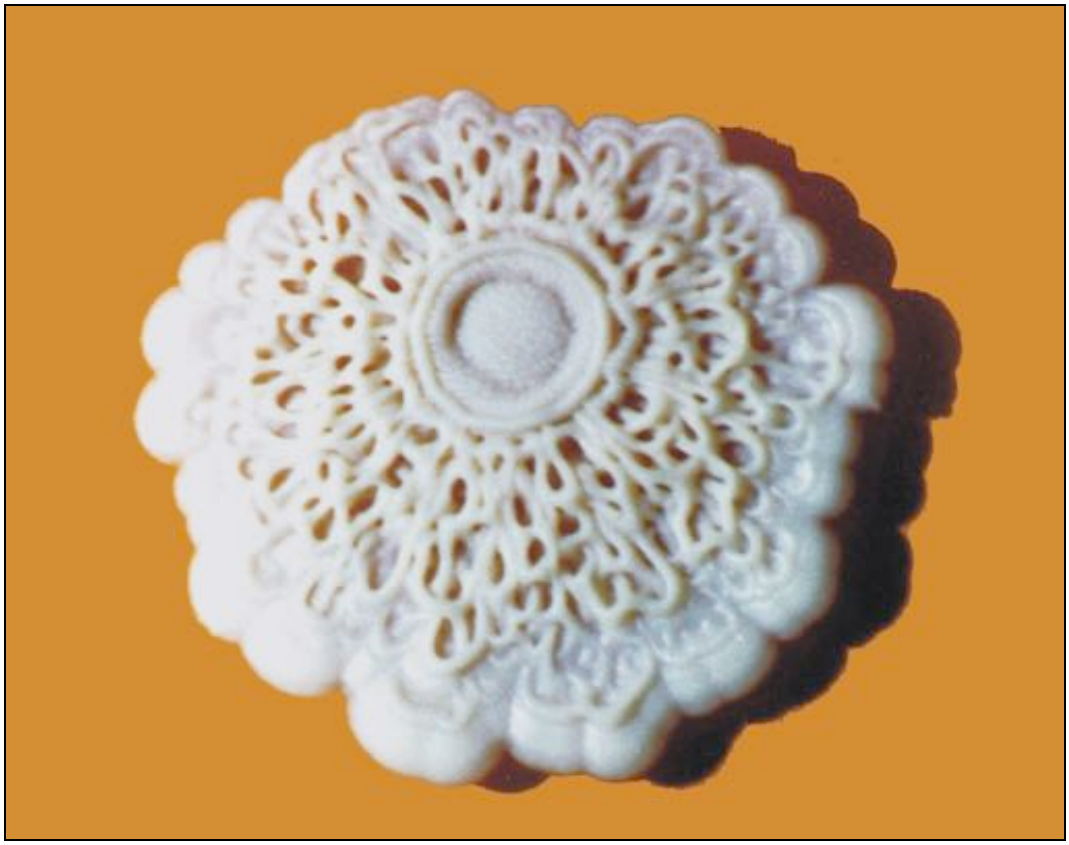
YEAST: PRODUCTS AND DISCOVERY



Proceedings of the 3rd Australian Conference on Yeast

The Vine Inn Conference Centre, Nuriootpa, Barossa Valley, South Australia, 4-6 April 2004.

Editors: Paul R. Grbin and Vladimir Jiranek

YEAST: PRODUCTS AND DISCOVERY

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http://www.australianyeastgroup.org/YPD/YPD_2004/

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Cover: *Saccharomyces bayanus* isolated from wine and cultured on YPD. Photograph by Jeff Eglinton, reproduced by permission from International Journal of Systematic Bacteriology [de Barros Lopes *et al.*, (1998) 48:279-286].

Welcome to the 3rd Yeast: Products and Discovery Meeting

Participants at the 2nd Yeast: Products and Discovery Meeting held at CSIRO, Parkville, Victoria from 27 – 29 November 2002.

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Australian Society for Biochemistry and Molecular Biology

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Yeast: Products and Discovery 2004

Vine Inn Conference Centre, Nuriootpa, Barossa Valley
Program: 4–6 April 2004

Saturday 3 April

15:00-18:00 Welcome at Coopers Brewery (sponsored by Coopers Brewery)

18:00-20:00 Coach travel to Vine Inn, Barossa Valley (optional)

20:00-22:00 Vine Inn - a la carte dinner menu (optional)

Sunday 4 April

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

9:10-10:40 **1. Yeast Cell Molecular Biology I**
Protein location, folding and assembly
Chaired by **John Wallace** (University of Adelaide)

Christina Mitchell (Monash University) - The role of the inositol polyphosphate 5-phosphatases in vesicular trafficking
Trevor Lithgow (University of Melbourne) - Targeting proteins to mitochondria
Alan Munn (University of Queensland) - The ins and outs of Vps4 - a conserved AAA-ATPase that buds vesicles into endosomes and viruses out of cells
Mayfebelle Reodica (University of New South Wales) - The contribution of mechanisms repressing meiosis and spore formation

10:40-11:00 Break

11:00-12:30 **2. Ecology and non-*Saccharomyces* yeast**
Chaired by **Graham Fleet** (University of New South Wales)

Graham Fleet (University of New South Wales) - The yeast ecology of wine grapes
Wieland Meyer (University of Sydney) - Has *Cryptococcus gattii* evolved into a more virulent genotype?
Xiaoming Zuo (Australian National University) - Isolation and identification of genes interacting with MGM101 in *Schizosaccharomyces pombe*
Sharon Guffogg (University of New England) - Stress response in novel antarctic yeast

12:30-13:30 Lunch

13:30-14:00 **XXIII International Conference on Yeast Genetics and Molecular Biology - Melbourne 2007**
Chaired by **Ian Macreadie** and **Ian Dawes**

14:00-15:30 **3. Yeast Cell Molecular Biology II**
Signalling and transport
Chaired by **Christina Mitchell** (Monash University)

Richard Gardner (University of Auckland) - Structure and function of the yeast *ALR1* magnesium transporter
Brent Kaiser (University of Adelaide) - Yeast assisted plant gene discovery
Brietta Pike (St Vincent's Institute) - Mdt1, a novel *Saccharomyces cerevisiae* protein, modulates DNA damage tolerance and G2/M cell cycle progression
Miles Barraclough (CSIRO-HSN) - Adaptive responses of *Saccharomyces cerevisiae* to the action of sulfa drugs

15:30-16:00 Break

16:00-17:30 **4. Biotransformations and bioproducts**
Chaired by **Peter L. Rogers** (University of New South Wales)

Peter Rogers (University of New South Wales) - Enzymatic biotransformation process for pharmaceutical production using pyruvate decarboxylase (PDC) from *Candida utilis*
Jean-Pierre Dufour (University of Otago) - A three-member gene family is responsible for the synthesis of short chain fatty acid esters during fermentation
Michael Patane (University of Western Sydney) - The development of a novel yeast based flavour enhancer
Daniel Cozzolino (AWRI) - Metabolic profiling using infrared (IR) spectroscopy

17:30-18:30 **Poster session (sponsored by Coopers Brewery)**
Chaired by **Paul Grbin** (University of Adelaide)

Monday 5 April

9:00-10:30 **5. Stress and aging**
Chaired by **Ian Dawes** (University of New South Wales)

Grant Stanley (Victoria University) – Ethanol, stress and working with yeast
Vince Higgins (University of Western Sydney) - The application of functional genomics to the study of industrial fermentation processes
Dewald van Dyk (Stellenbosch University) - The transcription factors Rme1p and Mss11p control invasive growth by modulating *FLO11* expression levels in *Saccharomyces cerevisiae*
Geoffrey Thorpe (University of New South Wales) - There is no such thing as a single form of oxidative stress

10:30-11:00 Break

11:00-12:30 **6. Brewers/bakers yeast**
Chaired by **Jon Meneses** (Coopers Brewery)

Paul Attfield (Macquarie University) - Monitoring heterogeneity - improving consistency of industrial yeasts
Philip Douglas (University of Adelaide) - Yeast strain mediated haze and filterability
Weidong Duan (RMIT) - Redox balance and the production of sulphite and sulphide by brewing yeasts

12:30-13:30 Lunch

13:30-15:00 **7. Yeast in medicine**
Chaired by **Ian Macreadie** (CSIRO-HSN)

Peter Iliades (CSIRO-HSN) - Employing model systems to investigate microbial drug resistance
Christine Hawkins (Murdoch Childrens Research Institute) - Modelling cell death pathways using yeast
Jan Swiegers (AWRI) - Engineering carnitine biosynthesis in *Saccharomyces cerevisiae* in order to increase its nutritional value

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Remainder of Sessions for Monday held at Yalumba Winery

15:00-15:30 Travel to Yalumba Winery

15:30-17:15 **Winery tour and tasting**
Hosted by **Geoff Linton**, **Louisa Rose & Simon Dillon** (Yalumba)

17:15-17:30 Break

17:30-18:45 **8. Wine yeast**
Chaired by **Paul Henschke** (Australian Wine Research Institute)

Louisa Rose (Yalumba) - Novel wine yeasts
Miguel de Barros Lopes (AWRI) - Wine yeast gene technology
Colin McBryde (University of Adelaide) - Adaptive evolution in enhancing wine yeast
Kate Howell (AWRI/University of New South Wales) - A genetic study to characterise the release of volatile thiols by *Saccharomyces cerevisiae*
Simon Dillon (Yalumba) - A quantitative chemical and sensory approach to characterising wine yeast for improved red wine colour and flavour

18:45-19:00 Break

19:00-23:00 **Dinner (sponsored by Yalumba Winery)** Yalumba Wine Room (includes wine and soft drinks) (optional)

Tuesday 6 April

9:00-10:30 **9. Yeast Cell Molecular Biology III**
Gene expression
Chaired by **Mary-Jane Gething** (University of Melbourne)

Jörg Heierhorst (St Vincent's Institute) - Budding yeast DNA damage response proteins as models for human cancer predisposition syndromes
Traude Beilharz (Victor Chang) - From transcriptome to proteome: the control of protein translation
Linda Palmisano (University of New South Wales) - The *LSM* gene set and its role in modulating splicing efficiency in the cell

10:30-11:00 Break

11:00-12:00 **10. Forum**
Chair by **John Wallace** (University of Adelaide)

Ian Dawes (University of New South Wales) - Fundamental yeast science. Opportunities and challenges
Isak Pretorius (AWRI) Bridging the gap between the idea and its transformation into commercial outcomes in yeast biotechnology

12:30-13:00 **Student prizes** (sponsored by ASBMB) and meeting close
Chaired by **Paul Henschke** (Australian Wine Research Institute)

13:00-14:00 Lunch
Vine Inn (optional)

14:00-16:00 Coach travel to Adelaide bus depot and airport (optional)

ORAL PRESENTATIONS

Yeast Cell Molecular Biology I - Protein location, folding and assembly
Chaired by **John Wallace** (University of Adelaide)

1–1

The role of the inositol polyphosphate 5-phosphatases in vesicular trafficking.

