beer and wine. Recently this association has become more sophisticate as *S. cerevisiae* is employed as a model research organism in cutting edge genetics and cell biology. As *S. cerevisiae* is the best characterized eukaryote it is now increasingly used as a model for evolutionary studies. Therefore, it is surprising that we know relatively of the general ecology and population biology of this species.

Recent work has uncovered the global population structure of *S. cerevisiae* and suggests that it may exist as a number of sub-groups that correlate with either geographic location or ecological niche. However, we have essentially no data regarding the roles that natural selection or dispersal have in shaping the *S. cerevisiae* species, nor its finer scale population structure.

I will present and discuss some of the Goddard lab's recent work regarding the ecology of *S. cerevisiae*.

Session 9. Molecular Biology: Gene Expression
Chaired by **Thomas Preiss** (Victor Chang Cardiac Research Institute)

9-1

What the length of the poly(A)-tail tells us about RNA metabolism

Traude Beilharz, Meghna Sobti, Jeni Yuan and Thomas Preiss.

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The addition of a poly(A)-tail to mRNA promotes its export from the nucleus and the recruitment of ribosomes for protein synthesis. The physical interaction of translation initiation factors bound to both the 5' cap structure and the 3' poly(A)-tail stimulates protein translation in a mechanism that is generally thought to include formation of a closed-loop mRNA configuration. The poly(A)-tail is deadenylated over an mRNA's life-time at a rate that correlates with function. Translation is proportional to the length of the poly(A)-tail. A transcript that is slowly deadenylated is a template for many rounds of protein translation, whereas a rapidly deadenylated transcript results in the synthesis of fewer protein molecules. Using affinity tagged-ribosomal proteins we show that a GFP reporter that is slowly deadenylated after a transcriptional pulse is three times more efficiently co-purified with ribosomes than a rapidly deadenylated GFP reporter. We propose that this difference in ribosome association stems from different extents of the closed-loop which is favoured by a long poly(A)-tail. We are directly testing this hypothesis by protein foot-printing.

On the other hand, non-coding transcripts may also carry a poly(A)-tail. Addition of a poly(A)-tail to some non-coding transcripts, such as structural RNA and cryptic unstable transcripts can result in their degradation. Others are stable, exported from the nucleus and co-fractionate with polysomes. To differentiate these two types of non-coding RNA we have hypothesised that the latter, stable variety will depend on the cytoplasmic deadenylase Ccr4 for poly(A)-tail shortening and thus accumulate poly(A)-tails of intermediate length, as seen with mRNAs in *ccr4-1* mutants. To test this hypothesis and to discover how prevalent such non-coding RNA are in the cytoplasm, we have fractionated adenylated RNAfrom wild-type and *ccr4-1* mutant cells based on the length of the poly(A)-tail using poly(U) chromatography. The composition (coding and no-coding) of short and long-tailed RNA fractions are currently being analysed by next generation sequencing.

9-2

The Ccr4-Pop2 mRNA deadenylase regulates morphogenesis in the human fungal pathogen *Candida albicans*

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Posttranscriptional mechanisms of gene expression play important roles in differentiation and development in lower and higher eukaryotes. The fungal pathogen *Candida albicans* undergoes developmental transitions from yeast to hyphal morphology and biofilm growth and this is essential in pathogenesis. Here we establish that the Ccr4-Pop2 mRNA deadenylase, an exonuclease which shortens mRNA poly(A) tails to regulate transcript stability and translation, is required for morphogenesis and biofilm formation in *C. albicans*. *ccr4* and *pop2* mutants present as big and round cells in yeast form, are prone to lysis and flocculate in culture. The mutants are sensitive to cell wall and cell membrane disrupting agents, are unable to undergo hyphal differentiation in response to serum and nutrient limitation and form defective biofilms. These phenotypes resemble *C. albicans* mutants in the RAM (regulation of Ace2 transcription factor and polarized morphogenesis) network, which controls cell wall and membrane biogenesis and filamentation. Transcriptome wide analysis showed that the RAM gene *CBK1* (cell wall biosynthesis kinase 1) is down-regulated in the absence of Ccr4-Pop2, whereas cell wall and membrane biogenesis genes are up-regulated possibly as a feedback mechanism to compensate for wall and membrane defects. The transcriptome profile of *ccr4* mutants is similar to cells with deficiency in the wall component beta-glucan and consistently the mutants have lower beta-glucans in their wall. Finally, the transcriptome wide profile of *ccr4* mutants indicates that the compensatory up-regulation of wall biosynthesis genes could be resulting from modulation of the Nrg1 transcriptional repressor.