Christina A. Mitchell[1], Fenny Wiradjaja[1], Alan L. Munn[2], Peter Mayinger[3] and Lisa Ooms[1]

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[2]Institute for Molecular Bioscience, Queensland Bioscience Precinct, The University of Queensland, St Lucia, QLD 4072, Australia.
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Phosphoinositides are membrane lipids which regulate many essential cellular functions including cell growth and apoptosis, cytoskeletal rearrangements and membrane trafficking. Seven distinct phosphoinositide signaling molecules are generated by the actions of specific lipid kinases and phosphatases that add or remove phosphates from the inositol ring. Phosphoinositides maintain the identity of membrane domains and facilitate membrane trafficking events by acting as a binding site on the membrane bilayer for proteins that regulate either vesicle formation or fusion. The inositol polyphosphate 5-phosphatases hydrolyze the phosphoinositide PtdIns(4,5)P_2 forming PtdIns 4-P. *Saccharomyces cerevisiae* contains four 5-phosphatases, Inp51-4p, three of which (1-3) contain two distinct enzyme domains, a 5-phosphatase and a Sac-1 catalytic domain. In addition yeast express a distinct SAC-1 gene which functions as a polyphosphate phosphatase principally hydrolyzing PtdIns 4-P forming PtdIns. Yeast 5-phosphatases demonstrate overlapping but not identical roles in membrane trafficking events, organelle dynamics, and actin cytoskeletal regulation. Single and double null mutants of the three INP1-3 genes are viable, any double knockout shows defects in endocytosis, vacuolar morphology and actin polymerisation, and a triple knockout is lethal due to an accumulation of PtdIns(4,5)P_2. The fourth 5-phosphatase, Inp54p, has been implicated in the regulation of secretion from the endoplasmic reticulum. To delineate the role of PtdIns(4)P and PtdIns(4,5)P_2 in regulating intracellular trafficking pathways, the functional relationship between Sac1p and Inp54p was examined by creating a double deletion mutant ∆sac1∆inp54. Loss of both Sac1p and Inp54p did not affect the total cellular phosphoinositide levels relative to ∆sac1 cells, however, a reporter protein that specifically binds PtdIns(4,5)P_2 was mislocalized in both ∆sac1 and ∆sac1∆inp54 mutants to the vacuolar membrane. ∆sac1∆inp54 mutants exhibited characteristics of vps mutants, including delayed transport to the vacuole, both from endocytosis and the biosynthetic route, compromised vacuolar function, and abnormal vacuolar morphology. These studies demonstrate the accumulation of PtdIns(4,5)P_2 on the vacuole membrane resulting from loss of lipid phosphatase activity, results in defects in vesicular trafficking.

1–2

Targeting proteins to mitochondria

Trevor Lithgow

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We use *Saccharomyces cerevisiae* as a model organism in our studies of intracellular protein targeting. We are interested to understand how proteins are targeted to mitochondria and in particular how membrane proteins are assembled into functional units. Integral proteins in the outer membrane of mitochondria control all aspects of organelle biogenesis, being required for protein import, mitochondrial fission and, in metazoans, mitochondrial aspects of programmed cell death. How these integral proteins are assembled in the outer membrane had been unclear. We know that two complexes in the outer membrane, the TOM complex and the SAM complex, are involved in recognizing newly made proteins for translocation through the outer membrane or insertion into the outer membrane. The structures of these complexes and the mechanisms they employ are under investigation using a combination of yeast genetics, biochemical assays, bioinformatics and direct structural analyses. The genes encoding at least two components of these complexes are essential for life and conditional yeast mutants have defects that arise from compromised targeting and insertion of proteins to the mitochondrial outer membrane.

1–3

The ins and outs of Vps4 - a conserved AAA-ATPase that buds vesicles into endosomes and viruses out of cells

Alan L. Munn

Institute for Molecular Bioscience, Queensland Bioscience Precinct, The University of Queensland, St Lucia, QLD 4072, Australia.

The multivesicular body (MVB) is a late endosome with numerous internal (intralumenal) vesicles. The intralumenal vesicles are formed by invagination of the limiting endosomal membrane in a process which is poorly understood. Some membrane proteins are incorporated into the intralumenal vesicles while others are excluded and remain on the limiting membrane - a selective process known as MVB sorting. MVB sorting is important for the rapid removal of activated signaling receptors (e.g. receptor tyrosine kinases) from the cytoplasm and inactivation of their signaling before their eventual proteolytic destruction in the lysosome. Vps4 (vacuolar protein sorting 4) is the key enzyme that drives MVB sorting and has been highly conserved during evolution - exhibiting 70% amino acid sequence identity from yeast to human. Yeast *vps4* mutant phenotypes are efficiently corrected by expression of human Vps4 showing that the function of Vps4 is highly conserved. Recent studies have shown that a range of enveloped viruses including the AIDS virus HIV-1 hijack the host MVB sorting machinery and use it to create new viral envelopes at the cell surface. Expression of HIV-1 surface glycoprotein (Gag) in yeast has recently been reported to cause efficient budding of empty viral envelopes containing HIV-1 Gag from the yeast cell surface suggesting these mechanisms are conserved in yeast. However, very little is known of how Vps4 functions and in particular how it cycles on and off intracellular membranes during MVB sorting. This talk will describe recent progress in understanding Vps4 through looking at its interactions with other proteins and the role of ATP binding and hydrolysis in regulating these interactions.

1–4

The contribution of mechanisms repressing meiosis and spore formation

Mayfebelle Reodica, Melissa J. Straffon and Ian W. Dawes

School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia.

For organisms undergoing developmental processes it is ideal that specific genes are repressed as well as induced at the correct time and to the correct level in a coordinated manner. The process of meiosis and spore formation (collectively known as sporulation) in *Saccharomyces cerevisiae* provides a convenient system to elucidate the contribution of gene repression mechanisms to a cellular developmental process.

This study has the broad aim of determining if mechanisms that repress sporulation genes grant an advantage to yeast during other stages of the life cycle. What would happen to a cell if the repression mechanisms that turn off sporulation-specific genes were over ridden or non-existent? Do the repressive mechanisms used by cells contribute towards cellular fitness or viability?

By ectopically expressing well-characterised meiosis-specific genes, this work specifically aims to elucidate whether the mechanisms of repression for sporulation genes grant an advantage to yeast cells during vegetative/mitotic growth. Considering the similarities between meiosis and mitosis such as DNA replication, the segregation of chromosomes and cytokinesis, the ectopic expression of sporulation-specific genes during vegetative growth seemed to be the most obvious and interesting direction to undertake initially.

Ectopic expression of sporulation genes was achieved by fusing a sporulation-specific gene downstream of a galactose-inducible promoter. Consequently, yeast cells can be engineered to synthesise sporulation-specific transcripts while undergoing mitotic division, provided the growth medium is supplemented with galactose, allowing the overriding of sporulation gene repression.

The relative fitness of several strains expressing different sporulation-specific genes was determined by a series of competition studies. Competitions were conducted as they have the advantage of detecting very small differences in fitness within the conditions tested. It was discovered that ectopic expression of the studied sporulation genes was not lethal. However, it was ascertained that active repression of certain sporulation-specific genes during vegetative growth is more advantageous for cellular fitness than others.

Ecology and non-*Saccharomyces* yeast
Chaired by **Graham Fleet** (University of New South Wales)

2–1

The yeast ecology of wine grapes

G.H. Fleet, A.L. Beh, C. Prakitchaiwattana, S.S. Bae and G.M. Heard

Food Science and Technology, School of Chemical Engineering and Industrial Chemistry, The University of New South Wales, Sydney, NSW 2052, Australia.

Yeasts are responsible for the alcoholic fermentation of grape juice into wine. The efficiency of this fermentation and wine quality are determined by the species and strains that grow throughout the fermentation. This ecology consists of the successive growth of various non-*Saccharomyces* species, as well as strains of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* that eventually dominate the fermentation. These yeasts originate from a combination of three sources (i) the grape (ii) the surfaces of processing equipment that come in contact with the grapes and grape juice and (iii) inoculated starter cultures. Grapes are a primary source of yeasts in wine production. Consequently, the ability to control and manage this ecology has important, practical implications in wine production. Although, over the last 100 years, many researchers have examined the yeasts associated with grapes, scientific understanding of this ecology is poor. It is widely accepted that species of *Hanseniaspora*/*Kloeckera* and *Metschnikowia* are predominant on wine grapes at the stage of maturity and harvest. Many researchers have not been able to isolate the principal wine yeasts, *S. cerevisiae* and *S. bayanus*, from wine grapes, raising interesting questions as to their origins in wine fermentations, especially those conducted in the traditional manner, without the use of starter cultures. We have conducted a comprehensive study of yeasts associated with wine grapes with the goals of determining (i) what species are present (ii) how their populations evolve during the stages of grape cultivation and (iii) what factors affect this ecology (e.g. vintage, grape cultivar, pesticide application, damage to grape berries). Grapes representing Cabernet Sauvignon, Shiraz, Merlot, Tyrian, Chardonnay, Semillon and Sauvignon Blanc from vineyards in the Hunter Valley, Mudgee and Griffith districts of NSW have been systematically examined over the 2001-2002 and 2002-2003 vintages. Yeasts associated with grapes were monitored using conventional cultural procedures and molecular analysis of DNA extracts by denaturing gradient gel electrophoresis. The yeast-like fungus, *Aureobasidium pullulans*, was the most prevalent species on all grape varieties at most stages of cultivation, including grapes at harvest. Some strains of this species were antagonistic to *S. cerevisiae* and other wine yeasts. In contrast to the literature, *Hanseniaspora*/*Kloeckera* and *Metschnikowia* species were infrequently found on healthy mature grapes and were more often, but not always, associated with grape berries that were physically damaged. *S. cerevisiae* and *S. bayanus* were only sporadically isolated, even after enrichment culture of grapes. The bacterium, *Bacillus thuringiensis*, was prominent on grapes and correlated with its application as a biological insecticide. It was not antagonistic towards *S. cerevisiae* or other yeasts. Damaged grape berries are probably the main source of indigenous non-*Saccharomyces* and *Saccharomyces* yeasts of wine fermentation.

2–2

Has *Cryptococcus gattii* evolved into a more virulent genotype?

Wieland Meyer

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Cryptococcus neoformans var. *gattii,* recently raised to species status as *C. gattii*, comprises serotypes B and C. It is a primary pathogen causing life-threatening disease mainly in hosts with normal immunity. Until recently it was considered to occur in tropical and sub-tropical climates. A world-wide survey of *C. gattii* isolates using PCR-fingerprinting/AFLP analysis has grouped all isolates into four major molecular/genotypes: VGI/AFLP4, VGII/AFLP5, VGIII/AFLP6 and VGIV/AFLP7. The molecular type VGII is of particular concern in view of a recent, ongoing, outbreak of cryptococcosis amongst people and a range of animal species on Vancouver Island, Canada. More than 80 cases of cryptococcosis in immunocompetent people (infection rate of $24/10^6$ population), with at least 4 fatalities and a much greater incidence in animals, including cats, dogs and porpoises, have been reported between 1999-2001. An extraordinary feature of this outbreak is that *C. gattii* VGII has not hitherto been recognised as an endemic in Canada and this area was considered well outside the geographical range of this organism. Environmental sampling on Vancouver Island has shown an association with native tree species (Douglas-fir, alder, maple and Garry oak) in the absence of eucalypts, a previously identified source. Strains of this specific subtype, VGII, have also been sporadically isolated from clinical, veterinary and environmental sources in Argentina, Australia, Brazil, Colombia, Greece and the USA. Unlike environmental investigations in Australia, high numbers of infectious propagules have been obtained from air samples in the most affected regions on Vancouver Island, suggesting that the fungus either undergoes sexual reproduction or increased haploid fruiting to produce basidiospores. Mating experiments have shown that the vast majority of the isolates are fertile and all belong to the α mating type, a surprising result in the light of Australian findings that mixed **a** and α populations are sterile. However recent studies of a small set of Australian VGII veterinary isolates revealed evidence of recombination, raising the concern that a new genotype is emerging with the potential to cause significant increases in the incidence of cryptococcosis in temperate climates.

2–3

Isolation and identification of genes interacting with MGM101 in *Schizosaccharomyces pombe*

Xiaoming Zuo, and Des Clark-Walker

Molecular Genetics and Evolution Group, Research School of Biological Sciences, the Australian National University, ACT 2601, Australia.

Mgm101p is a protein required for the replication of wild-type mtDNA in yeasts. To isolate and identify genes in *Schizosaccharomyces pombe* that encode proteins with the potential ability to interact with Mgm101p in vivo, a yeast two-hybrid system based on *GAL4* was utilised in this work. A construct, pGBKT7-SpMGM101-CT, containing the coding sequence for a trimmed conservative region of the C-terminus of the SpMgm101p, yielded a large number of positive colonies on selective plates, implying that this region of the SpMgm101p has the ability to directly bind DNA and interact with the Gal4p activation domain. Investigation by using pGBKT7-SpMGM101-C, which bears the complete C-terminal coding region of SpMgm101p, lead to the isolation of a *S. pombe* cDNA clone Y2HSP1. Sequencing of Y2HSP1 revealed that this clone encodes ribosomal protein small subunit 8 (Rps8p) in *S. pombe* that has the potential to be targeted into mitochondria. It is likely that Rps8p has a secondary function in mitochondria involved in the process of SpMgm101 mediated mtDNA maintenance.

2–4

Stress response in novel Antarctic yeast

Sharon Guffogg[1], Shanchita Khan[1], Walter Dunlap[2] and Kenneth Watson[1]

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[2]Marine Biotechnology, Australian Institute of Marine Science, Townsville, QLD 4810, Australia.

Yeasts isolated from extreme environments such as Antarctica, offer a unique opportunity to exploit characteristics not found in other microorganisms. The focus of this study was 60 yeast samples originating from the Vestfold Hills area located near the Australian Davis Base, Antarctica. Functional studies of these extremophiles using a combination of classical and molecular techniques provided a correlation analysis to be achieved verifying the placement of new species against positions of existing species. To date, 3 novel species have been identified as well a number of established species, not previously isolated from Antarctica.

One of the newly isolated Antarctic yeast was identified as *Rhodotorula mucilaginosa*, a highly pigmented red yeast. Novel growth characteristics of this Antarctic strain enabled further studies to investigate heat shock protein expression. Cell viability, measured after a 3 hr heat shock at 52ºC, declined sharply after 30 min of exposure. However, a mild thermal pre-treatment (37ºC for 1 hr) protected the cells against subsequent lethal temperatures. SDS-PAGE utilising ^{35}S-methionine labeling was used to identify heat shock induced proteins. Cells were subjected to UVA-radiation (355-375 nm, calibrated at 365 nm, measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) for 2, 3 and 4 hr and cell viability measured by serial dilution plate count. Cell viability of *R. mucilaginosa* measured as percent survivors, remained at ~100% after 4 hr exposure as compared with *Saccharomyces cerevisiae* with no surviving colonies after 2 hr. A number of other recently isolated Antarctic yeast produced similar results.

The coenzyme Q_8 ratio (ubiquinol: ubiquinone) of *R. mucilaginosa*, a marker of oxidative stress, was measured in cells by HPLC over a 4 hr period of exposure to UVA followed by a further 2 hr in the absence of UVA. The ubiquinol/ubiquinone ($CoQH_2$:CoQ) ratio varied from 54:45 at time 0 to 90:10 after 4 hours of UVA exposure and remained steady for a further 2 hours following the removal of the stimulus. The regulation of these processes to maintain sufficient levels of the reduced form of coenzyme Q appears to be a novel cellular response in *R. mucilaginosa* to UVA photooxidative stress not observed in other Antarctic or mesophilic yeast.

Yeast Cell Molecular Biology II - Signalling and transport
Chaired by **Christina Mitchell** (Monash University)

3–1

Structure and function of the yeast *ALR1* magnesium transporter

Jong-min Lee, Keith Richards, Salam Salih, Paul Donaldson[1], Guo Jun Liu[2], Donald K. Martin[2], Peter Ryan[3] and Richard Gardner

School of Biological Sciences or [1]School of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand.
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[3]CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

The main magnesium uptake system in yeast consists of two homologous genes, *ALR1* and *ALR2* (over-expression confers aluminium resistance). The ALR genes encode proteins whose C-terminal domains are homologous to the bacterial CorA family of magnesium transporters.

Electrophysiological analysis of *ALR1* in both yeast and *Xenopus* oocytes suggests that the protein acts as a channel and that it may mediate both inward and outward movement of magnesium ions into yeast cells. Mg-dependent inward currents are transient, operate only at high negative membrane potential, and are inhibited by aluminium.

Deletion analysis of the *ALR1* gene has identified the C-terminal region (including all of homology to the bacterial CorA genes) as essential for magnesium uptake. We undertook random PCR-based mutagenesis of the C-terminal half the *ALR1* gene. Mutations that completely or partially inactivate magnesium transport cluster within a small segment of the protein corresponding to the location of three putative transmembrane domains. Mutagenesis of the transmembrane region has allowed us to select mutants that show increased transport activity, as well as some with increased tolerance to aluminium, without any apparent alteration in the rate of magnesium uptake. These latter results provide additional support for our hypothesis that aluminium inhibits yeast growth specifically by inhibiting magnesium uptake.

3–2

Yeast assisted plant gene discovery

Brent N. Kaiser[1], Steve D. Tyerman[1] and David A. Day[2]

[1]Wine and Horticulture, School of Agriculture and Wine, The University of Adelaide, PMB#1, Glen Osmond, SA 5064, Australia.
[2]Biochemistry and Molecular Biology, School of Biomedical & Chemical Sciences, University of Western Australia, Crawley, WA 6009, Australia.

Yeast has become an integral tool of many research groups to functionally characterise genes encoding integral membrane transport proteins. Over the last decade, plant biologists have readily utilised the technology where a large number of novel membrane transport proteins have been identified and successfully expressed in yeast cells. As part of our research program, we have employed yeast heterologous expression systems to help identify and characterise plant genes encoding membrane transporters involved in the exchange of nutrients between legumes and their symbiotic nitrogen fixing partners *Rhizobium spp*. Within legume root nodules, nitrogen-fixing bacteria (bacteroids) are surrounded by a plant-derived peribacteroid membrane (PBM), which physically separates the infected plant cytosol and the symbiotic bacteroid. Consequently, a successful symbiosis is dependent on efficient transfer of nutrients across the PBM (for example carbon from photosynthesis supplied to the bacteroids in exchange for bacterial derived NH_4^+ that is released to the plant). Combined approaches using functional screens of soybean nodule cDNA libraries in yeast mutants and or functional testing of PCR amplified soybean genes homologous to known transport systems has enabled our group to identify novel genes encoding membrane transport proteins located on the PBM. In this presentation, I will be providing an overview of our previous and current research on the use of yeast systems in the identification of genes encoding for PBM proteins involved in ammonium, iron, zinc and molybdenum transport.

3–3

Mdt1, a novel *Saccharomyces cerevisiae* protein, modulates DNA damage tolerance and G2/M cell cycle progression

Brietta L. Pike[1], Suganya Yongkiettrakul[2], Ming-Daw Tsai[2], and Jörg Heierhorst[1]

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Proliferation control and cell survival require accurate genome propagation, which is continually challenged by DNA replication errors, cell metabolism by-products, and environmental DNA damaging agents. Eukaryotes from yeast to humans have developed highly conserved signal transduction 'checkpoint' pathways to cope with such threats. Importantly, checkpoint defects can lead to genetic instability, a major cause of cancer in multicellular organisms. Checkpoints monitor progression of a normal cell cycle, and in the presence of DNA damage prevent subsequent cell cycle transitions until preceding events are completed. These checkpoint pathways also have many roles in DNA metabolism, including regulation of repair pathway and telomere maintenance. Yeast studies have greatly aided our understanding of human checkpoint pathways through the characterisation of checkpoint proteins with human homologues.

We have identified a new *S. cerevisiae* protein involved in the DNA damage response as well as progression of normal cell cycles that we have termed Mdt1 (Modifier of DNA damage tolerance). Mdt1 was isolated based on its physical and genetic interactions with the central yeast DNA damage response regulator Rad53 (homologue of the human tumour suppressor protein Chk2). Mdt1 contains an N-terminal RNA-recognition motif and a C-terminal SQ/TQ-cluster domain, which is a characteristic checkpoint domain that function as phosphorylation targets for DNA damage kinases. Mdt1 is constitutively threonine-phosphorylated and further hyperphosphorylated in response to DNA damage *in vivo,* another feature of checkpoint proteins. Importantly, the DNA damage-Mdt1 hyperphosphorylation depends on the Mec1 and Tel1 checkpoint kinases, and Mec1 can directly phosphorylate a recombinant Mdt1 SQ/TQ domain fragment, demonstrating that Mdt1 is a checkpoint target. In the absence of DNA damage, *mdt1* deletion leads to delayed anaphase completion with an elongated cell morphology reminiscent of G2/M cell cycle mutants. In checkpoint mutant strains, *mdt1* deletion partially suppresses DNA damage hypersensitivity and generally improves DNA damage tolerance. *mdt1*-dependent and damage-dependent cell cycle delays are not additive suggesting that they act in a common pathway. Mdt1 genetically interacts with components of the telomere regulation machinery, suggesting a link to another crucial cellular function in the protection of DNA ends from degradation and fusions with other chromosome ends or DNA breaks, and importantly the maintenance of chromosome stability. Altogether, the data indicate that Mdt1 has important functions in cell cycle progression as a novel target of checkpoint pathways.