9-3

Cell-cycle sensing of oxidative stress in *Saccharomyces cerevisiae* by oxidation of a specific cysteine residue in the transcription factor Swi6p.

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Yeast cells begin to bud and enter S phase when growth conditions are favourable during G1 phase. When subjected to some oxidative stress, cells arrest at G1 delaying entry into the cell cycle allowing repair of cellular damage. Hence, oxidative stress sensing is coordinated with the regulation of cell cycle. We identified a redox-sensing cysteine residue in the cell-cycle transcriptional regulator of *Saccharomyces cerevisiae*, Swi6p, at position 404. Mutation of Cys404 to Ala abolished the ability of the cells to arrest at G1 upon treatment by lipid hydroperoxide. In a truncated form of Swi6p, the Cys404 residue was oxidised when cells were subjected to the oxidant. Microarray analysis revealed that mutation of Cys404 to Ala alleviated the wild-type suppression of the G1 cyclins Cln1p and Pcl1p when cells were exposed to lipid hydroperoxide. Deregulation of the G1-cyclins led to transition of cells from G1 to S-phase with emergence of buds despite the presence of oxidative stress. However, regulation for DNA replication was unaffected in the *C404A* mutant indicating the control for the S-phase events may be uncoupled in the mutant. In conclusion, oxidation of Cys404 serves as a molecular sensor of oxidative stress and inhibits entry into the cell cycle by suppression of G1-cyclin expression.

9-4

Title – to be advised

Alex Andrianopoulos

University of Melbourne, Australia.

POSTER PRESENTATIONS

P–1

Aromatic alcohol production by the wine related yeasts species *S. cerevisiae, H. uvarum* and *M. pulcherrima* and influence of aromatic alcohols on cell morphology.

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The yeast flora present on grapes is often dominated by species of *Hanseniaspora* and *Metschnikowia.* Interactions between yeasts and grapes are not well understood and thus little is known about why these yeast species dominate the grape berry surface. The reason may be that the species can adapt to a low nutrient environment and/or produce biofilms and thus adhere to the surface.

S. cerevisiae, though often not detected in vineyards it is important in winemaking, can change cellular morphology from unicellular to filamentous growth in response to low nitrogen availability. This change in morphology has been shown to be stimulated by the presence of the aromatic alcohols, phenyl ethanol and tryptophol. Phenyl ethanol and tryptophol are derived from amino acid catabolism in yeasts and are produced in higher quantities when nitrogen availability is low.

This study was initiated to investigate the production of aromatic alcohols by wine-related yeasts and to study how the presence of aromatic alcohols affects yeast morphology. Single- and mixed culture fermentations with varying ammonium concentrations were performed with *S. cerevisiae, H. uvarum* and *M. pulcherrima* to assess aromatic alcohol production. Morphological response of wild strains of *S. cerevisiae, H. uvarum* and *M. pulcherrima* to aromatic alcohols was investigated by subjecting the yeasts to aromatic alcohols in nitrogen limited environments.

P–2

Assessment of the total yeast diversity in a spontaneous Chardonnay ferment using 454-sequencing technology

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Spontaneous wine ferments exhibit a great diversity of yeasts, usually dominated in the beginning by non-*Saccharomyces* species and then later the ferments are taken over by *Saccharomyces* species. So far the estimates of diversity have relied on culture-based methods , where typically only some tens to hundreds of colonies are examined. Using 454-sequencing technology for 26S DNA assessment of total DNA extracted from each day of a natural ferment, an estimate of the total diversity of yeasts that are present in the ferment may be made. This will include species that may not be culturable, and will analyze tens of thousands of samples. Furthermore 454-sequencing allows parallel sequencing of different samples in one run due to so called tags or keys attached to the primer sequences for distinguishing between samples. Here, four spontaneous Chardonnay ferments from the Hawke's Bay region in New Zealand are assessed for their yeast community dynamics during the ferment. Total DNA of ferment samples from every day of these samples has been isolated, the divergent domains 1 and 2 of the 26S region are amplified with specially designed primers for 454-sequencing and the PCR products are then sent for sequence analyses. Six different primer tag's were used to enable the parallel sequencing of six different samples.

P–3

Removal of ochratoxin A in *Saccharomyces cerevisiae* liquid cultures

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The capacity for removal of ochratoxin A (OTA) during alcoholic fermentation was evaluated in batch system with one commercial strain and one wild strain of *Saccharomyces cerevisiae*.

Batch alcoholic fermentations were carried out in Yeast extract-malt extract broth (YM) medium with 18.0% glucose and OTA added to final concentrations of 3.48 and 4.95 ng/mL. The removal capacity of each yeast strain was examined after completion of fermentation in batch culture and after extended contact with yeast biomass. Removal capacity of yeast strains was examined in stationary phase cultures as well. Stationary phase yeast were studied with biomass harvested from the stationary phase of anaerobic fermentation, by incubation in phosphate buffer, with the addition of 5.00 ng/mL of OTA. Removal studies with stationary phase cells were performed with viable and non-viable cells inactivated with Na-Azide.

The study showed that in growing phase cultures, OTA removal was significant only after extended contact with yeast biomass; up to 29.7% and 25.4% for wild yeast ZIM 1927 and commercial yeast Lalvin EC-1118 respectively, but not during alcoholic fermentation. In stationary phase cultures, viable and non-viable cells were not significantly different in OTA removal from the medium. This demonstrated that OTA was not metabolized, but possibly adsorbed by the yeast cells. The presence of OTA in synthetic media had influence on yeast metabolism, causing higher volatile acidity production, by 0.08 and 0.13 g/L for Lalvin EC-1118 and ZIM 1927 respectively, and lower concentration of reducing sugar, by 0. 32 g/L, only for ZIM 1927.

P–4

Predicting the sensory impact of wine fermentation practices through targeted and non-targeted analyses of volatile aroma compounds

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Wine aroma and flavour is influenced by variations in fermentation practices, such as the choice of different fermentation yeast and the use of nutrient supplements to improve yeast performance. Targeted analyses of volatile aroma compounds have demonstrated that the sensory impact of such variations in practices can be partly explained by relative concentrations of acetate- and ethyl-esters, higher alcohols, and volatile fatty acids. Other classes of compounds containing sulphur, including polyfunctional thiols and mercaptans, have also proven to be important in driving sensory outcomes. Nonetheless, it remains difficult to predict the outcomes of new wine fermentation practices due to the highly variable fermentation substrate – grapes. Accounting for this variability requires a simultaneous scaling-up of experiment size, and scaling-down of fermentation size. Neither is compatible with formal sensory and targeted analyses of >50 volatile aroma compounds, due to the sample sizes required and the cost of conducting such analyses. In this study we compared various metabolomic approaches to this problem, with the aim to develop strategies for prediction of sensory outcomes from laboratory scale wine fermentations.

P–5

Effect of cold temperature fermentation on aroma compound production and gene expression in wine yeast

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Winemakers' report that white wines fermented at cold temperatures (10-18°C) are fruitier, with the current hypotheses attributing this character to changes in the balance of yeast-derived esters, decreased evaporation, or decreased production of compounds that reduce ester perception. Previous studies have obtained variable results regarding higher concentrations of aroma compounds in cold-fermented wines, with both yeast- and juice-specific effects.

This research examined the effect of cold fermentation on aroma production and gene expression of the commercial wine strain, Enoferm M2, in Sauvignon Blanc grape juice at 12.5°C and 25°C.

The cold-fermented wines had lower concentrations of aroma compounds (thiols, acetate esters, higher alcohols, fatty acids and ethyl esters), with the decreases in specific acetate and ethyl esters being proportional to that of their corresponding higher alcohols and fatty acids. These decreases suggest that cold temperature induces changes in upstream substrates and a global remodelling of central metabolism, rather than differential expression of genes responsible for ester biosynthesis.

Gene expression data showed that the genes most influenced by cold temperature during wine fermentation had roles in nutrient biosynthesis and transport, especially sulfur, iron and copper pathway genes. An additional down-regulation in lipid biosynthetic genes suggests that these differences in nutrient metabolism may be caused by altered membrane permeability or reduction in transport efficiency, which would also affect the flows into central metabolism.

Specific analysis of known aroma pathway genes showed that only five transcripts were down-regulated at cold temperature (*BAP1*, *CHA1*, *EEB1*, *PDC6* and *POT1*) and all changes were less than two-fold. These small changes are consistent with low aroma compound concentrations being a result of a shift in central metabolism, rather than cold regulation of aroma pathways *per se.* Work is underway to determine whether the cold-fermented wines taste fruitier using sensory analysis.

P–6

Transcription factor control of growth rate dependent genes in *Saccharomyces cerevisiae*: a three factor design

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Background
Characterization of cellular growth is central to understanding living systems. Here, we applied a three-factor design to study the relationship between specific growth rate and genome-wide gene expression in 36 steady-state chemostat cultures of *Saccharomyces cerevisiae*. The three factors we considered were specific growth rate, nutrient limitation, and oxygen availability.