3–4

Adaptive responses of *Saccharomyces cerevisiae* to the action of sulfa drugs

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Dihydropteroate synthase (DHPS) is a key enzyme in the folate biosynthetic pathway, catalysing the condensation of para-aminobenzoic acid (pABA) with 7,8-dihydropterin pyrophosphate (DHPPP). Mutations in DHPS have been associated with resistance to sulfa drugs in a number of organisms including two important human pathogens, *Pneumocystis carinii* and *Plasmodium falciparum*. Mutations resulting in amino acid substitutions at two highly conserved residues involved in substrate binding have been focused on in order to determine their affect on cell growth and viability. *Saccharomyces cerevisiae* was used as a model organism due to the difficulty of working with *P. carinii* and *P. falciparum*. A phenotypic analysis identified compromised growth due to a pABA requirement. This was seen in the double mutants but not the wild-type. Double mutant pABA prototrophs could be isolated at a frequencies between 1 in $1.5x10^3$ and 1 in $1x10^4$. Further selection on pABA-free media isolated double mutants with an adaptive response for growth in a pABA-free environment. Gene expression profiling identified a part of this response to be an increase in expression of pABA synthase. This study demonstrates that double mutations in DHPS confer a pABA requirement that can be overcome by an increase in expression of pABA synthase.

Biotransformations and bioproducts
Chaired by **Peter L. Rogers** (University of New South Wales)

4–1

Enzymatic biotransformation process for pharmaceutical production using pyruvate decarboxylase (PDC) from *Candida utilis*

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The chiral pharmaceuticals ephedrine and pseudoephedrine are currently produced commercially via the biotransformation of benzaldehyde and pyruvate to the intermediate (R)-phenylacetylcarbinol (PAC). This involves the addition of benzaldehyde to fermenting baker's yeast (which produces pyruvate) with the reaction being facilitated by the yeast pyruvate decarboxylase (PDC). The fermentative biotransformation introduces the chiral centre into the PAC with minimal energy requirements and at high chiral specificity.

For the fermentative process using *Saccharomyces cerevisiae,* concentrations of 10-22 g/l PAC in 10-20 hours have been reported with low PAC yields of 55-65%, due to significant conversion of the benzaldehyde to benzyl alcohol resulting from oxido-reductase activity in the yeast.

Our approach for process enhancement has focused on an enzymatic reactor, which uses partially purified PDC and excludes these latter activities in the yeast. Screening of filamentous fungi and yeasts for cell-free PAC production resulted in the identification of a number of efficient sources of PDC e.g. from *Rhizopus javanicus* and *Candida utilis,* with the yeast PDC showing less inactivation in the presence of benzaldehyde. Enzyme stabilization and pH control were identified as crucial factors for increasing final PAC levels. Using partially purified PDC, 50 g/l PAC was produced in about 30 hours in a simple batch enzymatic process, with no evidence of by-product benzyl alcohol formation.

Further process development has involved an aqueous/organic two-phase system designed to minimize the problems of enzyme deactivation and inhibition by the benzaldehyde that remains largely in the organic phase. Under optimized conditions using partially purified PDC from *C. utilis*, 141 g/l PAC was produced in the organic phase (octanol) in a period of 49 h, with an additional 19 g/l formed in the aqueous phase. Compared to the traditional fermentation process, the use of a 2-phase enzymatic bioreactor resulted in significantly increased yields and concentrations of PAC as well as enhanced PAC production per unit of PDC activity. This project that was initiated originally with ICI (Australia) has been developed subsequently in collaboration with BASF, Germany.

4–2

A three-member gene family is responsible for the synthesis of short chain fatty acid esters during fermentation

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Ester synthesis during fermentation mainly depends on yeast ester synthesis potential, i.e. the amount of available acetyl-CoA/acyl-CoA (an essential building block for yeast cellular components) and the level of ester synthase activities, the enzymes being synthesised during the growth phase. Biochemical evidence suggests that at least five enzymes are involved in the synthesis of esters within yeast. Ester hydrolyzing activities may play a determinant role on the final ester levels of products such as membrane filtered beer and bottle re-fermented beer.

Recently, scientists have taken advantage of the completed *S. cerevisiae* genome sequence database and the powerful tools of molecular biology to identify the corresponding genes and investigate the physiological role of ester synthesis. Recent rapid progress has provided insights not only into the regulation of cellular ester synthesis, but also into some general mechanisms of gene regulation. Three distinct AATase genes (*ATF1*, *LgATF1* and *ATF2*), responsible for the production of acetate esters, have been cloned from different yeast backgrounds. A fourth gene *EHT1* has been described as an ethanol hexanoyl-CoA transferase. In this work, we report the affect of *EHT1* disruption on the synthesis of esters.

Sequence comparisons revealed that *EHT1* belongs to a three-member gene family. Combination of simple, double and triple deletions did not affect growth. Disruption of one of the genes resulted in decreased levels of short chain acid esters, the decrease being accentuated with the number of disrupted genes. The results suggested an internal functional redundancy as the phenotype was increased with the number of disrupted genes. Analysis of the relationship between levels of short chain fatty acids and the corresponding ethyl esters strongly suggests that these enzymes may be involved in the removal of the toxic short chain fatty acids. Interestingly, disruption of the three-member gene family also stimulated the synthesis of the acetate esters.

4–3

The development of a novel yeast based flavour enhancer

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Flavours and flavour enhancers are used extensively within the food industry with most flavouring compounds derived by chemical synthesis, biological fermentation or botanical extraction. Even though these processes are constantly being improved, there are still problems associated with each method of manufacture. As a result, yeast and flavour manufacturers have combined their efforts focusing on the production of flavourings derived from microbial and yeast based fermentations. Currently, the manufacture of yeast extracts requires an autolysis to disrupt the yeast cells and a post-autolysis enzymic hydrolysis to produce 5′-nucleotides. The unit operations of fermentation, harvesting, autolysis and dehydration are extensive and generate a considerable waste stream that adds to the cost of production. A novel alternative technology has been developed for the generation of 5'nucleotides intracellularly whilst maintaining the viability of the treated yeast.

The project, with its origins in the pharmaceutical industry, involved several stages; the first was the extraction, purification and characterisation of the enzyme, 5′-Phosphodiesterase-1 from barley, which is used to produce the flavouring 5'-nucleotides; The next stage was the development of a delivery system comprised of liposomes engineered to be capable of delivering large molecules across the plasma membrane of yeast protoplasts; The final stage of the project involved verification of the encapsulation and endocytosis potential of the liposome bound enzyme to produce 5′-nucleotide flavour enhancers within "viable" yeast cells.

4–4

Metabolic profiling using infrared (IR) spectroscopy

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Recent advances in technology have brought about a revolution in the manner in which biological systems are visualized and queried. The generic term used to define the total biochemical composition of a cell or tissue sample at any given time is called "metabolome". In order to study the metabolome of biological samples, new analytical techniques were developed. Near infrared (NIR) and Fourier-transform infrared (FTIR) spectroscopy are physical-chemical analytical techniques, which use the vibrational characteristics of defined chemical bonds within molecules to obtain a "finger print" spectrum, with features associated with functional chemical groups in the sample. Multivariate analysis techniques are often used in conjuntion with vibrational spectroscopic techniques in order to determine physical or chemical sample properties from the spectra. To construct a multivariate model, the spectra and corresponding properties of many samples need to be measured in order to capture the variation in the sample properties. The fermentation supernatants of different *Saccharomyces cerevisiae* strains were scanned using a FOSSNIRSystems in 1 mm path length. Spectral data were exported into The Unscrambler for multivariate analysis using principal component analysis (PCA) and soft independent modeling class analogies (SIMCA). The results showed that NIR spectroscopy has the potential to differentiate between some strains of *S. cerevisiae* to a degree that is comparable with that obtained using other far more complex, expensive analytical techniques such as GC/MS.

Stress and aging
Chaired by **Ian Dawes** (University of New South Wales)

5–1

Ethanol, stress and working with yeast

Grant. A. Stanley

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Ethanolic fermentation is perhaps the oldest biotechnological process. Traditionally associated with the production of beverages, it now has an increasing role in the biofuel industry. Several factors impact on the productivity of this process, one of the major factors being that ethanol is toxic to the cell, its toxicity increasing as it accumulates in the culture broth. This has financial and logistic impacts on the fermentation process by slowing fermentation rates, reducing ethanol yields, escalating fermentation turnover periods and limiting microbial lifespan. This presentation will provide an overview of the research being conducted at Victoria University on ethanol tolerance in *Saccharomyces cerevisiae*.

The yeast research group at Victoria University is investigating ways of reducing the impact of ethanol toxicity on microbial performance. One of the aims of our research is to improve the inherent protective responses used by yeast to combat the inhibitory effects of low ethanol concentrations; these protective mechanisms are compromised at higher ethanol concentrations. Since relatively little is known about the ethanol stress response at the molecular level, our current investigation focuses on identifying and characterising the key genes associated with the cell's defence against ethanol stress. To do this, we are using gene array to follow transcriptome changes in yeast during ethanol challenge. Initial results comparing gene expression between ethanol stressed and unstressed cells identified a large number of genes with altered expression levels when exposed to ethanol stress. Although many of these genes could be grouped according to their role in the cell, it is likely that many do not have a major or direct role in promoting tolerance to ethanol. To identify the key genes that confer ethanol tolerance, we are also examining transcription in ethanol-stressed yeast in the presence of small quantities of acetaldehyde, which stimulates the adaptation rate to ethanol stress. We have also raised mutants with increased ethanol tolerance and these are currently being analysed to identify the genetic changes that confer this phenotype. Comparing the results from all three experimental approaches should identify a common pool of genes that potentially have a critical role in the protection and adaptation of yeast to ethanol stress. In addition to this, early microarray data identified a correlation between trehalose metabolism and cell adaptation to ethanol stress. This association is also currently being investigated. The results of this work can be used to design molecular probes for identifying cells with inherently high ethanol stress tolerance and to develop programs aimed at increasing microbial tolerance to ethanol stress, potentially leading to increased fermentation productivity and greater management of ethanol yields.

5–2

The application of functional genomics to the study of industrial fermentation processes

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Yeast are subjected to many types of stress and metabolic challenges throughout industrial fermentation processes. With the advent of new processes that increase yields or are more cost effective, different and increased demands have been placed on the yeast. These demands can lead to conditions that overwhelm the yeast defences causing defective fermentations. The dynamic nature of industrial fermentations often makes it difficult to determine the cause of these defective fermentations.

The sequencing of the yeast genome and analytical techniques enabling the quantification of expression levels of a large number of individual genes, has facilitated a major step forward in the ability to identify genes that have altered gene expression patterns in response to changing environmental conditions. This genome-wide expression technology was applied to the study of the response of an industrial yeast strain during an industrial fermentation process. Genes were identified as induced in the initial stages of a lager fermentation that gave insights into the conditions that were affecting the yeast and therefore important to the fermentation process.

5–3

The transcription factors Rme1p and Mss11p control invasive growth by modulating *FLO11* expression levels in *Saccharomyces cerevisiae*

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Invasive and filamentous growth of *Saccharomyces cerevisiae* occurs when the nutrient supply is limited, in particular when amino acids, nitrogen and/or carbon sources are at reduced levels. The GPI-anchored cell wall protein, Flo11p/Muc1p, plays an important role in this process, and is essential for substrate invasion. The transcriptional regulation of *FLO11/MUC1*, a gene that has one of the largest promoters in yeast, is under the control of a cascading network of signals. The network regulates the activity of the transcription factors, including the activators Flo8p, Msn1p, Mss11p, Ste12p, Tec1p and Rme1p, and the repressors Nrg1p, Nrg2p, Sfl1p and Sok2p. All these factors are shown to be directly required for regulation of *FLO11* expression but the genetic and functional relationships between them are not yet fully understood. Our data show that the central factor in the regulation of *FLO11 is* Mss11p. Most of the other factors investigated, whether activators or repressors, require the presence of Mss11p to affect *FLO11* transcription, whereas the presence of multiple copies of *MSS11* leads to a strong induction of the gene, even in strains carrying deletions in one or several activator genes. One exception to this rule is Rme1p (*R*egulator of *ME*iosis), a protein that acts through an 11 nucleotide stretch in the *FLO11* promoter to induce expression of the gene and appears to act independently of Mss11p. Rme1p was previously shown to bind to similar Rme1p response elements (RRE) in the promoters of *IME1* (Inducer of MEiosis) and the G_1-cyclin encoding gene, *CLN2*. Mutation of the *FLO11* RRE results in the promoter not being responsive to increased *RME1* gene-copies. Our genetic data also suggest that Rme1p works independently of most other activators and repressors, suggesting that it acts as a central switch between several nutrient-dependent cellular differentiation pathways.

5–4

There is no such thing as a single form of oxidative stress

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Aerobically growing cells are exposed to reactive oxygen species (ROS) generated during normal metabolism. ROS include hydrogen peroxide (H_2O_2), the hydroxyl radical (OH) and the superoxide anion (O_2^-), which can damage many cellular constituents. In respiring cells, the primary source of ROS is leakage of electrons from the mitochondrial respiratory chain. When cellular defence mechanisms are unable to cope with existing ROS, oxidative stress occurs.

In order to identify cellular functions required for tolerance of oxidative stress, the collection of all viable single gene deletion mutants in *S. cerevisiae* was screened for sensitivity to oxidative stress. In total, approximately 4500 deletion strains were tested for sensitivity to five compounds that affect the redox balance of the cell, each of which induces a different form of oxidative stress. The screen identified 456 mutants that were sensitive to at least one oxidant, and these mutants were ranked in order of their sensitivity. Many genes identified were not previously known to have a role in oxidative stress resistance. There was also little correlation between mutant sensitivity and the reported transcriptional response of each gene to a given oxidative stress condition. More surprisingly, no two oxidative stress conditions had a similar profile of sensitive mutants, showing that no single oxidant is representative of a general oxidative stress.

Brewers/bakers yeast
Chaired by **Jon Meneses** (Coopers Brewery)

6–1

Monitoring heterogeneity - improving consistency of industrial yeasts

Paul Attfield

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Manufacturers and end-users of yeasts seek to improve the quality and economics of yeast-dependent processes by improving yeast performance in production plants, and improving yeast strain potential (genetics and physiology).

Microbiogen is a yeast technology company specialising in rapid analyses of yeasts to assist in quality issues and process improvements during manufacture, and development of non-GM yeast strains via high throughput, markerless classical genetics.

Most of the current knowledge of microbial physiology and molecular functions is derived from analyses of biomass. For example, traditional assays of dry weight, turbidity, metabolism (e.g. fermentation and respiration), metabolites, enzymes (native or reporter activities), proteins and gene transcripts rely upon sampling many millions of cells in concert. Whilst providing useful information the traditional analyses do not inform about potential heterogeneity within microbial cultures. Occurrence of heterogeneity is however, important in the use of yeasts in industrial processes, where predictability and consistency of performance is sought. Fluorescence-based analytical techniques offer the ability to measure various physiological properties of cells. When coupled with flow cytometry, fluorescence offers the means to analyse single cells at rates of 1000s per sec. Thus, it is possible to obtain important information about the overall population as well as individual cells making up that population.

Microbiogen is utilising fluorescence-based techniques together with flow cytometry to study heterogeneity of industrial yeast samples. Analyses of yeasts from brewing plants and of dried yeasts for baking, wine, and distilling, indicate substantial cell-to-cell variations within samples. Recognition and quantification of the cell-to-cell heterogeneity in industrial yeast samples provides a useful diagnostic aid for optimising quality in yeast manufacturing and management processes.

6–2

Yeast strain mediated haze and filterability

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The invertase enzyme is usually reported to be located intracellularly and/or in association with the cell wall of *Saccharomyces cerevisiae*. Recent work with numerous industrial strains indicates that invertase is in fact also able to be released into the supernatant without loss of cell integrity. This enzyme is of importance to brewing given that sucrose comprises typically 5% of wort carbohydrates, but can represent as much as 30% when sucrose is used as an adjunct. As a glycoprotein, invertase is also significant to the wine industry as it has been show to act as an inhibitor of the formation of visible haze in this product.

A survey by this laboratory of 30 strains has shown a wide variation in the degree of release of invertase into the medium. We have sought to correlate this variability to the following parameters: 1) filterability of the fermented, cell-free product; 2) chill haze of the filtered product; 3) the forced chill haze of this filtered product after 48 hours at 60°C; and 4) the total protein released into the culture supernatant. While a clear link between these parameters and the amount of extracellular invertase activity was not seen, a strong strain dependence was apparent.

6–3

Redox balance and the production of sulphite and sulphide by brewing yeasts

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Sulphate is assimilated for S-amino acid biosynthesis, via 2 NADPH-dependent, reductive steps to sulphite and sulphide. If the assimilation pathway terminates at $S^{=}$ or SO_2, it operates as an electron sink. Could it be that the availability of reduced coenzyme trims the flux of sulphate through the assimilation pathway? Not forgetting that feedback mechanisms also operate to reduce the activity of various steps in the sequence. Sulfur assimilation must compete for reducing equivalents with other reductive pathways - the ergosterol pathway, fatty acid and volatile ester production. These in turn, are affected by the nutritional status of the wort, and the yeast's physiology. Studies with knockout mutants using rapid micro-fermentation techniques showed that glutamine, asparagine and arginine alter the $NADP^{+}$/NADPH ratio, presumably through oxidative deamination activity. Redox control overlays the general nutritional effects that determine the growth - dependent 'pull' for biosynthetic S requirements. Redox balance explains the nutrient control of SO_2/H_2S poise in brewing yeasts, the timing of volatile production - that is the differential appearance of sulphur dioxide and hydrogen sulphide in the brew, and shows how informed selection of malt specification can avoid the production issues associated with nuisance S compounds.

Yeast in medicine
Chaired by **Ian Macreadie** (CSIRO, Health Sciences & Nutrition)

7–1

Employing model systems to investigate microbial drug resistance

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Drug resistance of pathogenic organisms is a continual process of evolution in response to drug pressure. In order to study microbial drug resistance of organisms that are difficult or impossible to culture, model systems need to be employed. The pathogen, *Plasmodium falciparum*, that causes the most severe form of malaria and kills 2 million people p.a., mostly in the third world, is treated with antifolate drugs such as the sulfonamides. Sulfonamides are also used to treat immunocompromised AIDS patients suffering from an often fatal pneumonia caused by *Pneumocystis jiroveci*. This disease also kills 2 million patients p.a. worldwide. These two organisms are either difficult or impossible to culture. Therefore, suitable *S. cerevisiae* and *E. coli* model systems have been utilised to investigate drug resistance mechanisms and to screen for more effective drugs. These studies showed that gene amplification of the sulfonamide drug target could cause drug resistance. Further, a dual mode of action was demonstrated for sulfonamides, one mode previously being unrecognised. Consequently, this has led to the search for a new drug target. Mutations in the sulfonamide drug target were shown to cause drug resistance either directly or through a secondary adaptive response. Model systems have further enabled the screening of existing sulfonamides for more effective candidate drugs. In addition, structural studies of drug targets have been initiated that may lead to in-silico screening of chemical compounds or alternatively, the rational design of novel drugs.

7–2

Modeling cell death pathways using yeast

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The focus of our laboratory is to identify and functionally characterise components of apoptotic pathways – the pathways that control the survival and death of cells in metazoans. Tight control of apoptosis is essential for normal development and to avoid a host of diseases, from cancer to neurodegenerative disease. We have reconstituted apoptotic pathways from mammals and nematodes in the yeast *Saccharomyces cerevisiae*. Yeast offer many advantages for exploring apoptotic mechanisms. Compared to mammalian or insect cell culture systems, yeast lack endogenous apoptotic pathway components, and are easy to grow, transform, assay and screen. Unlike bacteria, yeast are eukaryotic, thus increasing the likelihood that proteins undergo normal post-translational modifications and sub-cellular localisation. Our reconstitution of apoptotic pathways has exploited the finding that many active caspases (a family of apoptotic proteases) are lethal to yeast. We have successfully assembled the nematode (EGL-1 - CED-9 - CED-4 - CED-3) and apoptosome (Apaf-1 - Caspase-9 - Caspase-3) caspase activation pathways in yeast. These strains permit analyses of the activity of defined mutants, functional testing of candidate homologues of apoptotic regulators and screening for novel pathway components.