Results
We identified 268 growth rate dependent genes, independent of nutrient limitation and oxygen availability. The transcriptional response was used to identify key areas in metabolism around which mRNA expression changes are significantly associated. Among key metabolic pathways, this analysis revealed *de novo* synthesis of pyrimidine ribonucleotides and ATP producing and consuming reactions at fast cellular growth. By scoring the significance of overlap between growth rate dependent genes and known transcription factor target sets, transcription factors that coordinate balanced growth were also identified. Our analysis shows that Fhl1, Rap1, and Sfp1, regulating protein biosynthesis, have significantly enriched target sets for genes up-regulated with increasing growth rate. Cell cycle regulators, such as Ace2 and Swi6, and stress response regulators, such as Yap1, were also shown to have significantly enriched target sets.

Conclusions
Our work, which is the first genome-wide gene expression study to investigate specific growth rate and consider the impact of oxygen availability, provides a more conservative estimate of growth rate dependent genes than previously reported. We also provide a global view of how a small set of transcription factors, 13 in total, contribute to control of cellular growth rate. We anticipate that multi-factor factorial designs will play an increasing role in elucidating cellular regulation.

P–7

Further characterisation of an adaptively evolved wine yeast strain

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We have used adaptive evolution as a means of developing optimised wine yeast. Employing a sequential batch fermentation system allowed for the cultivation of populations of yeast for many generations under suboptimal conditions where a multi-stressor environment were imposed. The adaptively evolved strain FM16-C7 was generated utilising these techniques and demonstrates more efficient fermentation of sugars under suboptimal conditions than its parent. We are currently employing a number of techniques (genomic sequencing, transcriptomics, metabolomics and phenotype arrays) to further characterise this strain in order to gain insight in to the mechanism of shortened fermentation duration as well as to better understand what other phenotypic changes may have occurred.

P-8

Mapping the metabolic inputs of 'Brett' taint

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'Brett' taint, or the accumulation of spoilage compounds due to the presence of *Brettanomyces* and *Dekkera* yeast is widely accepted to occur via the enzymatic breakdown of *p*-coumaric and ferulic acids to give 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol and 4-vinylguaiacol. However, with other hydroxycinnamic acid conjugates present in the grape berry, can it be assumed that *p*-coumaric and ferulic acids are solely responsible for the evolution of 'Brett' taint compounds?

In addition to the hydroxycinnamic acids, the presence of esterified conjugates have also been confirmed in the grape berry in the form of glucose and tartaric acid esters, as well as a number of anthocyanin bound glucose esters. This work into alternative grape derived metabolic inputs to 'Brett' taint, has already shown that acetylated glucose esters of the hydroxycinnamic acids in fermentation experiments with *Brettanomyces* are metabolised to give 4-ethylphenol and 4-ethylguaiacol.

Conversion rates for the acetylated glucose esters are as high as 20% with a single strain and 42% with a co-innoculation, compared with 52% and 65% respectively for the free acids. These results confirm that the potential for 'Brett' taint goes beyond the hydroxycinnamic acids, and extends to the aforementioned conjugates.

Understanding all of the metabolic inputs present in the grape berry would give adequate information to be able to pre-determine the potential for the production of Brett taint in the resulting wine. This would require confirmation of potential precursors in the presence of *Brettanomyces* and *Dekkera*, as well as a sufficient method of quanitification once confirmed.

P–9

Enhancing yeast ethanol tolerance for biofuel production

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The decrease of fossil fuel availability has created a high demand for alternative fuels, including bioethanol produced by yeast fermentation of carbohydrate. Relatively low yields of ethanol can be a major problem in this bioconversion. Approaches to increase efficiency in the fuel ethanol industry include improving yeast metabolic flux and fermentation rate and selection of yeast with higher ethanol tolerance. This study has an alternative approach, aiming to enhance ethanol tolerance of several strains of *Saccharomyces cerevisiae* through modification of growth medium composition. It focuses specifically on two important components which have been shown to positively affect yeast stress tolerance; inositol and L-proline. Various concentrations of these compounds are being added to laboratory scale fuel ethanol fermentations to determine levels that potentiate the highest ethanol tolerance and productivity of the yeast.

The authors would like to acknowledge the support of the Ministry of National Education of the Republic of Indonesia, for provision of a postgraduate scholarship to SI.

P–10

Saccharomyces cerevisiae genes involved in membrane stability modulate accumulation of flavour compounds during fermentation

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Flavour production in white wines is mediated by the yeast *Saccharomyces cerevisiae*, which releases volatile aroma compounds from odourless precursors in grapes and produces secondary metabolites consisting primarily of esters, higher alcohols and carboxylic acids. In an ongoing search for novel flavour-active genes we examined the transcriptomes of two commercial wine yeast with significantly different flavour profiles. Gene ontology searches were conducted upon genes exhibiting greater than 2-fold differences in transcript level between the strains, with most common ontologies involving transport, metabolism and biosynthesis of amino acids, fats, sterols and fatty acids. Selected deletion strains from the Euroscarf collection were screened for impact upon volatile aroma compound formation, revealing a subset of genes involved in membrane stabilisation that significantly reduced the concentration of several higher alcohols and esters when absent. Constitutive expression of these genes in a multi-copy plasmid enhanced accumulation of these compounds, providing strong evidence for their role in modulating 'flavour'. These results have lead us to pursue the influence of membrane composition on flavour compound production and release from the yeast cell.

P-11

Commercial yeast inoculation influences consumer preference and shelf life of Sauvignon Blanc wines

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Alcoholic wine fermentation using specific *Saccharomyces cerevisiae* wine yeast strains is an effective means of enhancing wine aroma, through modulation and production of hundreds of volatile aroma compounds. Different wine yeast strains have been shown to modify the concentration of some key flavour compounds of Sauvignon Blanc wines, notably volatile thiols, and give rise to different wine aroma profiles. The questions remain as to whether the wine aroma changes brought about by yeast strains are sufficient to affect wine quality as perceived by consumers (1), and whether these changes are persistent in wine over time (2).

Sauvignon Blanc wines were made using different yeast strains, including two- and three-yeast co-inoculations and single-strains. Wines were analysed for their chemical composition and sensory profiles. To answer the first question (1), wines from four yeast inoculums which showed large sensory differences were subjected to consumer testing by 120 consumers, with differences in overall liking found. Four clusters of consumers were identified, with one group strongly preferring the two-yeast co-inoculated wine with an intermediate sensory profile, while another group most liked the wine made using the three-yeast co-inoculation.

For the second question, wines made with different yeast strains from two consecutive vintages (2005 and 2006) were cellared for three-years in glass bottles under screw cap closures, prior to analysis. The results showed that there were differences among both sets of wines in the chemical and sensory profiles. Wines made using strains that showed high initial concentrations of volatile thiols and continued to show greater levels of thiol-related sensory attributes after three years in bottle.

Overall, the study has shown that the yeast strains used to conduct fermentation cause sufficient flavour differences to affect consumer preference, and that even after extended storage the sensory effects are retained.

P–12

Breeding cold tolerant wine yeasts

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A breeding programme has been underway at the University of Auckland to produce improved wine yeast strains (*Saccharomyces cerevisiae*) that are better able to tolerate fermentation at cold temperatures. The goal of the cold tolerance breeding is to obtain novel strains that can produce Sauvignon Blanc wines with superior qualities - in particular wines with higher production of fruit aromas and lower production of sulfur off-flavours. We are using a non-GM approach that involves inter-mating of parent yeast strains followed by selection of progeny with improved growth and fermentation ability at low temperatures. The two most promising new yeasts have been dried down and underwent commercial trials in the 2009 vintage.

P–13

The role of yeast in the generation of the powerful odorant damascenone in wine

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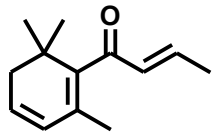
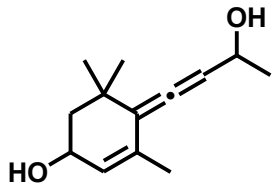
β-damascenone, **1** is an important flavour and aroma compound with a low aroma detection threshold of 2 ng/L in water and 50 ng/L in model wine. It has a characteristic fruity 'stewed apple' aroma and has been identified in many different foods and beverages such as tobacco, coffee, honey, tomatoes as well as in alcoholic beverages such as wine.



1

We have shown previously that damascenone forms under acidic hydrolysis conditions from the allenic diol **2**. It is the origin of this diol **2** which forms the basis of this work but it is the role of yeast in the generation of damascenone that forms the focus of this project.

A variant of *Saccharomyces cerevisiae* has long been used as a chemical reagent to reduce carbonyl functional groups. The ketones **3** and **4** were chosen as potential precursors of diol **2** as it was felt that, under fermentation conditions, these substrates would be metabolised by the yeast and would be reduced to give the diol, which ultimately produces damascenone.