7–3

Engineering carnitine biosynthesis in *Saccharomyces cerevisiae* in order to increase its nutritional value

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L-Carnitine is involved in energy metabolism in eukaryotic organisms. In particular, it facilitates the β-oxidation of fatty acids in the peroxisome and mitochondria. In humans, carnitine deficiency can be a debilitating disease generally caused by a mutated carnitine transporter. In recent times, carnitine has been used for the symptomatic treatment of chronic fatigue syndrome, heart disease and Alzheimers disease. It is also widely used as an additive to baby milk formulae, sport drinks and weight-loss supplements. Unlike most other eukaryotic organisms, *Saccharomyces cerevisiae* is not able to biosynthesise carnitine. In fact, carnitine is essential for growth on non-fermentable carbon sources when the glyoxylate cycle citrate synthase is deleted. However, the filamentous fungus *Neurospora crassa* is able to biosynthesise carnitine from lysine in a five step process. Trimethylated lysine is converted by four enzymes to L-carnitine; the first enzyme is trimethyllysine hydroxylase (TMLH) and the last enzyme is butyrobetaine hydroxylase (BBH). We have cloned the genes encoding TMLH and BBH and heterologously expressed them in yeast. Both enzymes were functional and the yeast could biosynthesise carnitine endogenously from the precursor butyrobetaine. A carnitine biosynthesising strain of *S. cerevisiae* would increase the nutritional value of foods such as bread and beverages such as wine and beer.

Wine yeast
Chaired by **Paul Henschke** (The Australian Wine Research Institute)

8–1

Novel wine yeasts

Louisa Rose

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With the increasing volume of premium wine being made in Australia and around the world, winemakers are looking to make wines with more complexity, mouthfeel, and fruit expression to set their wines apart from the many competitors that sit beside them on the shelf. The premium wine drinker is also changing, looking for subtle and "new" characters in their wines that complement their lifestyles, particularly those parts associated with food.

The use of novel yeasts is one winemaking technique that can influence the characters and flavours in wine, helping to build layers, texture, balance and integration in the final product. Although many Australian winemakers are somewhat hesitant to use novel yeasts in fear of "off flavours", volatility and stuck ferments, at Yalumba we have used them with very good success, and continue to use and evaluate them both in our commercial wines, as well as the experimental stage.

This presentation will outline the details of some of the experimental work performed, the protocols, the results to date, and challenges for future work and understanding.

8–2

Wine yeast gene technology

Miguel de Barros Lopes

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An increasing gap between wine produced and wine consumed has lead to a growing demand for cost-effective production of wine with minimised resource inputs, enhanced quality, increased health benefits and low environmental impact. Yeast molecular biology is being employed in an attempt to further understand and improve various aspects of winemaking. A brief overview of the international scientific research effort in to developing genetically improved wine yeast will be presented.

8–3

Adaptive evolution in enhancing wine yeast

Colin McBryde, Miguel de Barros Lopes[1] and Vladimir Jiranek

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Adaptive evolution defines a set of mutations that occur in response to a specific challenge and are advantageous to the cell (3). The technique is performed by serial or continuous cultivation of a population for many generations under conditions to which it is not optimally adapted (1, 2). We have used this technique to isolate adaptively evolved mutants of two strains of *Saccharomyces cerevisiae* under extended anaerobic fermentation. We used a sequential batch fermentation system, whereby parental cultures were inoculated at low cell densities into chemostats containing chemically defined grape juice media. Isolates were taken from the fermentation cultures every 50 generations and screened in small scale fermentations under conditions of anaerobic stress. Fermentation kinetics were monitored and extracts were analysed in terms of the production of major metabolites. We found that after two to three hundred generations, isolates appeared to have adapted to the imposed conditions. Under anaerobic conditions, mutants were able to catabolise the supplied sugar within a shorter period. In addition, glycerol concentrations at the end of fermentation by these mutants were also higher than the respective parental strain. Changes in transcriptional regulation have been analysed employing microarray technology. Preliminary data indicates that differences between the two strains may be attributable to a differential transcriptional regulation of oxidative stress genes. We propose that adaptive evolution offers a versatile method to isolate commercial wine yeast strains, which are highly tailored to the stressful conditions of a typical wine fermentation.

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8–4

A genetic study to characterise the release of volatile thiols by *Saccharomyces cerevisiae*

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Volatile thiols are important contributors to wine varietal aroma. A non-volatile precursor for one of the most potent thiols, 4-mercapto-4-methylpentant-2-one (4MMP) has been identified as a cysteine-bound conjugate that is released by *S. cerevisiae* during the alcoholic fermentation of grape juice. Cleavage of the precursor by an unknown yeast enzymatic mechanism results in the volatile 4MMP being released. In this study, *S. cerevisiae* deletion strains were assayed for their ability to release 4MMP from the synthesised non-volatile conjugate. The volatile thiol is released when a carbon sulfur bond is cleaved; therefore genes which encode or are predicted to encode C-S lyases were selected. Four of the deletion strains showed a reduced capacity to release 4MMP. Involvement of these genes in release of 4MMP in wine conditions was confirmed by deleting the genes in industrial wine yeast. The corresponding genes were cloned into yeast and bacterial expression plasmids. Overexpression studies and purification of the corresponding enzymes will clarify the role that each enzyme has in the release of 4MMP.

8–5

A quantitative chemical and sensory approach to characterising wine yeast for improved red wine colour and flavour

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The grape-derived anthocyanins and various derivatives contribute to the colour of red wine. Red wine colour depends on many factors, including the grape variety and viticultural, fermentation and maturation conditions, but little information is available on the role(s) of yeast. Yeast strains that enhance the content of anthocyanins and other desirable phenolic compounds during alcoholic fermentation of grape must may be useful for improving the colour and flavour of red wine made from low phenolic musts. An understanding of the mechanism(s) involve may also lead to the development of better red wine yeast. From previous studies we have demonstrated that yeast are able to consistently influence wine colour and phenolic content and, on this basis, ranked 17 *Saccharomyces cerevisiae* strains into three distinct groupings. One representative strain from each group, denoted ‘low’, ‘medium’ and ‘high’ colour yeast, was selected to quantitatively determine the effect of yeast strain on the chemical, phenolic and sensory properties of resultant wines. Shiraz fruit sourced from Berri, South Australia, during the 2003 vintage was divide into duplicate 700 kg lots and alcoholic fermentation was initiated with the presumptive ‘low’, ‘medium’ and ‘high colour’ yeast. The three yeast strains affected wine phenolic profile and 9 of the 11 aroma and flavour attributes determined. Study of the relationship between wine composition and sensory attributes will provide clues to the role of yeast in these changes.

Yeast Cell Molecular Biology III - Gene expression
Chaired by **Mary-Jane Gething** (University of Melbourne)

9–1

Budding yeast DNA damage response proteins as models for human cancer predisposition syndromes

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DNA damage response pathways are crucial for the maintenance of genome stability and the prevention of cancer. DNA damage response pathways are remarkably conserved throughout eukaryotic evolution, which allows us to utilize budding yeast as a simple model organism to efficiently study basic mechanisms involved in carcinogenesis in more complex human cells.

We have analysed the function of an N-terminal protein-interaction domain called FHA domain of the *Saccharomyces cerevisiae* Rad53 and Dun1 kinases, which are similar to the human CHK2 kinase. CHK2 mutations are associated with the Li-Fraumeni multi-cancer syndrome and breast cancer predisposition. Using site-directed mutagenesis to introduce single point mutations into the chromosomal *RAD53* locus, we have shown that the FHA1 domain is essential for DNA damage-dependent Rad53 activation by upstream kinases and subsequent cell cycle arrest during metaphase. The Rad53 FHA1 domain is also essential for inhibition of late DNA replication origins during DNA damage in S phase without affecting Rad53 activation, indicating that it links activated Rad53 to downstream effectors. Despite these crucial cellular functions, FHA1 domain mutation leads only to moderately increased DNA damage hypersensitivity as long as it is "buffered" by additional cell cycle checkpoint pathways. However, combination of the FHA1 mutation with similar mutations in the second Rad53 FHA2 domain or gene deletions in two Rad53 activating pathways (Rad9 and Rad17) leads to dramatically increased DNA damage hypersensitivity. These results could explain why mammalian CHK2 deletion by itself has only modest DNA damage response effects, despite increased cancer risk.

We have also used yeast as an experimental tool to isolate a novel human DNA repair protein called ASCIZ in a two-hybrid screen for CHK2 FHA domain-interacting proteins. ASCIZ is the human orthologue of yeast Mdt1 (see abstract by Pike et al.) and forms characteristic DNA damage-induced nuclear foci that function in homologous recombination repair of gapped single-stranded DNA intermediates generated by the mismatch repair pathway. The identification of a novel conserved DNA damage response protein family should provide additional powerful tools for the detailed analysis of cancer-related DNA repair defects using yeast as a model system.

9–2

From transcriptome to proteome: the control of protein translation

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To appropriately respond to the challenge of changing environments, yeast cells launch complex regulatory networks to maintain homeostasis. A typical response to change might be simplified to the following three step generic pathway: *a)* cellular perception of change through a phosporylation cascade, *b)* activation of transcription factors which change the cell's transcriptional out-put, *c)* translation of this altered transcriptome in the cytosol to produce the appropriately changed proteome buffering the impact of change in the environment. The advent of accessible genome-wide technologies has allowed the individual aspects of such regulatory networks to be probed. We are investigating the control of protein translation after induction of stress by monitoring the transcripts associated with actively translating ribosomes using microarray analysis. Comparing polysome associated mRNA (actively translated messages) with transcripts associated with non-translating ribosomes, we have made the striking observation that there is a homo-directional amplification of the transcriptional change at the level of translation after induction of stress. That is, messages up-regulated in the transcriptome are also more efficiently translated than other messages and visa/versa. This implies that the messages subject to transcriptional change are differentially marked, causing them to be differentially interpreted by the ribosome leading to their differential translational fitness. Whether this molecular mark is *in cis* or *in trans*, is the subject of our current investigations. The length of the poly(A) tail appended to newly synthesised messages is a *cis* element known to influence translational fitness. Using poly(U) sepharose to fractionate mRNA based the length of its poly(A) tail, we are determining firstly, the steady state poly(A) length of the transcriptome (where each transcript is expected to have a defined tail-length) and secondly, whether this tail-length is linked to the rate of transcription, translation and turnover of each transcript in response to change.

9–3

The *LSM* gene set and its role in modulating splicing efficiency in the cell

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The spliceosome is a large macromolecular structure, essential for the correct processing of most pre-mRNA in eukaryotic organisms. It consists of five small nuclear ribonuclear proteins (snRNPs) which together contain five snRNAs and more than a hundred proteins. Each snRNP is believed to contain seven small core proteins which form a ring structure to which specific snRNAs bind and with which splicing factors associate. Two types of core proteins have been identified in the spliceosome - Sm and 'like-Sm' or LSm proteins.

This study examined the regulation of Lsm proteins, at the level of transcription and mRNA stability in *Saccharomyces cerevisiae* grown on different carbon sources, and thus at different growth rates. A number of *LSM* genes were found to be coordinately regulated in a manner which correlated with changes in the splicing requirements of the cell. Experiments using mutant cells with *LSM1, LSM6* or *LSM7* deleted confirmed the coordinate gene set and inferred a manner by which this coordinate expression may be achieved. A novel *in vivo* splicing assay was also developed. Results show that the observed changes in expression of the *LSM* gene set also correlate with the splicing capacity of the cell under these conditions, indicating an additional level of regulation for all genes with introns in this organism.