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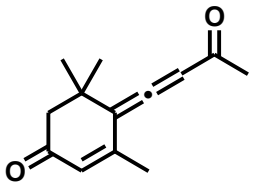

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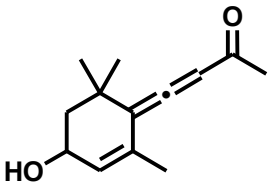

4

This study investigates the generation of damascenone under fermentation conditions from the precursor forms and also looks at the fate of damascenone under fermentation conditions. The biotransformation of the precursors **3** and **4**, during model fermentations will be presented, along with some fermentation studies investigating the action of yeast on damascenone.

P–14

Selection of yeast strains for optimal fermentation of Queensland Verdelho wines

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Verdelho is a variety for which Queensland is becoming well known. The objective of this pilot project was to trial commercially available yeasts for optimal development of regional character of Queensland Verdelho wines. Three commonly used white wine yeasts (EC1118 and QA23 from Lallemand, VL3 from Laffort Oenologie), two new hybrids (AWRI 1502 and AWRI 1503 from Mauri Yeast Australia) and a co-inoculation (VIN13 and NT116 from Anchor Yeast) were used to prepare small experimental batches of wine from Granite Belt Verdelho grapes. At the end of fermentation, most strains resulted in residual sugar of 3 to 4.5 g/L although VL3 and AWRI 1503 seemed to become stuck at significantly higher levels (17.8 and 12.9 g/L respectively). This led to production of around 0.6% less ethanol and seems to reflect problems with fructose assimilation, as most of the residual sugar was fructose. Whether this is related to the small fermentation volumes remains to be determined. When the wines were presented to winemakers and judges for sensory evaluation, feedback on all wines was positive. The scores from the 20 point scoring system were statistically similar. Wines were evaluated at bottling and 6 months later, and seem to have improved with some time in bottle. While some sensory differences were noted, the standard show judging criteria used for evaluation did not help to differentiate the wines. Further trials will be undertaken with larger ferment volumes, enabling sufficient wine to be produced for trained panel sensory evaluation for further discrimination of sensory profiles.

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P–15

Characterization of yeast communities in spontaneous New Zealand Pinot Noir fermentations

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The species and natural succession of yeasts in spontaneous Pinot Noir fermentations in New Zealand is completely unknown. Spontaneous ferments are conducted by a succession of non-*Saccharomyces cerevisiae* yeast before *Saccharomyces sp* completes the fermentation. This work has determined the various yeast species present in Pinot Noir fermentations from four key areas in New Zealand (Central Otago, Marlborough, Martinborough and Kumeu). We used molecular methods (by analyzing the Internal Transcribed Spacer region (ITS) of the ribosomal repeats and the divergent domains 1 and 2 of the 26s Ribosomal subunit) to enumerate and characterize 96 random isolates from freshly crushed juice from each region. DNA was extracted from single colonies in order to initially analyze the ITS region, employing the Polymerase Chain Reaction (PCR). Ultimately the DNA sequence of the ITS region was determined in order to unambiguously identify each isolate to species level. A minimum of 13 and a maximum of 16 different species have been identified thus far, including *Candida albicans, Candida zemplinina, Hanseniaspora uvarum, Issatchenkia terricola*, *Lachancea thermotolerans, Metschnikowia chrysoperlae, Metschnikowia pulcherrima, Pichia anomala, Pichia fermentans, Pichia kluyveri, Saccharomyces boulardii, bayanus, cerevisiae*, and *uvarum, Torulaspora delbrueckii, Unidentified Metschnikowia.* The Kumeu ferments were followed and their population dynamics determined until the ferment was complete. The identified yeast will then be used to conduct micro-fermentations with thermovinified and sterilized Pinot Noir juice in order to identify any strains that may be of commercial interest. Any positive contributions to final wine aroma and flavour will then be determined by an experienced panel of judges.

P–16

A spontaneous commercial Marlborough Savignon Blanc un-ravelled

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New Zealand Savignon Blanc (SB) is world famous for its sharp and distincitve aromas. This wine is usually characterised by high levels of volatile sulfur compounds called thiols which impart passionfruit/grapefruit notes to the wine. Kumeu River (KR) winery consistantly produces unique high quality SB every vintage, one big difference between this winery and other major producers is that Kumeu River Sauvignon blanc ferments are spontaneous. Spontaneous fermentaion of wine is carried out by natural isolates of many different species and/or strains of yeast occuring in the juice that possibly interact with one another to produce wine of complex character. Therefore the question is: what are the yeast species and or species-interactions that is making spontaneous SB ferment at KR unique yet retaining typical NZ SB characters?

To answer the above question, the vintage of 2008 was followed closely; SB must and wine samples were taken before, during and at the end of ferment for analysis. Change in the natural yeast community diversity was monitored using standard molecular biology techniques along with tracking the evolution of different compounds like thiols, esters and other soluable metabolites which are characteristic of New Zealand SB. The aim of this analyses was to obtain patterns of change in yeast diversty and co-relate it to change in levels of impact compounds thus underpining key changes during a ferment that drives Kumeu River SB wine quality.

Molecular biology techniques unveiled approximately 16 different species of yeast. The dominant yeast that drove the fermentation of sugars to ethanol were natural isolates of *Saccharomyces. bayanus* which is in sharp contrast to most inoculated ferments which use *S.cerevisiae*.*S.cerevisiae* were very rare in this ferment. Chemical analyses of aroma compounds revealed unusually high amounts of 4-mercapto-4-methylpentan-2-one (4MMP) in the wine compared to other Marlborough SB, yet the levels of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were about average for this varietal. There was a significant shift in the quantity and type of soluable metabolites during fermentaion. Evolution of key aromas co-incided with increase in temperature of fermentation, increased consumption of sugars and prevalance of *S.bayanus* as the dominant yeast in the fermentation. Previous studies have shown that *S.bayanus* strains are capable of producing more 4 MMP compared to their *S.cerevisiae* counterparts (1). This could explain the high amounts of 4 MMP seen in the wine which added uniqueness yet the 3MH and 3MHA levels maintained the distinctiveness of a Marlborough SB.

(1) Dubourdieu et al. (2006) Am. J. Enol. Vitic. 57:81-88.

P–17

Molecular and physiological characterisation of a fission yeast isolate used in industrial ethanol production.

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Concern regarding the use of fossil fuels contributing to global warming has led to exploration of renewable fuel sources. Indeed, bioethanol production from plant biomass hydrolysates, such as molasses, is now an important fossil fuel alternative. CSR Ethanol (Australia) produces approximately 60 million litres of bioethanol annually from the fermentation of molasses in a process that uses an uncharacterised fission yeast isolate. To better understand the CSR Ethanol fission yeast and identify possible areas for improving its industrial performance we have performed an initial molecular and physiological characterisation of the isolate. Sequencing of the rDNA ITS regions identified the CSR industrial strain as a *Schizosaccharomyces pombe* isolate that shows divergence from the standard *S. pombe* laboratory strain. Growth and morphological analysis showed that the industrial isolate had increased resistance to a variety of stresses including high temperature and the DNA damage drug hydroxyurea. Preliminary fermentation data demonstrated that the CSR Ethanol fission yeast isolate grew quicker on molasses and showed a higher overall ethanol yield compared to the standard *S. pombe* laboratory strain. Overall, this study highlighted areas where optimisation may lead to an improved industrial fermentation processes and established a new experimental system in which to study the regulatory and metabolic pathways important for the industrial fermentation of sugars to ethanol in fission yeast.

P-18

Identification of wild type *Saccharomyces bayanus* strains from New Zealand vineyards and wineries

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Saccharomyces bayanus is a species that occurs in grape juice and wine and is an excellent fermentor at low temperatures – cold-fermenting lager yeasts are a hybrid between *S. cerevisiae* and *S. bayanus*. We are interested in isolating strains that would be useful in low temperature wine fermentations. Grape juice and wine samples were collected from wineries in the Marlborough region of New Zealand at different times during the wine making process, including post harvest and during uninoculated fermentations. Samples were cultured on YPD and colonies identified using RFLP and sequencing of the ITS region. To further characterise the Saccharomyces bayanus colonies we sought to develop a DNA fingerprinting approach using microsatellites. Microsatellite repeats were selected from the *S. bayanus* genome sequence (CBS 7001) using a new bioinformatics programme to identify polymorphic loci. Primers for ten loci were selected and combined in a multiplex PCR reaction. Although some strain identification was possible, the results overall were disappointing - only a small number of alleles were detected for each locus, and the alleles did not appear to differ in size as a result of variation in the number of tandem repeats. Selected strains of *S. bayanus* have been analysed and show wide phenotypic variation in terms of sulfite tolerance and fermentation ability. Eight individual Marlborough strains were crossed with ACY 338, a genetically marked strain derived from the sequenced *S. bayanus*, and tetrads from the F1 hybrids were dissected. Two hybrids were sterile, with less than 5% viable progeny, and may represent polyploids or have some other incompatibility with *S. bayanus*. However, four strains showed at least 75% viability of the F2 progeny, and gave rise to tetrads that segregated 2:2 for the genetic markers. These strains can therefore be unambiguously classified as *S. bayanus*.