Forum
Chaired by **John Wallace** (University of Adelaide)

10–1

Whither yeast research?

Ian W. Dawes

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Yeasts have been research tools for more than a century, both to underpin their uses in biotechnology, and also, for *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* as model eukaryotic organisms for basic cell and molecular research. Their pre-eminence as research tools stems from the extensive knowledge of their biochemistry and genetics and the rapidity with which they can be used for genetic, biochemical and cellular research. This, and the early sequencing of the *Saccharomyces cerevisiae* genome has meant that this organism has become a paradigm for all of the major sequencing programs that followed.

This has led to a very rapid generation of useful tools and resources for genome-wide analyses of cellular processes, and major advances in the understanding of many cellular functions. The current challenge for many yeast researchers is how to maintain international competitiveness in the face of high throughput, expensive technology and very collaborative international research programs. This contribution is aimed at reviewing the newer technologies available for yeast research, the current trends in the field and the strengths and weaknesses of yeast research from the national perspective. The intention is to initiate discussion of where future research may go and ways to improve the impact of our research on the Australian and international scene.

10–2

Bridging the gap between the idea and its transformation into commercial outcomes in yeast biotechnology

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Wine research is inspired not only by the quest for understanding but by the expectation of providing insights that lead to innovation, which is the most powerful dynamo of technological progress. This is the conviction of *The Australian Wine Research Institute*, where we believe this dynamo of innovation promotes the cost-effective production of wine with minimised resource inputs, improved product quality, increased health benefits, and low environmental impacts. Research into yeast biotechnology illustrates how the AWRI is working to bridge the gap between a scientific idea and putting it into commercial practice; in other words how AWRI's science can help produce a better product for industry and the consumer.

Yeasts have enjoyed a long and distinguished history in fermentation and their industrial importance has extended into many commercially important sectors, including food, beverages, biofuels, chemicals, industrial enzymes, pharmaceuticals, agriculture and the environment. However, just as the Stone Age did not end because mankind ran out of stones, yeast-based industries will, despite the abundance of traditional approaches to fermentation, continue on a path of technological progress. Yeast research and development will continue to contribute to this and we anticipate greater and more urgent demand for *designer* yeasts for a wide variety of existing and new products. To meet this expectation, it is inevitable that yeast researchers will regularly cross the hypothetical boundary between fundamental and applied research. How we as researchers transcend this boundary will, in turn, determine how successful yeast research programs will be in efficiently bridging the gap between theoretical science and research developments that can be applied to new ideas and knowledge that have commercial outcomes. It will, therefore, become even more challenging for yeast researchers to keep striving for academic excellence, while at the same time helping to develop products and processes that could enhance the international competitiveness of a particular yeast-based industry.

Even though it is clear that modern yeast research cannot function optimally within the limitations of a conventional, one-dimensional band in which applied science remains on the distant side of the gap, we do not believe that, whatever the new demands, they should be allowed to erode the fundamental scientific base because commercial outcomes will always rely on basic research to create new knowledge. To bridge the gap, what is needed is neither "Bohrian" basic research nor "Edisonian" trial-and-error, but rather an inclusive "Pasteurian" approach where research is directed towards increasing fundamental understanding in a way that responds to the needs of the application end, and this needs to happen at the basic stages of problem selection and experimental design. In such a scenario, targeted research and synergistic partnerships between practitioner and scientist will become even more important. The metaphor for these partnerships, therefore, is not the relay race in which the baton is passed from the researcher to the practitioner. At the AWRI, we see ourselves more as players in a rugby team passing the ball back and forth, moving the full distance to the goal line as a unit with the practitioners. It is in this way that basic and applied wine research continually enlighten one another – and how we at AWRI bridge the gap between science and commerce.

POSTER PRESENTATIONS

Chaired by **Paul Grbin** (University of Adelaide)

P–1

Identifying and characterising ethanol tolerance genes in brewing yeast

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During industrial fermentations the brewing yeast *Saccharomyces cerevisiae* is exposed to a range of stress conditions including temperature shifts, pH extremes, osmotic stress and ethanol accumulation. Of these stresses, ethanol accumulation constitutes a major inhibitory factor; as levels of ethanol increase during fermentation yeast cell growth is inhibited and viability is reduced, compromising fermentation performance and ethanol yield.

S. cerevisiae acclimatises to ethanol stress, but to a limited extent. Improving yeast tolerance to ethanol will enable yeast cells to better survive under the stress conditions associated with ethanol production and possibly lead to higher ethanol yields and process productivity. One of our strategies to achieve this end is to identify and characterise the genetic mechanisms involved in yeast acclimatisation to ethanol stress.

In this investigation, an industrial brewing yeast strain of *S. cerevisiae* was subjected to chemical mutagenesis using the mutagen ethyl methane sulfonate (EMS). A mutant strain with increased ethanol tolerance was selected by adaptive evolution using a chemostat operating over many generations under high ethanol stress conditions. The ethanol tolerant variant was isolated and the phenotype confirmed. The method used and the phenotype of the mutant will be described.

The next stage of the project will be to identify genes that confer increased ethanol tolerance in the mutant. This will involve analysing gene expression profiles of ethanol tolerant variants and the parent strain under ethanol stress and control (unstressed) conditions. It may also be necessary to create a gene library to isolate genes that confer increased tolerance. Genes identified as potentially conferring ethanol tolerance will be tested for their phenotype using knockout and rescue strains under ethanol stress.

P–2

Trehalose metabolism and its role in the ethanol tolerance in *Saccharomyces cerevisiae*

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The performance of yeast in fermentation processes is often compromised due to the inhibitory effects of accumulating ethanol. Finding a means of improving ethanol tolerance in yeast will therefore be of importance to industries such as breweries, wineries and bioethanol manufacturers. Trehalose, a disaccharide of glucose found in plants, yeast cells and many other microorganisms, has been associated with tolerance to a range of stresses including osmotic pressure, freezing, thawing, dehydration and exposure to ethanol.

Studies at Victoria University have shown that the genes *TPS1*, *TPS2* and *TSL1*, which encode enzymes for trehalose biosynthesis, are highly up-regulated in yeast during ethanol stress. This supports previous studies that implicate trehalose synthesis in enabling yeast cells to acclimatise to ethanol stress. The role of the trehalose synthesis pathway in ethanol stressed yeast cells however is yet to be determined. In our investigation, growth profiles of *S. cerevisiae* strains with gene knockouts for either *TPS1*, *TPS2* or *TSL1* have been compared under various growth conditions. All three knockouts showed essentially the same growth characteristics as the parent under ethanol stress when grown in YPD medium. The same knockouts and the parent were then grown in minimal medium at a range of ethanol concentrations, and in this case the knockout for *TPS2* showed increased sensitivity to ethanol. We are currently working towards defining the role of this gene in ethanol tolerance in yeast.

P–3

Evidence of genome loss in interspecific hybrids of S*accharomyces* yeast

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Saccharomyces cerevisiae, *Saccharomyces cariocanus* and *Saccharomyces paradoxus* are closely related species of yeast belonging to the *Saccharomyces* sensu stricto complex. They can mate, but produce infertile offspring as they are incapable of undergoing a functional meiosis. However, such hybrids can efficiently propagate asexually via mitosis. Hybrid formation results in the creation of novel genomes, not only by combining two sets of divergent genes, but also by accelerating chromosomal modifications (such as gene silencing, gene loss or intergenomic translocations). Results in plants and animals have demonstrated that rDNA gene silencing, and occasionally rDNA gene loss, is widespread in interspecific hybrids. Hybrids of *S. cerevisiae* X *S. cariocanus* and *S. cerevisiae* X *S. paradoxus* were produced using rare mating. PCR-RFLP screening showed that some hybrids were found to be missing the *S. cariocanus* rDNA locus. Further experiments, mainly carried out using the PCR-RFLP method, have shown that these hybrids lack DNA from the rDNA locus to the right end of the chromosome, indicating that recombination occurred at the rDNA repeats. *S. cerevisiae* X *S. paradoxus* hybrids were also shown to have lost this section of Chromosome XII. Studies are being performed to determine whether hybrids that have lost the rDNA sequences have a selective advantage.

P–4

Comparative proteomics: Differential protein expression in a winemaking strain of *Saccharomyces cerevisiae*

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Extensive phenotypic variation can exist between individuals of a single species. Elucidating the genetic basis for this variation, especially in the post genome era, has received considerable attention in view of its importance in a broad range of objectives including predicting human disease, defining biodiversity and improving agricultural yield. *Saccharomyces cerevisiae* wine yeast strains have been selected over thousands of years of winemaking for properties that include fast growth in high sugar grape juices, high yield and tolerance to ethanol and, more recently, sulfite resistance and the biosynthesis of flavour and aroma compounds at concentrations beneficial for wine quality. However, the genes that contribute to these favourable properties have not yet been elucidated. By using a comparative proteomic approach, differences in protein expression as well as possible modifications were observed in a wine strain compared to a laboratory strain. This is the first known comparison of an *S. cerevisiae* commercial wine strain to an *S. cerevisiae* laboratory strain using proteomics. Proteins identified using mass fingerprinting (MALDI-TOF) include glycolytic enzymes, proteins involved in amino acid metabolism, and signalling proteins. Interestingly, there is the same level of transcript for each of these strains at the same cell number during fermentation, indicating post-transcriptional mechanisms may differ between strains. Furthermore, the metabolome of a deletion mutant of one of the genes that was overexpressed at the protein level in the wine strain, SHM2, revealed that despite no changes to fermentation rate or growth, there is substantial changes to the metabolic profile.

P–5

Application of genome-wide expression analysis to identify molecular markers useful in monitoring industrial fermentations

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The ability to monitor the progression of large and expensive industrial lager fermentations is paramount to a quality end-product. Analysis of yeast gene expression using molecular markers that indicate stress conditions, have been applied to monitor the progression of some fermentations. These are very useful in alerting the brewer to the possibility of problems with the fermentation, but the lack of specificity of the markers has not made it possible to identify the nature of the difficulties. We used genome-wide expression analysis to identify molecular markers useful in the detection of fermentation conditions that are deficient in zinc. Zinc depletion causes the phenomenon known as 'stuck brews' where the lack of zinc causes yeast fermentation activity to cease. Genome-wide expression analysis of an industrial yeast strain identified the homologues, *YOR387*c and *YGL258w*, as highly inducible in zinc-depleted conditions. Induction was specific for zinc deficiency and was dependent on the Zap1p transcriptional activator. The results indicate that these sequences may be valuable molecular markers for detecting zinc deficiency in industrial fermentations.