P–19

Understanding the mechanisms which allow *Candida apicola* to grow in an Italian wine with high sugar and ethanol (Vino cotto).

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Vino cotto is a wine produced in Central Italy (Marche and Abruzzo regions) after a prolonged fermentation (> 45 days) of cooked grape must. After cooking, where the grape must is heated to 95°C for 48 hours, the must becomes dark and reaches a high sugar concentration (55%). After fermentation, the ethanol concentration is generally 15% w/v. The activity of osmotolerant non-*Saccharomyces* yeasts in these fermentations is considered important for the final aromatic profile of wines. The aim of this study is to examine two different strains of *Candida apicola* isolated from a vino cotto fermentation, *C .apicola* 480 and *C. apicola* 201, for their different behaviours in relation to resistance to osmotic stress and ethanol concentration. We analysed the physiological response of these two yeasts under different growth conditions by varying sugar concentration (glucose, fructose and sucrose at 2%, 20%, 40%, 60% w/v), ethanol (0%, 8%, 14%, 20%) and glycerol (0%, 2%, 7%, 12%). We found different growth profiles between these two strains; *C. apicola* 201 can tolerate up to 60 % of glucose and fructose while *C. apicola* 480 tends to be inhibited at 20% glucose and fructose. *C.apicola* 480 can grow in 20% ethanol while *C. apicola* 201 can only tolerate 8% ethanol. The two strains show a similar response when glycerol and sucrose concentrations are varied. We aim to understand the molecular mechanisms which allow *C. albicans* 201 and 480 to grow in high sugar must. Future work includes analysis of the presence, structure and function of gene HGT4 (*C. albicans),* encoding a high-affinity glucose sensor that may play a role in efficient sugar transport. The present work hopes to describe new yeast species which may be used as novel starter cultures for the production of Vino cotto.

P–20

Regulation of sulfur metabolism during wine fermentation by nutrient supplements

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Volatile sulfur compounds play a primary role in wine aroma. Many sulfur compounds are associated with negative descriptors, whereas some are regarded to contribute positive aromas to wine. Hydrogen sulfide (H_2S), a highly volatile compound which imparts a 'rotten egg' aroma, is considered a major off-flavour in wine due to its low aroma threshold. During fermentation H_2S is formed by yeast as an intermediate in the biosynthesis of the sulfur containing amino acids cysteine and methionine. This pathway involves the reduction of sulfate (the most abundant sulfur source in grape must) into sulfide and incorporation of sulfide into an amino acid precursor. Insufficient biosynthesis of the amino acid precursor leads to the liberation of H_2S. Since the amino acid precursor is derived from nitrogen metabolism, H_2S formation during wine fermentation is closely linked to the nitrogen content of grape must. In this study, we have tested the effect of different yeast nutrients on sulfur metabolism. Nutrient sources included a commercial organic yeast nutrient preparation and a source of inorganic nitrogen in the form of diammonium phosphate, which is a commonly added nutrient during wine fermentation. Our results showed clear effects of the nutrient source on production of H_2S. In addition, gene expression profiling revealed nutrient-specific regulation of genes involved in sulfur metabolism pathways that was not linked to the nitrogen content of the medium. Further research is needed to determine the role of nutrients in sulfur metabolism and H_2S production.

P–21

Ionomic profiling of *Saccharomyces cerevisiae* under oxidative stress conditions

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Following the example of genomics, transcriptomics, proteomics and metabolomics, the total composition of metal and non-metal ions in an organism has been coined as 'ionomics'. Since 'ionome' homeostasis is required for an array of biological functions like transport signalling, enzymatic activity and stress responses, the ion composition in an organism is critical to its physiological function and survival. To better understand the changes that oxidative stress cause in the cellular ion profile, inductively-coupled plasma optical emission spectrometry (ICP-OES) was used to measure the element content in *Saccharomyces cerevisiae* treated with hydrogen peroxide, cumene hydroperoxide, linoleic acid hydroperoxide, a superoxide-generating agent (menadione), a thiol-oxidising agent (diamide, diazine-dicarboxylic acid-bis(dimethylamide), a thiol sulfenic group modifying agent (dimedone (5,5-dimethyl-1,3-cyclohexanedione), and a reactive nitrogen species (peroxynitrite). The levels of zinc, iron, copper, manganese, potassium and magnesium were significantly lower when cells were treated with some oxidants while aluminium, sodium and calcium were elevated by others. No general trends in differential ion composition were evident between the different oxidant treatments highlighting the specificity of cellular responses to different reactive oxygen species.

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