P–6

The protein import receptor Tom20 from *Arabidopsis thaliana* is targeted to mitochondria in *Saccharomyces cerevisiae*

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The vast majority of proteins destined for location in a mitochondrial compartment make their way there via a series of receptors and pore forming units known collectively as the TOM complex. There are at least three different proteins in the *S. cerevisiae* TOM complex that are known to act as receptors for mitochondrial proteins; Tom70, Tom22 and Tom20. Genome sequencing has revealed homologs to each of these TOM proteins in several vertebrate and invertebrate species. A recent examination of the *Arabidopsis thaliana* TOM complex revealed that the pore forming TOM subunits have been conserved through plant evolution, but the receptor TOM subunits present in fungi and animals are absent in plants. In their place is an ~20kDa protein named AtTom20. The plant TOM putative receptor protein shares no significant sequence similarity to the Tom20 of non-plant species and is anchored in the outer membrane of mitochondria via it's C terminus, the opposite orientation of non-plant Tom20s.

Despite these differences, *A. thaliana* Tom20 is targeted to the outer mitochondrial membrane when expressed in *S. cerevisiae*. Here we show the preliminary steps taken towards demonstrating the convergent functions of plant and non-plant Tom20s, and how *S. cerevisiae* can be exploited as a model system for the study of heterologous proteins.

P–7

The role of acetaldehyde in promoting yeast tolerance to ethanol stress

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During fermentation yeast cells are physiologically challenged by osmotic stress, changes in pH and exposure to increasing levels of ethanol. Yeast subjected to these stresses have reduced cell growth and diminished viability, consequently limiting productivity. Although the principle causes of decreased fermentation performance in ethanol-stressed cells are yet to be identified, it is known that yeast acclimatise more quickly to ethanol stress in the presence of small quantities of acetaldehyde; the biochemical processes underpinning this effect are however unknown. A key objective of our work is to identify the mechanisms associated with the acetaldehyde-mediated acclimatisation of yeast to ethanol stress, to facilitate the development of: yeast strains with improved ethanol tolerance and/or strategies for that brewers can use to improve ethanol tolerance in yeast.

This project has focused on comparing gene expression in *Saccharomyces cerevisiae* during ethanol stress in the presence and absence of acetaldehyde. Yeast was inoculated into four different flasks containing either: a rich medium; medium with added acetaldehyde; medium with ethanol; and medium containing both acetaldehyde and ethanol. Samples were taken during the period of adaptation to the ethanol stress (i.e. growth lag period). Gene array analysis of the samples identified some significant changes in gene expression caused by the presence of acetaldehyde in the ethanol-stressed samples. There was a significant up-regulation of genes associated with protein biosynthesis, supporting the observed stimulatory effect of acetaldehyde on the growth of ethanol-stressed cells. Acetaldehyde also caused the up-regulation of genes associated with the carbon metabolism in both unstressed and ethanol-stressed cells. Whether this accounts for its growth-stimulatory effect in ethanol-stressed yeast is currently under investigation.

P–8

Improved yeast viability and vitality of Castlemaine yeast strain 1152B1 with yeast dilution harvesting system

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Castlemaine yeast has traditionally been used to produce beers such as the XXXX brands *Bitter* and *Gold*. Over the last 18 months extensive research work has been carried out to establish what stresses yeast are exposed to and what can be done to improve viability and vitality. Due to the move from gravity brewing to high gravity brewing, the alcohol content of the yeast supernatant has increased to around 8% (v/v) that is indicative of the liquid medium that the 1152B1 strain tries to survive in. Protease studies were carried out to ascertain the amount of protease enzyme the yeast secretes due to yeast cell stress whilst exposed to a potentially toxic environment. The high levels of yeast protease confirmed that the alcohol content is a contributing factor and that diluting the alcohol by around 30% would significantly reduce the protease activity and hence the stress levels on the yeast cell. A project is now underway to engineer and implement a dilution system that will automatically dilute the yeast slurry *en route* from fermenter to storage. This will be accomplished by a communication loop between the controlling valve and the Aber unit that will monitor the viability of the yeast slurry in line during transfer.

P–9

Ethanol tolerant mutants of *Saccharomyces cerevisiae*

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Yeast performance is compromised during alcoholic fermentations due to byproduct inhibition and ethanol is arguably the product with the greatest impact, acting as a potent chemical stress. This stress decreases fermentation productivity and limits the final ethanol yield. The characteristic signs of cell stress are reduced cell growth rate and viability, and an increased growth lag period. This is accompanied at a molecular level by the induction of stress response genes, some of which undoubtedly play a role in acclimatizing yeast cells to ethanol stress. The yeast research group at Victoria University is currently identifying the key genes involved in this acclimation with the aim of 'designing' yeast with increased ethanol tolerance or modifying fermentation processes to improve ethanol productivity and yield.

Our investigations into the stress response of *S. cerevisiae* have included the generation of chemically- and spontaneous-derived ethanol-tolerant mutants that were isolated using adaptive evolution in chemostats, with ethanol providing a selective pressure. Compared to the parent strain, these mutants have considerably higher acclimation rates to non-lethal ethanol concentrations and greater tolerance to lethal ethanol concentrations. In batch culture, the mutant and parent strains grew similarly in the absence of ethanol stress although the mutant strains grew to higher final populations. The acclimation rate of the mutants to 6.5% v/v ethanol was 47% higher than the parent strain and their specific growth rates in the presence of this ethanol concentration were 18% higher than the parent strain. An ethanol concentration of 9% v/v was found to be lethal to the parent strain yet the mutants grew slowly at this ethanol concentration. Cultures of the parent strain were totally non-viable after exposure for 2 hours to 18% v/v ethanol, yet at this ethanol concentration cultures of both mutants had substantial viable populations after exposure for 24 hours. To characterise the molecular basis of this acquired ethanol tolerance, we are currently using microarrays to identify differences in transcription between the parent and mutant strains in the absence and presence of ethanol stress.

P–10

The *Saccharomyces cerevisiae* alcohol acetyl transferase Atf1p is localized in lipid particles

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The yeast alcohol acetyl transferase I, Atf1p, is responsible for the major part of volatile acetate ester production in fermenting *Saccharomyces cerevisiae* cells. Some of these esters, such as ethyl acetate and isoamyl acetate, are important for the fruity flavors of wine, beer and other fermented beverages. In order to reveal the subcellular localization of Atf1p and further unravel the possible physiological role of this protein, *ATF1::GFP* fusion constructs were overexpressed in brewer's yeast. The transformant strain showed a significant increase in acetate ester formation similar to that of a *ATF1* overexpression strain, indicating that the Atf1p-GFP fusion protein was active. UV fluorescence microscopy revealed that the fusion protein was localized in small, sphere-like organelles. These organelles could be selectively stained by the fluorescent dye nile red, indicating that they contained high amounts of neutral lipids and/or sterols, a specific characteristic of yeast lipid particles. Purification of lipid particles from wild type and mutant cells confirmed that the Atf1p-GFP fusion protein was located in these organelles. Furthermore, a clear alcohol acetyl transferase activity could be measured in the purified lipid particles of both wild type and transformed cells. The localization of Atf1p in lipid particles may indicate that Atf1p has a specific role in the lipid and/or sterol metabolism that takes place in these particles.

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LATE ABSTRACTS

P–11

Genetic diversity of *Brettanomyces*/*Dekkera* strains isolated from Australian wines

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Descriptors such as “medicinal”, “barnyard” and “Band-aid®” have been used to describe the aroma of wines tainted with 4-ethylphenol (4EP), the compound generally equated with “brettiness” of wine. The origin of 4EP in wine has been linked to the *Brettanomyces*/*Dekkera* genera of yeast, particularly the species *Dekkera bruxellensis*.

Classification of the *Brettanomyces*/*Dekkera* genera has been revised in recent years through the advent of molecular typing techniques, with a number of former species grouped together as *D. bruxellensis*. There is, however, little information regarding strain variability within this species and it is possible that genetically divergent strains may exhibit altered metabolic capacity to synthesise 4EP from its coumaric acid precursor.

In this study, amplified fragment length polymorphism (AFLP) was applied to examine the genetic diversity of *Dekkera* strains in Australian wine. This technique has been demonstrated to successfully discriminate yeast strains with a high degree of resolution and reproducibility.

Significant genetic divergence was observed between the 43 isolates typed, with some strain groupings exhibiting only 58% similarity to one another. Despite this relatively large difference in genetic similarity, according to internal transcribed spacer-polymerase chain reaction-restriction fragment polymorphism (ITS-PCR-RFLP) and morphological characteristics all strains were from the *D. bruxellensis* species. In comparison, strains of *Saccharomyces cerevisiae* exhibit around 97% similarity based on AFLP data.

A majority of isolates (70%) were highly similar based on AFLP and could be grouped together as one strain (DB 26). This strain was predominant across 13 different wine regions, while other strains were only evident in particular wine regions or even wineries. Further studies will attempt to elucidate the relationship between genetic divergence and metabolic characteristics, particularly the ability to synthesise 4EP under various fermentation conditions.

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Effect of ammonium supplementation of a Chardonnay must on wine aroma

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Yeast are subjected to a variety of stresses during the wine alcoholic fermentation, including nitrogen limitation. Nitrogen stress has important implications for wine quality. Ammonium (in form of diammonium phosphate, DAP) is typically added to grape must before and during fermentation to reduce the risk of stuck and sluggish fermentation and to prevent H_2S formation. The repercussion of this practice on the formation of other volatile compounds and its impact on the sensory quality of wine is not yet well understood. In this study a low nitrogen Chardonnay must (160 mg YAN/L) was supplemented with ammonium to obtain a medium (320 mg YAN/L) and high (480 mg YAN/L) concentration of assimilable nitrogen. The effect of these must nitrogen concentrations on the chemical and sensory characteristics of the resulting wines was studied. Increasing must nitrogen content increased yeast biomass and decreased fermentation rate. The wine derived from medium nitrogen must relative to the low nitrogen must contained an increased concentration of esters and acids, but a decreased content of higher alcohols. The wine produced with the high concentration of nitrogen showed a similar trend in the concentration of aroma compounds but, in comparison with the wine from the medium nitrogen must, had a higher concentration of ethyl acetate and acetic acid, whereas there was a decrease or no change for the remaining compounds. The impact of these changes on the sensory characteristics of wines was also determined. The control low nitrogen wine was rated high in the “stale beer” “cheesy” and “sweaty” descriptors. The medium nitrogen wine showed a reduction of these aromas and an increase in floral and fruity attributes. The high nitrogen wine was also rated low for the “stale beer” “cheesy” and “sweaty” attributes but had the highest rating for “acetic” and “nail polish remover”. These results, which provide quantitative chemical and sensory data, reinforce the importance of optimizing the nitrogen content of white wine musts.